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Impact of Fluoridated Dental Products on Color Change of Bleached Tooth Enamel: An In Vitro Study

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Abstract

Aims: To assess the impact of fluoridated dental products (Toothpaste, mouth rinse, and fluoride varnish) on bleached tooth enamel's color when used before, after, or before and after the tooth bleaching process.

Method: A total of (64) bovine permanent incisors were prepared and divided into two main groups: Group (A) was treated before and after the bleaching. Group (B) was treated after bleaching only. The bleaching process used an Opalescence boost of 40% hydrogen peroxide. Each main group was divided into (4) subgroups: (A1, B1) Control, (A2, B2) treated with FluorKIN mouthrinse, (A3, B3) treated with FluorKIN toothpaste, (A4, B4) treated with Proshield Fluoride Varnish.

A spectrophotometer was used to evaluate color change ΔE.

Results: There was a statistically significant increase in ΔE after bleaching and no change after treatment, and there was no statistically significant difference between the group receiving bleaching only and the group receiving preventive protocol before bleaching.

Conclusion: According to the ΔE means results in the present study, the fluoride treatment protocols had no impact on color change when used before, after, or before and after dental bleaching.

Introduction:

Healthy, white, clean teeth can help enhance life, increase self-confidence, and decrease complaints. This depends on two factors: maintaining oral health through various methods, like tooth-brushing and flossing and using cosmetic treatments to whiten teeth (1).

Both intrinsic and extrinsic stains are possible. Stains intrinsic to the tooth might be detected in the dentin underneath the enamel or in the enamel itself.

Tetracycline incorporation, a range of metabolic illnesses, high fluoride intake during tooth growth (fluorosis), and other systemic and metabolic variables are all potential causes (2).

Extrinsic staining appears on enamel and exposed dentin, particularly on hard-to-clean surfaces and those with thick pellicle layers by direct adsorption of both organic and inorganic chromophores to the tooth (mainly if there is a rough tooth surface) or integrated into biofilm, pellicle, and/or calculus (2).

The safest and most efficient technique to whiten teeth is tooth bleaching. Today's procedures for whitening teeth at home use trays and have low amounts of hydrogen or carbamide peroxide. In contrast, dental professionals put high concentrations of hydrogen peroxide or carbamide peroxide during in-office bleaching methods (3). Despite the positive color change, peroxide-based whitening has been linked to adverse side effects, including demineralization, erosion, and tooth sensitivity (4). A decrease in microhardness and increased surface roughness values was observed as an adverse effect following the bleaching application (5, 6).

The bleached enamel surface is susceptible to stains or discoloration from dark or colored fluids such as tea, coffee, juices, wines, and cola-based soft drinks. While some acidic solutions contain ethanol and/or pigments, others contain substances that may accelerate demineralization. Additionally, frequent use of tobacco products, artificial food colorings, and certain beverages are thought to be the primary causes of teeth discoloration, staining, and darkening (7). An increase in enamel surface roughness promotes the adhesion of Streptococcus mutans to tooth enamel (8). For these reasons, the damaged enamel surface must be recovered after bleaching.

The erosive effects of bleaching techniques utilizing sodium fluoride-based (NaF) materials have been studied using various strategies (4). Due to fluoride stimulation fluoride and calcium deposition on enamel surfaces, it was the first and most widely utilized substance in after-bleaching treatment (9). Fluoride therapy could reduce the deleterious effects of bleaching agents. Calcium fluoride is created through the incorporation of fluoride ions into demineralized regions. Fluoride ions can also replace the enamel's apatite hydroxyl groups, producing fluorapatite (9). This significantly lowers mineral loss and restores microhardness (10) and roughness (11). It is possible that fluoride's mineralizing capability, when used before to bleaching, could affect tooth enamel permeability, or possibly prevent H_2O_2 from penetrating the tooth structure and lessen the bleaching effect. Additionally, some fluoride product ingredients may cause teeth darkening when used after bleaching, which would reduce the effectiveness of the whitening process (10).

The following study aims to assess the impact of fluoridated dental products (toothpaste, mouth rinse, and fluoride varnish) on bleached tooth enamel's color when used before, after, or before and after the tooth bleaching process.

Materials and Methods

This study protocol was conducted *in vitro*, submitted, and approved by the local Ethics Committees, REC reference no. (UoM.Dent/A.DM.76/22) Research Ethics Committee of Collage of Dentistry University of Mosul, Nineveh, Iraq. This in vitro study was conducted from 28 /Dec /2022 to 23 /Sep /2023 at the Department of Pedodontics, Orthodontics, and Preventive Dentistry at College of Dentistry, University of Mosul.

Teeth Samples Collection

Bovine incisors recently extracted were obtained from the slaughterhouses of Kirkuk governorate. The teeth were cleaned and washed with tap water and then stored in 0.1% thymol solution (Switzerland) at 4ºC until use (12, 13). Among all the collected teeth, only Sixty-four teeth with no cracks, stains and developmental defects were taken for color change analysis.

Teeth Samples Preparation

The roots were cut using a straight Diamond sectioning disc bur with a low-speed engine and continuous water cooling to prevent enamel damage after the teeth were thoroughly cleaned and polished with non-fluoridated pumice (Argentina) (14). The crowns of teeth were embedded in blocks of auto-polymerized cold-cure acrylic resin (Acrosun, Betadent com. Iran) (15) using 15 mm high cylindrical plastic tubes with flat, parallel upper and lower borders, the outer labial surface of the teeth is pointed upward. Each tooth's labial surface was ground and polished ten times in one direction with grit paper (grit 400, 600) (Saudi Arabia company) to create a flat and uniform enamel surface specimen for surface roughness testing (14). Every sample was sanded using the same technique with new carbide silicon abrasive paper (11). The samples were placed in an ultrasonic cleaner for 10 minutes to remove residues (16). By measuring the distance between the incisal edge, the cementoenamel junction, and the mesiodistal dimension, the middle third of the labial surface of the crown was marked using a caliper. On the labial surface of the samples, a (10×7 mm^2) adhesive tape was applied in the middle of the crowns (15). The crowns were painted with acid-resistant nail polish; after the samples had dried, the tapes were removed to reveal the enamel surface (15), figure 1.

Bleaching procedure

All bleaching procedures were done using Opalescence Boost, Ultradent, USA (40% Hydrogen peroxide) per the manufacturer's instruction—application of bleaching product considering the maximum duration suggested by the manufacturer (3 x 20 minutes). The two syringes of bleaching material come together; one syringe contains 1.1% sodium fluoride and 3% potassium nitrate with a chemical activator, and the second contains hydrogen peroxide (HP). Mixing materials was done by pressing the plunger of one syringe to push all content into the other; this was repeated 50 times to ensure good mixing. The concentration of HP after mixing is 40%.

A thin layer (0.5 -1 mm) of HP gel was applied to the enamel surface and left for 20 minutes with periodically checking and re-applying areas that had been thinned or needed replenishing; then this layer was washed with water and lightly air-dried; this procedure was repeated two additional times. The total period of the bleaching procedure was 1 hour.

Treatment Cycle

A total of (64) bovine permanent incisors were prepared and divided into two main groups: Group (A) was treated before and after the bleaching. Group (B) was treated after bleaching only. Each main group was divided into (4) subgroups: (A1, B1) Control, (A2, B2) treated with FluorKIN mouthrinse, (A3, B3) treated with FluorKIN toothpaste, (A4, B4) treated with Proshield Fluoride Varnish. During the experiment, all prepared samples were stored in deionized water (14, 17). Initially, all groups were transported for color analysis performed at the baseline. Each subgroup in group A (A1 D.D.W., A2 Mouthrinse, A3 Toothpaste, and A4 Fluoride Varnish) was treated with the corresponding remineralizing agent before the bleaching process and preserved in deionized water, the samples in subgroups (B1 D.D.W., B2 Mouthrinse, B3 Toothpaste, and B4 Fluoride Varnish) were bleached with 40% hydrogen peroxide as manufacturer instruction then preserved in deionized water, after that all groups were transported for color analysis.

After analysis, subgroups (A1 D.D.W., A2 Mouthrinse, A3 Toothpaste, and A4 Fluoride Varnish) were bleached with 40% hydrogen peroxide and preserved in deionized water. In contrast, subgroups (B1 D.D.W., B2 Mouthrinse, B3 Toothpaste, and B4 Fluoride Varnish) were treated with the corresponding remineralizing agent and preserved in deionized water, and all groups were transported for color analysis.

Subgroups (1A D.D.W., 2A Mouthrinse, 3A Toothpaste, and 4A Fluoride Varnish) were treated again with corresponding remineralizing agents after the bleaching process and preserved in deionized water and transported for color analysis.

Following the completion of the remineralization cycle, all samples were thoroughly cleaned with distilled water before being restored to deionized water.

Subgroup 1 (Control group) Samples were immersed in distilled water with daily refreshment of solution during the experiment.
Subgroup 2 (Fluor KIN mouthwash) Samples were immersed in 10 ml of 0.05% NaF mouthwash (Fluor KIN mouthwash) for five minutes every 12 hours (twice a day), then without rinsing, samples were immersed in deionized water (18) , figure 2.
Subgroup 3 (Fluor KIN toothpaste) Specimens were treated with Fluor KIN toothpaste every 12 hours for five minutes, then the paste was wiped by soft cot-ton without rinsing, followed by immersing specimens in deionized water (18, 19) , figure 3.
Subgroup 4 (Proshield varnish) Using a micro brush, the varnish was applied thinly, and the specimens were kept in deionized water, figure 4. After six hours, the varnishes were delicately removed with a scalpel blade, being careful not to touch the enamel surface. (20). All samples were rinsed with deionized water for a minute while not scratching the surfaces before beginning the treatment and bleaching cycle (21, 22).

***Color analysis* (ΔE, L*, a*, b*)**
Using a spectrophotometer (Vita Easyshade 5, Germany), the color of the tooth was measured using the Commission International de l'Eclariage (CIE-L*a*b*) color system, which captured the color of the tooth and analyzed it three-dimensionally into L* value: lightness (0-100), a* value: green-red chromacity (-150 to +100), and b* value: blue-yellow chromacity (–100 to + 150) (23).
The aperture of the spectrophotometer is 5 mm, and it is constant for all samples; the device had been calibrated before beginning and between each measurement using a calibration block found on the device's charging base. The samples were gently dried using a drying paper without dehydration, and the spectrophotometer's aperture was fixed on the exposed enamel window at a right angle to the tooth surface (24) , figure 5. Three measurements were done for each sample, and these measures' mean were taken.
To compare the variations in tooth color at various time intervals in relation to the baseline, the color changes (ΔE) after bleaching and after treatment were measured using the following equation: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where ΔL, Δa, and Δb are the differences in the L*, a*, and b* values respectively.
Therefore, $\Delta E_{\text{after bleaching}} = [(L_{\text{Baseline}} - L_{\text{bleaching}})^2 + (B_{\text{Baseline}} - B_{\text{bleaching}})^2 + (A_{\text{Baseline}} - A_{\text{bleaching}})^2]^{1/2}$, and $\Delta E_{\text{after treatment}} = [(L_{\text{Baseline}} – L_{\text{after treatment}})^2 + (B_{\text{Baseline}} - B_{\text{after treatment}})^2 + (A_{\text{Baseline}} - A_{\text{after treatment}})^2]^{1/2}$ (23).

Results

Before applying any test, the first step was determining whether the data were normally distributed. Kolmogorov-Smirnova and Shapiro-Wilk tests were used to do this, and because the data were normally distributed, parametric tests and one-way ANOVA were chosen.

Color Change (ΔE)
Table (1) displays mean ΔE and standard deviation for all the subgroups in group (A) and Duncan's multiple range test of (ΔE1, ΔE2, and ΔE3) mean values in each group, In all subgroups, there was an increase in ΔE2 value after bleaching and remain with no difference after final treatment ΔE3.
Figure (6) shows the ΔE means values at ΔE1 (After initial treatment and Baseline), ΔE2 (After bleaching and Baseline), and ΔE3 (After final treatment and Baseline) of group A. After the first treatment, all groups had little increase in ΔE values.
After bleaching, all groups highly increased the ΔE values with slight differences among them.
After the final treatment, the Mouthrinse group had the maximum increase in ΔE value. Fluoride varnish, toothpaste, and control group had the minimum increase in ΔE value with slight differences among them.
Table (2) illustrates the comparison among (ΔE1 and ΔE2) mean values in each group by Independent sample T-test, and the

results showed that there were no statistically significant differences $p > 0.05$ in all groups between ΔE 1 and ΔE 2. Figure (7) shows the ΔE means values at ΔE1 (between baseline and after bleaching) and ΔE2 (between baseline and after treatment) of group B, which concluded that after bleaching, all groups had a high increase in the ΔE means values with slight variation among them.
After treatment, the high increase in the ΔE means values remain at the same level with slight variation after remineralization.

Discussion

Bovine Teeth Substituted Human Teeth

Enamel samples for the current study were taken from incisors of cows. Since the composition, hardness, and tensile strength of bovine teeth are remarkably similar to those of human teeth, they have long been used to replace human teeth so the bovine teeth could be utilized as an excellent alternative to human teeth in dental researches (25).
Despite the fact that the shade of bovine enamel is lighter than the shade of human enamel (26), young bovine teeth are regarded as a workable model for analyzing bleaching procedures (27, 28).
Bayrak, et al. (29) concluded that due to the similarities in chemical and physical characteristics, such as composition and hardness, between bovine and human teeth, human teeth have long been substituted in experiments. Furthermore, the use of bovine teeth leads to more standardized test conditions because the composition of bovine teeth varies less than that of human teeth. The size of bovine teeth helps ensure enough enamel surfaces, and the chemical composition of bovine enamel and its response to erosive attack are comparable to those of human enamel. Additionally, using bovine specimens is advantageous because one bovine incisor can yield up to four or five specimens.

Color Study Assessment

In the present study, color measurement was done using a VITA Easy shade ® spectrophotometer; the instrumental color analysis offers a potential advantage over other methods. This device generates spectral color space readings that are not only precise but also quick, repeatable, and reliable. The VITA Easyshade® spectrophotometer also has an advantage over laboratory spectrophotometers and colorimeters, which need a flat surface and are therefore not recommended for measuring the spectral color of teeth. Additionally, laboratory spectrophotometers cannot measure the natural teeth's translucency (30).
The CIE Lab color system coordinates (L*, a*, b*) used in the current study locate the object in a 3D color space and quantify the differences in lightness and chromaticity (ΔL*, Δa*, Δb*) for the total color difference (ΔE) calculation. The statistical analysis showed similar results for the total color difference (ΔE) and the parameters L*, a*, and b* in isolation (31).
According to the current study, all groups in this investigation showed a significant change in ΔE values after dental bleaching, consistent with the Vieira-Junior, et al. (32) study. A decrease in the b* coordinate and an increase in the L* coordinate were linked to the bleaching effect. The bleaching process had no noticeable impact on the a* value, indicating a less yellowish and lighter color for the tooth, supporting the efficacy of the whitening procedure (10). The mean values of the total color change (ΔE) following in-office dental bleaching exceeded 4.2 units, the recommended standard values for the clinical acceptability of color differences (33, 34).
It is possible that fluoride's mineralizing capability, when used before bleaching, could affect tooth enamel permeability or possibly prevent H_2O_2 from penetrating the tooth structure and lessen the bleaching effect. In the present study, applying fluoridated mouth rinse, toothpaste, and varnish before the bleaching procedure did not affect ΔE promoted by bleaching. Additionally, research examining desensitizing products with 2% sodium fluoride before bleaching with 35% H_2O_2 found no impact on the ΔE (35). Another study conducted by Crastechini and Borges (10) revealed that the ΔE induced by bleaching was not

affected by the previous application of calcium silicate/phosphate fluoride gel.

Additionally, fluoride applied to teeth after bleaching may cause discoloration, which will reduce the effectiveness of the whitening. However, the current study showed no statistical difference in mean results of final ΔE (between final treatment and baseline) among all subgroups in the group (A and B). This means fluoride concentration did not affect color change after in-office dental bleaching. After remineralization, the means of general color change (ΔE) were larger than 4.2 units, which is recognized as a standard value of clinical acceptability of color difference, and the color analyses of all groups revealed an increase in L* value (black-white axis) and a decrease in b* values (blue-yellow axis), these results came in agreement with Vieira and Vieira-Junior (34) study which claimed that the addition of F had no impact on the effectiveness of bleaching.

Despite the remineralizing procedures, all bleached groups showed statistically similar ΔE overall. According to the results of the current study, the application of remineralization fluoridated products before, after, or before and after the bleaching procedure did not affect the bleaching efficacy since the obtained final results of general color change (ΔE) were greater than 4.2 units (34).

Our results disagreed with Morrier's (36) study, which concluded that fluoridated products should not be used to treat lesions on the canines and incisors because they can stain teeth.

Despite the green-colored mouth rinse that was used in the present study, the ΔE results of the mouth rinse subgroup were not statistically significantly different from toothpaste and fluoride varnish subgroups after final treatment indicating that the effectiveness of whitening treatment was unaffected by the use of colorful mouth rinse following in-office tooth bleaching. These results agreed with Vieira-Junior and Ferraz (32) that the use of mouth rinses containing colors does not limit the effect of tooth whitening.

The present study showed a slight decrease in ΔE values of mouth rinse, toothpaste, and fluoride varnish subgroups after the final treatment. Despite this reduction was not statistically significantly different from ΔE results after bleaching, it indicates that the remineralization had some effect on tooth color. A study by Kim, et al. (37) suggested that mineral loss after bleaching will produce a porous substructure induced by an altered light-scattering mode within the enamel structure, producing a whitish appearance.

Conclusions

Fluoridated agents used in the present study did not affect color change (ΔE) results when used before, after, or before and after in-office dental bleaching. They did not interfere with the whitening effect of the bleaching agent.

Fig. (1): (A) and (B) A widow of exposed enamel surface after drying of nail varnish and removing of adhesive tape.

Fig. (2): Samples were immersed in a glass containing (Fluor KIN Mouthrinse) and left 5 min before rinsing with water.

Fig. (3): The paste was left on samples for 5 min before rinsing with water.

Fig. (4): Samples were coated with a thin layer of varnish using a fine brush for 6 hours.

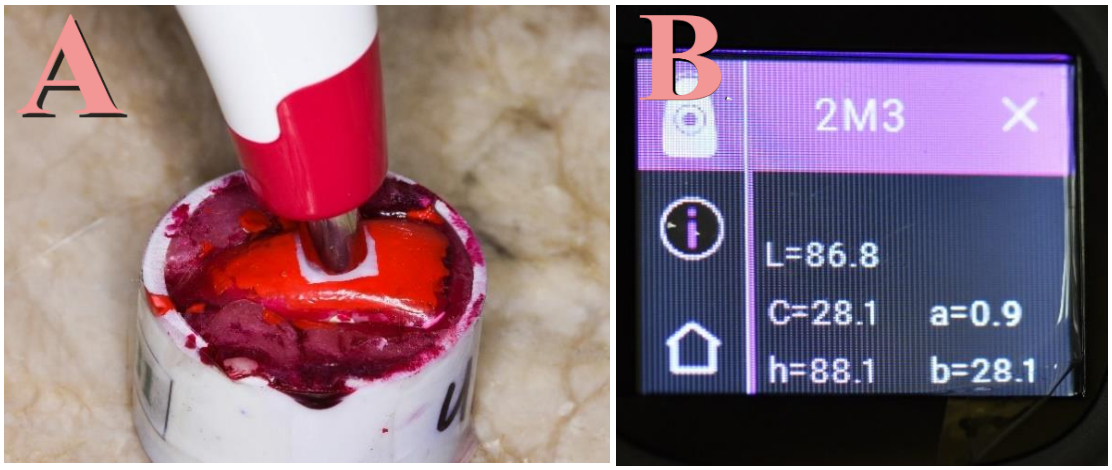

Fig. (5): (A) Aperture was fixed at a right angle to the tooth surface, **(B)** CIE lab values showed in Vita easyshade screen.

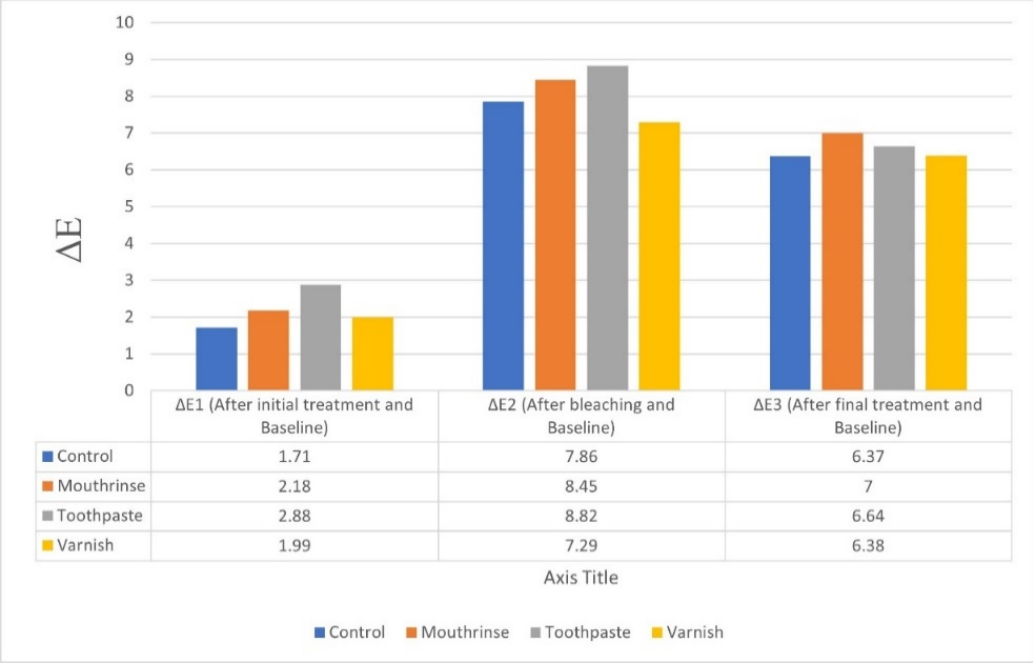

Fig. (6): Show the ΔE means values at ΔE1 (After initial treatment and Baseline), ΔE2 (After bleaching and Baseline) and ΔE3 (After final treatment and Baseline) of group A.

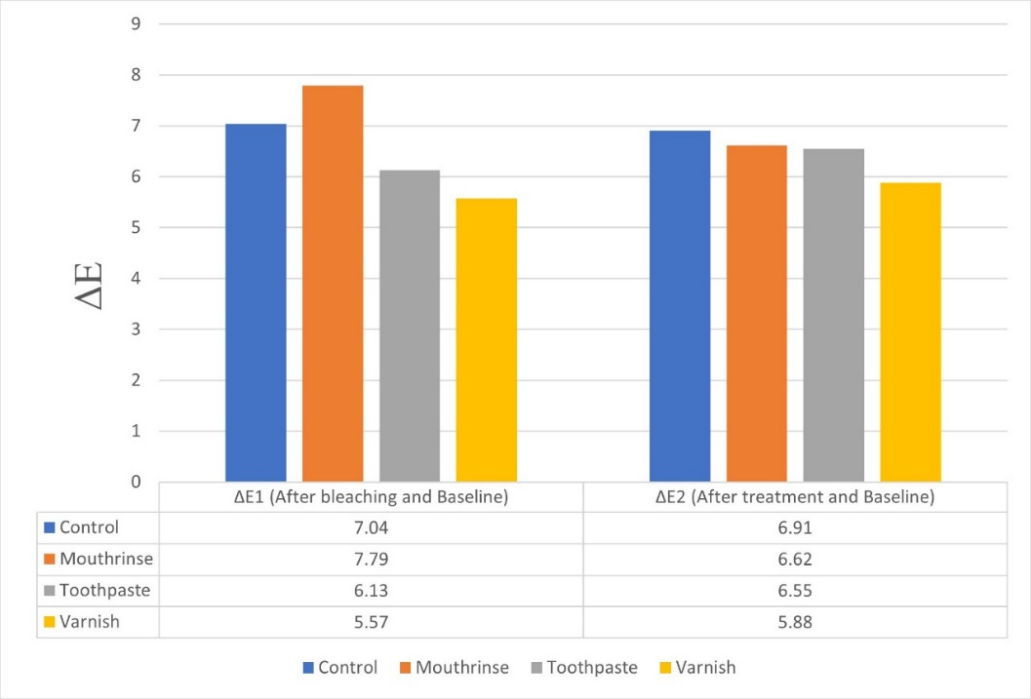

Fig. (7): shows the ΔE means values at ΔE1 (between baseline and after bleaching) and ΔE2 (between baseline and after treatment) of group B.

Table (1): Mean values, Standard deviation, and Duncan's Multiple Range tests between mean values of (ΔE1, ΔE2, and ΔE3) in each subgroup of group A.

Group A		Control	Mouthrinse	Toothpaste	Varnish
ΔE1 (After initial treatment and Baseline)	Mean	1.7122 Ab	2.1843 Ab	2.8874 Ab	1.9994 Ab
	N	8	8	8	8
	SD	1.25792	1.26998	2.03096	1.18443
ΔE2 (After bleaching and Baseline)	Mean	7.8685 Aa	8.4507 Aa	8.8228 Aa	7.2908 Aa
	N	8	8	8	8
	SD	2.98514	1.61035	2.44585	1.99402
ΔE3 (After final treatment and Baseline)	Mean	6.3797 Aa	7.0079 Aa	6.6490 Aa	6.3826 Aa
	N	8	8	8	8
	SD	2.08188	3.25986	3.12503	2.94584
Total	Mean	5.3201	5.881	6.1197	5.2243
	N	24	24	24	24
	SD	3.41904	3.46498	3.51015	3.13811

N: Number of the specimens, Std. Deviation: Standard Deviation. Different small letters indicate statistically significant differences within the same column (Vertically). Different capital letters indicate statistically significant differences within the same raw (Horizontally).

Table (2): ΔE Independent samples t-test for group (B).

Group B		N	Mean	t-value	sig	SD	SE Mean
Control	ΔE 1	8	7.0429	0.117	0.909	2.22728	0.78746
	ΔE 2	8	6.916	0.117	0.909	2.12367	0.75083
Mouthrinse	ΔE 1	8	7.7919	1.154	0.268	2.01455	0.71225
	ΔE 2	8	6.6259	1.154	0.268	2.02805	0.71702
Toothpaste	ΔE 1	8	6.1387	-0.337	0.741	2.69864	0.95411
	ΔE 2	8	6.5563	-0.337	0.741	2.22829	0.78782
Varnish	ΔE 1	8	5.5783	-0.574	0.575	0.76777	0.27145
	ΔE 2	8	5.8831	-0.574	0.577	1.29198	0.45678

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