

Comparative Effects of Three Bleaching Techniques on Tooth Discoloration Caused by Tea

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Article Info	ABSTRACT					
<i>Article type:</i> Original Article	Objectives: This study aimed to investigate the impact of three different bleaching protocols on tooth discoloration caused by tea.					
	Materials and Methods: Forty extracted sound premolars were cleaned, disinfected, and their initial color parameters were measured (T1). The teeth were					
<i>Article History:</i> Received: 01 Jun 2022 Accepted: 24 Dec 2022 Published:24 Jul 2023	then immersed in boiled tea solution for 24 hours, and their color was measured again (T2). The samples were divided into four groups of 10 teeth each. Group A was treated with an in-office bleaching gel followed by 30 seconds of light curing. Groups B, C, and D were respectively treated with 0.24M oxalic acid followed by bleaching gel, 5.25% sodium hypochlorite followed by bleaching gel, and a combination of 0.24M oxalic acid, 5.25% sodium hypochlorite, and bleaching gel. The color was measured once more after the interventions (T3). Data were					
* Corresponding author:	analyzed using two-way repeated measures ANOVA, paired sample t-test, and Tukey's test.					
Dental Research Center, Research Institute for Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences Email: <u>Amir gh th@yahoo.com</u>	Results: The mean color change (ΔE_2) between T2 and T3 was significantly higher in group D compared to group A (P<0.05). However, there was no significant difference in color change between groups A, B, and C. Additionally, there was no significant difference in color change between groups B, C, and D. The groups also showed significant differences in ΔL (P=0.007), with the only significant difference found between groups B and C (P=0.001).					
	Conclusion: The combined use of sodium hypochlorite and oxalic acid followed by in-office bleaching gel is more effective than bleaching alone in correcting tooth discoloration caused by tea. This protocol can further reduce yellow coloration.					
	Keywords: Tooth Bleaching; Hydrogen Peroxide; Oxalic Acid; Sodium Hypochlorite; Tea					

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INTRODUCTION

Today, there is a heightened focus on tooth discolorations due to the growing importance of facial esthetics and a beautiful smile. Tooth discolorations have different types based on their etiology, which affect treatment planning. Generally, discolorations can be categorized as intrinsic or extrinsic. Extrinsic types are often caused by tooth staining and can be treated through effective tooth cleaning and prophylaxis. Intrinsic discolorations, on the other hand, involve deeper layers of the tooth structure and require more complex procedures for correction. It's important to

Copyright © 2023 The Authors. Published by Tehran University of Medical Sciences. This work is published as an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (http://creativecommons.org/licenses/by-nc/4). Non-commercial uses of the work are permitted, provided the original work is properly cited. note that extrinsic discolorations can turn into intrinsic if left on the tooth surface for an extended period of time [1].

Tea stain is a common cause of tooth discoloration, as the polyphenol compounds in black tea penetrate the external tooth surface and cause discoloration [2,3]. There have been several techniques developed to correct this type of discoloration.

When selecting a bleaching agent, it is crucial to consider the chemical components responsible for the discoloration. Oxalic acid was the first chemical agent used for tooth bleaching [4]. In a patent by Reinhardt [5], oxalic acid was combined with manganese salts to enhance the performance of manganese compounds as a bleach catalyst. At present, it is widely used for elimination of contaminations caused by iron products in many industries such as the ceramic industry and wood-pulp bleaching in the paper industry [6,7].

Penumatsa and Sharanesha [8], Cárdenas Flores et al [9] and Belkhir and Douki [10] used sodium hypochlorite for the same purpose. However, they first etched the enamel, and then used sodium hypochlorite. Subsequently, they coated the enamel surface with resin materials, and reported more favorable results. Ghousia et al [11] recommended demineralization and deproteinization of teeth prior to bleaching to improve the efficacy of the procedure and enable better penetration of bleaching agents. Following the introduction of chlorine compounds and hydrogen peroxide for bleaching. their application for tooth whitening increased as well [1].

The available evidence does not provide a clear answer regarding which of the mentioned compounds is more effective in correcting tooth discoloration caused by tea. Therefore, the purpose of the present study was to compare the efficacy of three different tooth bleaching protocols in addressing teainduced tooth discoloration. The aim is to determine the most effective protocol for this specific purpose. Our null hypothesis states that there is no significant difference between the three protocols being considered.

MATERIALS AND METHODS

This in vitro, experimental study evaluated 40 caries-free human premolars freshly extracted as part of orthodontic treatment. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.1398.031). The inclusion criteria were teeth with no caries, restorations, developmental defects, enamel cracks, or coronal discoloration. The detection was carried out by a ×2.5 magnifier (Camar, Tehran, Iran). After selection by convenience sampling, the attached soft and hard tissue residues were debrided by a curette. Next, they were cleaned with a prophy brush and pumice paste to eliminate superficial debris and external stains. The teeth were then immersed in 0.5% chloramine T solution for 1 week for disinfection. After rinsing, they were stored in 0.9% sterile saline (0.9% sodium chloride; Daroupakhsh, Tehran, Iran) at room temperature until the experiment (not more than three months). The teeth were then randomly divided into four groups (N=10) and were digitally photographed. Afterwards, their baseline (T1) color parameters were determined by a spectrophotometer (X-rite Ci6x, Tucson, AZ, USA) according to the CIE L*a*b* color space. The spectrophotometer was calibrated prior to use with the calibration tiles provided by the manufacturer. Red dental wax was used to fix the teeth in a standardized position for imaging of their labial surface against a gray background.

Staining:

The apex of all teeth was coated with colorless nail varnish to prevent dye penetration into the root canal. After recording the baseline (T1) color parameters of the teeth, black tea bags (Golestan, Tehran, Iran) were immersed in boiling water with a ratio of 2g per 100 mL and boiled for 5 minutes. The obtained solution was filtered and according to the methodology of Sulieman et al [12], the teeth were immersed in the tea solution at room temperature for 24h to simulate the chronic tooth discoloration caused by tea. Empty tea bags were used to suspend the teeth in the solution and for coding. Next, the teeth were rinsed with water and polished with a rubber cup and pumice paste to remove external stains. Color parameters of the teeth were measured again (T2) under the same conditions as T1, and the L*, a* and b* color parameters were recorded. The teeth then underwent bleaching according to the following methods.

Protocols:

Group A: Pola Office Plus (SDI, Australia) bleaching gel containing 37.5% hydrogen peroxide was applied on the external tooth surface four times for 8 minutes, according to the manufacturer's instructions. The teeth were monitored during this time and after 4 minutes, the gel on the tooth surface was agitated. The teeth were subjected to halogen curing light (Demetron LC, Kerr, Orange, CA, USA) at a 1-2mm distance for 30 seconds with a constant light intensity of 600 mW/cm² to accelerate the hydrogen peroxide reactions.

Group B: In this group, 0.24M oxalic acid solution in water (3%) was prepared [13], by adding 3.026g (126.07g/mol) oxalic acid dihydrate powder (Merck, Germany) to an adequate amount of water to obtain 100 cc oxalic acid solution. The pH was adjusted to 3, by gently adding ammonium hydroxide and constant measurement. This solution was applied on the external tooth surface for 5 minutes [14]. The teeth were then rinsed with distilled water for 1 minute and 37.5% Pola Office Plus bleaching gel was applied on the external tooth surface as explained for group A. Group C: In this group, 5.25% sodium hypochlorite solution was applied on the external tooth surface for 5 minutes. The teeth were then rinsed with distilled water for 1 minute. Next, 37.5% Pola Office Plus bleaching gel was applied on the external tooth surface as explained for group A.

Group D: In this group, 0.24M oxalic acid solution in water (3%) was initially prepared, and applied on the external tooth surface for 5 minutes. Afterwards, the teeth were rinsed with distilled water for 1 minute and 5.25% sodium hypochlorite solution was applied on the external tooth surface for 5 minutes. Subsequently, the teeth were rinsed with distilled water for 1 minute. Finally, 37.5%

Pola Office Plus bleaching gel was applied on the external tooth surface as explained for group A.

Following these procedures, all teeth were rinsed with distilled water for 1 minute and stored in saline for 1 week to reverse the effect of dehydration caused by bleaching. Then, the teeth underwent spectro-photometry, and their L*, a* and b* color parameters were measured (T3). Accordingly, the color change value (Δ E) of each tooth was calculated at different time points (T1, T2 and T3) using the formula:

$$\Delta E = \sqrt{\left[(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2\right]}$$

For statistical analysis normal distribution of data was evaluated by the Kolmogorov-Smirnov test. Paired sample t-test was used to compare the L*, a*, and b* color parameters before and after tea staining. Changes in L*, a*, and b* color parameters and ΔE were analyzed using two-way repeated measures analysis of variance (ANOVA). Pairwise comparisons were performed by Tukey's test and P<0.05 was considered statistically significant.

RESULTS

Table 1 presents the measures of central dispersion for the L*, a*, and b* color parameters in the four groups at different time points. The Kolmogorov-Smirnov test confirmed the normal distribution of data (P=0.200). Thus, paired sample t-test was applied to compare the L*, a*, and b* color parameters before and after tea staining.

The results showed that all parameters changed significantly after tea staining (P<0.001). Table 2 shows the mean change in ΔE , a*, b* and L* (Δa^* , Δb^* and ΔL^*) in the four groups between T1-T2 (Δ_1) and T2-T3 (Δ_2). The mean $\Delta E_2 \pm$ S.E (after bleaching) was 9.93±0.5 in group A, 11.17±0.41 in group B, 12.46±0.49 in group C, and 13.32±0.45 in group D.

The findings from two-way repeated measures ANOVA revealed a significant difference in all color parameters after bleaching as compared to the staining-related parameters (P<0.001).

Time points	Color parameters	Group A		Group B		Group C		Group D	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
T1	L*	72.78	1.97	74.45	2.13	73.12	3.13	75.28	3.51
	a*	0.83	0.53	0.75	0.92	1.01	0.85	0.39	0.5
	b*	12.87	1.79	12.52	2.92	12.43	2.52	11.02	1.81
T2	L*	65.76	3.58	66.44	2.7	66.35	3.4	67.75	2.98
	a*	3	0.9	3	1.69	3.34	1.47	2.96	1.27
	b*	13.89	1.61	14.84	3.47	15.28	2.74	14.68	2.3
Т3	L*	73.81	2.76	74.99	2.05	75.82	2.54	77.35	2.98
	a*	0.36	1.01	0.11	0.79	0.15	0.72	-0.63	0.54
	b*	9	2.07	8.69	4.1	8.14	1.54	6.32	1.78

Table 1. Measures of central dispersion for the L*, a*, and b* color parameters in the four groups at different time points

Table2: Mean and standard error values of the color parameters in the study groups

	ΔΕ		Δа		Δ	b	ΔΙ		
	1	2	1	2	1	2	1	2	
Group A	7.49±1.13	9.93±0.5	2.17±0.25	-2.63±0.33	1.02±0.48	4.89±0.52	-7.01±0.82	8.04±0.46	
Group B	8.84±0.57	11.17±0.41	2.25±0.48	-2.88±0.47	2.32±0.71	-6.14±0.58	-8.00±0.32	8.54±0.5	
Group C	7.39±0.77	12.46±0.49	2.33±0.3	-3.18±0.39	2.84±0.58	7.13±0.56	-6.77±0.36	9.47±0.51	
Group D	9.05±0.77	13.32±0.45	2.57±0.34	3.58±0.28	3.66±0.42	8.36±0.47	7.52±0.54	9.59±0.44	

Different bleaching protocols had significant effects on ΔE (P=0.045). Pairwise comparisons of the groups by the Tukey's HSD test showed no significant difference between groups A, B and C (P=0.414). The difference between groups B, C and D was not significant either (P=0.437). However, group D had a significantly higher ΔE than group A (P=0.025). Moreover, the interaction effect of the two variables (time and bleaching protocols) was significant (P=0.014).

The groups were also significantly different regarding ΔL (P=0.007). According to Tukey's HSD test there was no significant difference between groups A, B and D (P=0.097). Similarly, groups A, C and D did not show a significant difference (P=0.061). However, the difference between groups B and C was significant (P=0.009). The interaction effect of the two variables (time and bleaching protocols) was not significant (P=0.462).

The groups were not significantly different regarding Δa (P=0.232) and the interaction effect (time and bleaching protocols) was not significant (P=0.577).

The groups were not significantly different regarding Δb (P=0.598). However, the interaction effect (time and bleaching protocols) was significant (P=0.001).

DISCUSSION

This study compared the efficacy of three different bleaching protocols by using oxalic acid, hydrogen peroxide and sodium hypochlorite for correction of tooth discoloration caused by tea.

In the current investigation, spectrophotometry was chosen as the method for color assessment of the teeth due to its accuracy and reliability. Unlike digital cameras, spectrophotometry is not affected by environmental lighting conditions, ensuring consistent and dependable results [15,16]. Tooth bleaching was performed using oxalic acid, a known reducing agent. Oxalate (C2O42-) has the ability to chelate metal cations [14] and is commonly found in the form of oxalate dihydrate (H2C2O4.2H2O). Oxalic acid is widely used as the reactive agent in many formulations of iron oxide solvents [17], thanks to its excellent performance. Furthermore, the subsequent removal of oxalic acid is relatively easy [17,18]. A study on the composition of tooth whitening gels prepared with natural fruit juices reported small amounts of oxalic acid in their composition [19]. Also, tooth whitening chewing gums contain organic acids such as oxalic acid [20]. Lo Giudice et al [21] used a combination of oxalic acid and bleaching gel to improve the efficacy of light-activated bleaching. They confirmed the optimal efficacy of light-activated bleaching, which also supports our methodology.

Since dissolution of oxalate solution depends on its pH, and maximum speed of reaction occurs at a pH of 2.5 to 3 [6], the pH of oxalic acid solution in the present study was adjusted at 3 by using ammonium hydroxide (forming oxalate hydroxide). According to Lee et al [6] ammonium hydroxide is the best product to control the pH of oxalate solution. The concentration and application time of oxalic acid were selected according to previous studies [13,14].

Sodium hypochlorite was also used in this study [5]. In clinical setting, the gel form of sodium hypochlorite should be preferably utilized due to its easier handling and greater control over it [22]. However, it was used in the form of solution in the present study due to its in vitro design. Gupta et al [23] applied sodium hypochlorite alone for correction of tooth discoloration caused by fluorosis and showed that it was only effective for removal of mild stains.

In the present study, the teeth were stained in tea solution according to the methodology of Sulieman et al [12]. After staining in tea, all teeth showed significant changes in L*, and a* color parameters (P<0.001) such that they became significantly darker and the redness of teeth increased in the red-green axis. The b* parameter also increased, indicating greater yellowness in the yellow-blue axis. The mean Δ E1 indicated a clinically significant color change.

Black tea has high amounts of polyphenol compounds such as theaflavin (C29H24O12) and thearubigin (C43H34O22), which have

phenolic rings with double bonds, and are responsible for the yellow-red color of black tea [24]. These compounds bond to tooth surface proteins and cause tooth discoloration [2,3,25]. Black tea is dark, red and yellow and can cause a reduction in L* and an increase in a* and b* color parameters. The effects of tea on tooth color reported in our study are in agreement with the results of many previous studies [12,26-30].

The coloring agents in tea solution are polar [2], which match the whitening agents used in this study; i.e., water, the hydroxyl ions in hydrogen peroxide bleaching gel, sodium hypochlorite, and oxalic acid are similarly polar [31]. Thus, they would probably have optimal efficacy for correction of tooth discoloration caused by tea. However, it should be noted that the bleaching results may not be exclusively related to removal of tea stains. Other stains and even intrinsic molecules in the tooth structure such as dentin amino-acids might have been affected by the bleaching process as well [32]. Thus, interpretation of results should be done with caution.

In all groups, application of bleaching agents (without considering the type of materials) significantly increased the $\Delta E2$ (P<001) and the L* parameters (P<0.001) which was in agreement with the results of studies that used this bleaching agent for treatment of conventional discolorations. According to former investigations, the L* parameter is responsible for most of the color correction in bleached discolored teeth [33-35]. The a* parameter significantly decreased in all groups (P<0.001), which was in line with similar studies [35,36]. However, according to the guidelines of the American Dental Association [37], and a study by Luo et al [33] changes in the a* parameter are often smaller and occurs slower during the bleaching process. The significant changes in this parameter observed in our study was probably due to the in vitro study design since such changes can be detected faster and more accurately in vitro. Also, it has been indicated that a* and b* parameters significantly change due to discolorations caused by tea, which

could be reversed through bleaching procedures [26]. The b* parameter significantly decreased in our group A (P<0.001), which confirms this point. According to Gerlach et al [35] increase in L* and decrease in b* indicate color correction and whitening of teeth.

The mean change in L^* parameter (ΔL_2) (P=0.868) and the mean ΔE_2 (P=0.414) in group B were not significantly different from the corresponding values in group A. Since iron oxide is usually not a common compound in black tea [2, 3], this outcome does not have any controversy with the studies revealing the bleaching effect of oxalic acid on iron oxides [6, 7]. Although, oxalic acid has been effective in removing tea stains in industries and patents, no significant effect of its addition to the bleaching process was seen in this study [38]. ΔE_2 in group C, was not significantly different from group A (P=0.470), which indicates that application of sodium hypochlorite followed by Pola Office Plus was not more effective than the use of Pola Office Plus alone for bleaching of tea-stained teeth. Slightly higher ΔE_2 was also noted in group C, which may be due to the use of sodium hypochlorite. The bleaching effects of sodium hypochlorite on tea stains was also noted in the study of Juwita et al [39]. However, their study was performed on heat cured acrylic resins.

The ΔE_2 in group D, was significantly different from group A (P<0.001). Despite the slightly higher ΔE_2 in group D, compared with group C, this difference was not significant (P=0.437). Furthermore, according to the results obtained in group D, it may be concluded that use of oxalic acid has a synergistic effect with sodium hypochlorite and the bleaching gel.

The mean change in the L* parameter (Δ L2) was found to be significantly different between groups B and C (P=0.009). This indicates that applying sodium hypochlorite before the bleaching gel has a greater impact on the brightness of teeth discolored by a tea solution compared to using oxalic acid before the bleaching gel. This finding aligns with a previous study by Juwita et al [39]. However, there were no significant differences in Δ E2 between groups B and C (P=0.437), suggesting that the changes in a* and b* parameters did

not result in a significant increase in $\Delta E2$ in group C.

The mean change in the a* parameter ($\Delta a2$) was similar across all groups (P=0.232), indicating that the application of oxalic acid and/or sodium hypochlorite before the bleaching gel did not have a significant effect on the redness (a* parameter) reduction of teeth discolored by a tea solution. However, groups C and D showed a slight, non-significant decrease in the changes of the a* parameter, suggesting a possible role of sodium hypochlorite in reducing the level of redness in teeth discolored by a tea solution, which is consistent with the findings of Juwita et al [39].

Similarly, the mean change in the b* parameter (Δ b2) was similar across all groups (P=0.598), indicating that the application of oxalic acid and/or sodium hypochlorite before the bleaching gel did not have a significant effect on the yellowness (b* parameter) reduction of teeth discolored by a tea solution. However, groups C and D showed a slight, nonsignificant decrease in the changes of the b* parameter, suggesting a possible role of sodium hypochlorite in reducing the level of yellowness in teeth discolored by a tea solution, which is in line with the findings of Juwita et al [39].

In summary, the use of sodium hypochlorite and oxalic acid in combination with Pola Office Plus proved to be more effective in bleaching tea-discolored teeth compared to Pola Office Plus alone. Similar synergistic effects have been noted in other studies, such as the combination of in-office and at-home bleaching being more effective than in-office bleaching alone. Phosphoric acid has also been used to enhance the efficacy of hypochlorite in bleaching fluorosed teeth, and hydrogen peroxide and sodium hypochlorite have been found to have synergistic disinfecting effects. Additionally, a combination of diluted hydrogen peroxide and oxalic acid with potassium permanganate has been reported for melanin removal. It is important to note that this study was conducted in a laboratory setting, and therefore caution should be exercised when generalizing the results to a clinical setting. Further clinical trials are needed to validate these findings.

CONCLUSION

Application of sodium hypochlorite and oxalic acid followed by in-office bleaching gel is more effective for correction of tooth discoloration caused by tea, compared with the gel alone.

CONFLICT OF INTEREST STATEMENT

None declared.

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