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Internal bleaching with calcium oxalate and laser irradiation for managing discolorations induced by mineral trioxide aggregate

Sara Majidinia[1], Farzaneh Ahrari[2*], Melika Hoseinzadeh[2], Navid Ramezanian[3], Arsalan Shahri[1] and Seyyedeh Zahra Jamali[4*]

Abstract

Aim This in vitro study investigated the effects of incorporating 1%, 3%, and 5% calcium oxalate into 15% hydrogen peroxide (H_2O_2), with and without laser activation, on the whitening of teeth discolored by mineral trioxide aggregate (MTA).

Methods The pulp tissue of 80 bovine incisors was removed, and an MTA plug was placed at 2 mm below the cementoenamel junction. After nine months, the samples were randomly divided into eight groups ($n=10$). Groups 1 to 4 were treated with 15% H_2O_2 gel containing 0, 1, 3, or 5% calcium oxalate, respectively. The same gels were applied in groups 5 to 8 but activated with an 810 nm diode laser (2 W, continuous wave). The teeth were incubated for five days, followed by the second gel application. Tooth color was evaluated at baseline (T1), after MTA discoloration (T2), and after the first (T3) and second (T4) gel applications, using the CIELAB system to measure color changes (ΔE).

Results The mean ΔE_{2-3} and ΔE_{2-4} differed significantly between groups ($P=0.002$ and $P=0.040$, respectively). After the first and second gel applications, ΔE values were significantly higher in groups 2 (H_2O_2 + 1% calcium oxalate) and 6 (H_2O_2 + 1% calcium oxalate + laser) than in groups 1 (H_2O_2), 5 (H_2O_2 + laser), and 8 (H_2O_2 + 5% calcium oxalate + laser) ($P<0.05$).

Conclusions Incorporating 1% calcium oxalate into 15% H_2O_2 can enhance the whitening of teeth discolored by white MTA. Laser activation did not further improve the outcome of internal bleaching in teeth with MTA discoloration.

Keywords Calcium oxalate, Dental bleaching, Dental pulp chamber, Diode lasers, Hydrogen peroxide, Mineral trioxide aggregate, Tooth discoloration, Tooth bleaching agents, Laser bleaching, Internal bleaching

*Correspondence:
Farzaneh Ahrari
Farzaneh.Ahrari@Gmail.com; Ahrarif@mums.ac.ir
Seyyedeh Zahra Jamali
zahrajamali7073@yahoo.com
[1]Dental Materials Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
[4]Department of Cosmetic and Restorative Dentistry, Dental Materials Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran

Introduction

Mineral trioxide aggregate (MTA) is commonly used in endodontic treatments, including apexification, managing perforations, vital pulp therapy, and regenerative endodontic procedures (REPs) due to its excellent biocompatibility and sealing properties [1]. One key drawback of MTA application is crown discoloration, which can lead to patient dissatisfaction, especially anterior teeth [2].

Multiple hypotheses have been suggested to explain the mechanism of tooth discoloration after applying MTA. One initial hypothesis suggested that the oxidation of heavy metals, such as iron or magnesium, is responsible for the discoloration associated with grey MTA [3]. Therefore, white MTA, which contains minimal FeO, MgO, and Al2O3 in its structure, was introduced to reduce discoloration. However, color changes still happened after using white MTA, although less intensely than with grey MTA [4]. Therefore, bismuth oxide, the radiopacifier in MTA, was implicated as the key contributor to tooth discoloration in white MTA [3]. Bismuth oxide binds with calcium silicate hydrate and is gradually released as it degrades over time [5]. Released bismuth oxide can cause tooth discoloration, primarily by interacting with dentin collagen to form a black precipitate [6]. Additionally, it may react with carbon dioxide and produce bismuth carbonate, thus leading to tooth discoloration [3]. The exposure of bismuth oxide to light irradiation and higher temperatures may also cause discoloration in MTA or calcium-enriched mixture (CEM) cement [3].

Internal bleaching is a practical, conservative, and cost-efficient therapy for managing discoloration in non-vital teeth. However, this treatment method has potential adverse effects, such as external cervical root resorption, dentine morphological changes, and collagen degradation [7, 8]. To minimize these risks, researchers are exploring methods to reduce the duration of treatment and concentration of bleaching agents.

A suitable approach to address discoloration from metal oxides is to use chelating agents such as oxalic acid, which effectively binds metal cations [9] facilitating the removal of insoluble ionic compounds from the substrates [10]. Oxalates are widely used in various industries, such as ceramics and paper bleaching, to remove metal contaminations [11, 12]. Thereare a few studies about the effect of oxalic acid on improving bleaching outcomes [9, 13]. Studies on the use of oxalic acid in dentistry have mainly focused on its ability to reduce tooth sensitivity after whitening [9, 14]. Potassium oxalate, when applied to dentin, decreases nerve transmission and forms calcium oxalate crystals that block dentinal tubules [14]. However, little evidence exists on the bleaching effect of oxalate compounds in dental practice.

Another promising method to enhance the whitening process is laser irradiation, which acts through photothermal or photochemical processes. The photothermal theory states that the laser can heat the bleaching agent and speed up the chemical reactions. According to the photochemical theory, the laser directly interacts with the tooth's surface, causing photo-oxidation of pigment molecules and breaking them down chemically. The laser may also interact with the components of the bleaching gel and enhance the generation of reactive oxygen species that further degrade the chromogenic molecules [15].

Previous studies on laser-assisted bleaching primarily examined in-office procedures, revealing faster whitening effects and fewer complications in laser assisted bleaching than in conventional techniques [16–19]. However, there is limited and controversial evidence regarding the efficacy of lasers in the internal bleaching of non-vital teeth [20, 21]. Furthermore, there have been no studies examining the effectiveness of internal bleaching with calcium oxalate and diode laser irradiation for MTA-induced tooth discoloration. Therefore, this study aimed to investigate the effects of incorporating 1%, 3%, and 5% calcium oxalate into a 15% hydrogen peroxide (H2O2) gel, with and without laser activation, on teeth discolored by white MTA.

Materials and methods

The protocol for this in vitro study was approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1403.017).

Sample preparation

Eighty bovine incisor teeth were obtained from a slaughterhouse. The samples were immersed in 0.5% chloramine-T for one week. Each tooth was examined under a stereomicroscope at 20x magnification to confirm the absence of cracks, fractures, or enamel defects. A putty index was made to standardize the area for color measurement on each tooth. The initial color of the tooth cervical area (T1) was recorded using a colorimeter (CR-400; Konica Minolta, Osaka, Japan). The colorimeter was calibrated before each measurement session using the calibration plate provided by the manufacturer.

Staining procedure

An access cavity was prepared on the palatal side, and the pulp tissue was removed using a #40 Hedstrom file (Mani Inc., Tochigi-Ken, Japan). To simulate the thickness of a human tooth, dentin was removed from the access cavity using a high-speed round bur until the buccal surface measured 3 mm. The final thickness was verified with a digital caliper.

An MTA plug (Angelus Dental, Londrina, Brazil) was placed 2 mm below the cementoenamel junction (CEJ).

A moist cotton pellet and a temporary filling material (Cavisol Temporary Filling, Golchai Co., Iran) were placed over the MTA plug. The samples were then incubated for 9 months at 37 °C in 100% humidity. After this period, the tooth color was measured again (T2) using the putty index (Fig. 1).

Preparation of bleaching gel containing calcium oxalate

To prepare 100 cc of internal bleaching gel containing 15% H_2O_2 and 1%, (3%, or 5%) calcium oxalate, 43 cc of 35% H_2O_2 gel (Merck, Darmstadt, Germany) was mixed with 0.1 wt% silica (Merck, Germany) and 3 wt% Carbopol (Lubrizol, Ohio, USA). The mixture was then combined with 37 cc of water, 8 wt% glycerol (Merck, Darmstadt, Germany), and 1 wt% (3% or 5%) calcium oxalate powder (Merck, Darmstadt, Germany). To adjust the pH and achieve the desired gel consistency, 1.6 cc of a solution containing 50 wt% polyethylene glycol 6000 (PEG) (Merck, Darmstadt, Germany) and 0.5 M Tris (Merck, Darmstadt, Germany) was added to the mixture. The pH range of the prepared gel was between 5 to 6. A red pigment (methyl red; Merck, Germany) was then added to serve as the photosensitizer for the diode laser. Methyl red is most effective at the pH range between 4.4 and 6.2, corresponding to the bleaching gel's pH [22]. Therefore, 1 cc of methyl red solution was added to 5 cc of the H_2O_2 gel.

Sample allocation and bleaching procedure

The samples were randomly divided into eight groups as follows ($n = 10$):

- Group 1: 15% H_2O_2.
- Group 2: 15% H_2O_2 + 1% calcium oxalate.
- Group 3: 15% H_2O_2 + 3% calcium oxalate.
- Group 4: 15% H_2O_2 + 5% calcium oxalate.
- Group 5: 15% H_2O_2 + Laser.
- Group 6: 15% H_2O_2 + 1% calcium oxalate + Laser.
- Group 7: 15% H_2O_2 + 3% calcium oxalate + Laser.
- Group 8: 15% H_2O_2 + 5% calcium oxalate + Laser.

In groups 1 to 4, the MTA plug was removed from the access cavity, and the access cavity was filled completely with the bleaching gel, followed by a temporary restorative material. The samples were incubated for 5 days, and the tooth color was recorded (T3).

In groups 5–8, the process was similar to that explained in groups 1 to 4, but the gel was activated with a gallium-aluminum-arsenide (GaAlAs) diode laser (DoctorSmile, Lambda S.p.A., Italy). The laser emitted a wavelength of 810 nm and was set at the power of 2 W and continuous wave (CW) mode, using a non-contact bleaching handpiece at the approximate distance of 1 mm from the gel. The laser was irradiated three times for 30 s each, at one-minute intervals between irradiations [18]. The samples

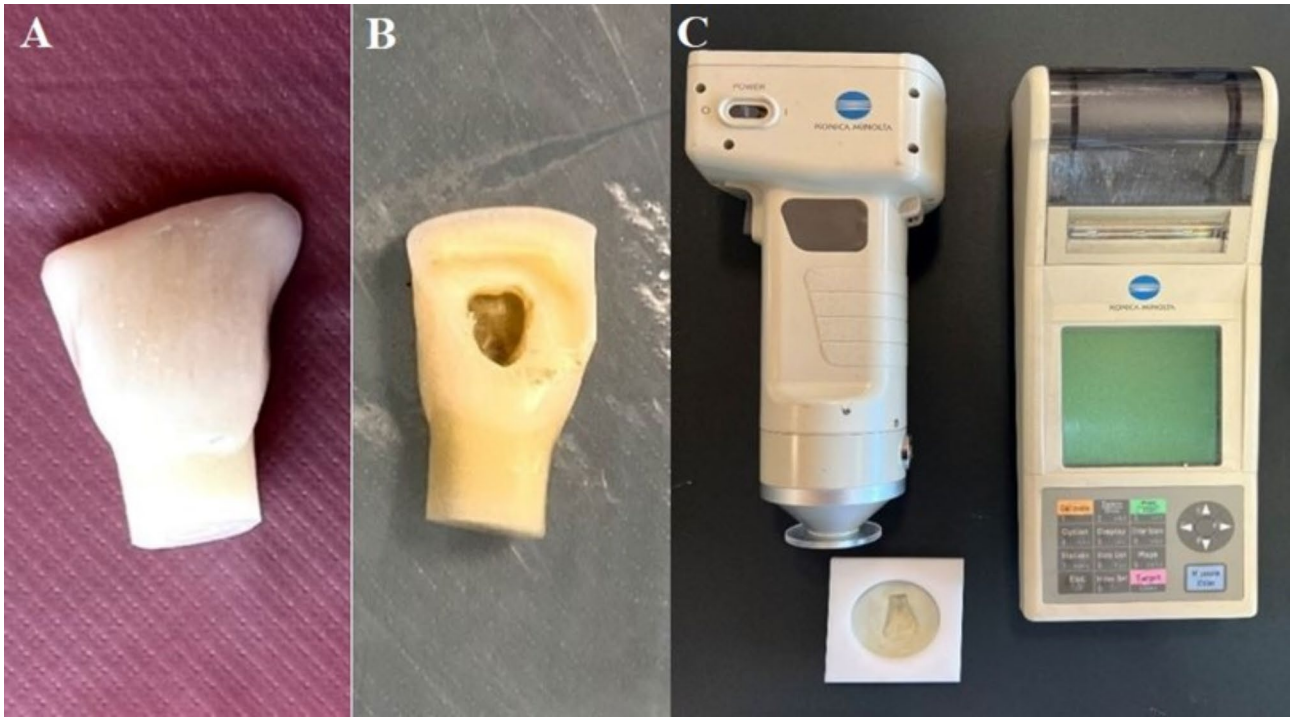

Fig. 1 (**A**) Intact bovine tooth; (**B**) Coronal discoloration following MTA application; and (**C**) A silicone impression used for assessing color change with a Chroma Meter CR-400

Table 1 Comparing mean ± standard deviation of color change (ΔE) values among the groups

Experimental groups		ΔE_{1-2}		ΔE_{2-3}		ΔE_{2-4}		ΔE_{3-4}	
		Mean ± SD	95% CI for Mean	Mean ± SD	95% CI for Mean	Mean ± SD	95% CI for Mean	Mean ± SD	95% CI for Mean
1	15% H_2O_2	3.94 ± 2.32	2.28 to 5.60	7.65 ± 2.21 [a]	6.06 to 9.23	7.54 ± 2.77 [a]	5.55 to 9.53	1.50 ± 0.54	1.11 to 1.89
2	15% H_2O_2 + 1% calcium oxalate	3.64 ± 1.25	2.74 to 4.54	10.81 ± 1.54 [b]	9.70 to 11.91	9.79 ± 1.95 [b]	8.40 to 11.19	2.06 ± 0.85	1.45 to 2.67
3	15% H_2O_2 + 3% calcium oxalate	4.19 ± 1.98	2.77 to 5.60	8.13 ± 2.09 [ab]	6.63 to 9.62	8.15 ± 1.36 [ab]	7.18 to 9.13	1.56 ± 0.85	0.94 to 2.17
4	15% H_2O_2 + 5% calcium oxalate	3.61 ± 0.65	3.03 to 4.18	8.18 ± 1.75 [ab]	6.92 to 9.44	8.59 ± 1.49 [ab]	7.52 to 9.65	1.33 ± 0.66	0.86 to 1.81
5	15% H_2O_2 + Laser	4.40 ± 2.40	2.68 to 6.12	7.70 ± 1.98 [a]	6.28 to 9.11	7.59 ± 0.94 [a]	6.38 to 9.28	1.67 ± 0.51	1.30 to 2.05
6	15% H_2O_2 + 1% calcium oxalate + Laser	4.10 ± 2.60	2.23 to 5.96	10.15 ± 1.59 [b]	9.01 to 11.30	9.55 ± 1.48 [b]	8.48 to 10.61	1.42 ± 0.84	0.81 to 2.03
7	15% H_2O_2 + 3% calcium oxalate + Laser	4.93 ± 1.20	4.06 to 5.79	8.73 ± 2.54 [ab]	6.92 to 10.55	8.38 ± 1.80 [ab]	7.46 to 10.59	1.54 ± 0.76	0.99 to 2.08
8	15% H_2O_2 + 5% calcium oxalate + Laser	3.94 ± 2.32	2.28 to 5.60	7.73 ± 2.15 [a]	6.06 to 9.23	7.34 ± 2.51 [a]	5.55 to 9.53	1.50 ± 0.54	1.11 to 1.89
P value		0.851		0.002*		0.040*		0.460*	

*Different lowercase letters represent a significant difference among the groups at $P < 0.05$.

were incubated for 5 days, followed by color measurement (T3).

In all groups, the pulp chamber was rinsed after the first gel application and the bleaching procedure was repeated. The surface color was measured after the second gel application (T4).

Color change assessment

The tooth color was measured using the CIELAB (Commission International de l'Eclairage L*a* and b*) color space system. In this system, L* represents the degree of lightness (ranges from 0: black to 100: white), while the a* value indicates the degree of red/green (+a: red, -a: green), and the b* value corresponds with the degree of yellow/blue (+b: yellow, - b: blue). The color change was calculated using the following formula:

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}$$

The color change between the different stages were defined as follow:

- $\mathbf{\Delta E_{1-2}}$: Color change between baseline (T1) and 9 months after MTA application (T2).
- $\mathbf{\Delta E_{2-3}}$: Color change between T2 and the first bleaching session (T3).
- $\mathbf{\Delta E_{2-4}}$: Color change between T2 and the second bleaching session (T4).
- $\mathbf{\Delta E_{3-4}}$: Color change between T3 and T4.

Sample staining, gel application, laser irradiation, and color assessment were performed by one operator (Z.J.) to ensure standardization of the experiment.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of the data. One-way ANOVA and Tukey post hoc test were performed to compare color changes between the treatment stages among the groups. All statistical analyses were conducted using SPSS software version 26 (IBM Inc., Armonk, NY, USA) with a significance level set at $P < 0.05$.

Results

Table 1 presents the mean and standard deviation of ΔE values between different time points in the experimental groups. ANOVA indicated no statistically significant differences among the groups in ΔE_{1-2} ($P = 0.851$) and ΔE_{3-4} ($P = 0.460$). However, significant between-group differences were observed in ΔE_{2-3} and ΔE_{2-4} ($P = 0.002$ and $P = 0.04$, respectively). The highest ΔE_{2-3} value was observed in the group 2 (15% H_2O_2 + 1% calcium oxalate: 10.81 ± 1.54) and the lowest in the group 1 (15% H_2O_2: 7.65 ± 2.21). For ΔE_{2-4}, the highest and lowest values were observed in the group 2 (15% H_2O_2 + 1% calcium oxalate: 9.79 ± 1.95) and group 8 (15% H_2O_2 + 5% calcium oxalate + laser: 7.34 ± 2.51).

Pairwise comparisons revealed that ΔE_{2-3} and ΔE_{2-4} were significantly greater in group 2 (15% H_2O_2 + 1% oxalate) and group 6 (15% H_2O_2 + 1% oxalate + laser) as compared to group 1 (15% H_2O_2), group 5 (15% H_2O_2 + laser), and group 8 (15% H_2O_2 + 5% oxalate + laser) ($P < 0.05$). No significant differences were found between the other groups ($P > 0.05$).

Discussion

This study investigated whether adding 1%, 3%, or 5% calcium oxalate to 15% H_2O_2 gel, with or without laser activation, could improve the efficacy of internal bleaching in teeth discolored by white MTA. In bleaching agents, H_2O_2 is commonly used at concentrations ranging from 3 to 50%. However, higher concentrations are associated with adverse effects, such as mucosal irritation, cervical root resorption, and dental hard tissue morphological changes [23]. The 15% H_2O_2 gel provides a good balance of efficacy and safety for bleaching treatments [24, 25]. Therefore, 15% H_2O_2 gel with or without calcium oxalate was used in the present study for internal bleaching of teeth with MTA-induced discoloration.

In this study, bovine teeth were used due to certain challenges associated with human teeth. Obtaining human teeth in sufficient quantity and quality can be difficult since they are often extracted because of extensive defects. Bovine teeth are chosen over other non-human dental hard tissues because of their availability, uniform composition, higher surface area and their similarity to human teeth, particularly regarding calcium content [26]. Bovine teeth have been used effectively in studies assessing microleakage, remineralization, hardness, and thermal expansion [26–28]. According to Franchini Pan Martinez et al. [29], there is the possibility for replacement of human by bovine teeth in studies concerning microleakage, organic and inorganic tooth content, coefficient of thermal expansion, spectrofluorometry, hardness, and radiodensity. The use of bovine teeth, however, is associated with some limitations, including variations in morphology, radiodensity, and mechanical properties compared to human teeth. Furthermore, bovine teeth have greater thickness, which may hinder accurate color assessment. In this study, dentin was carefully removed from the access cavity using a high-speed round bur until the buccal surface measured 3 mm, simulating the thickness of a human tooth. Favoreto et al. [30] reported that the degree of color change and hydrogen peroxide penetration is comparable between human and bovine teeth, supporting the use of bovine teeth in bleaching experiments.

This study used ΔE to evaluate color changes, which is a standard metric in bleaching studies. A $\Delta E = 3.3$ is the clinically acceptable color change threshold [31, 32]. ΔE value between 3 and 8 is moderately perceptible color change in the human eye, whereas ΔE values more than 8 are highly perceptible color change [33, 34]. In this study, all groups showed $\Delta E_{1-2} > 3.3$, indicating a perceivable discoloration following MTA application. The color change of the teeth improved following the first (ΔE_{2-3} range = 7.65 to 10.81) and second (ΔE_{2-4} range = 7.34 to 9.79) gel applications in all groups. The ΔE_{3-4} varied between 1.33 and 2.06, which is not clinically noticeable. These imply that that a 5-day internal bleaching period effectively improved discoloration caused by MTA, and repeating the bleaching process did not yield better outcomes.

The highest ΔE values after the first gel application were observed in the H_2O_2 + 1% calcium oxalate (10.81 ± 1.54) and H_2O_2 + 1% calcium oxalate + laser groups (10.15 ± 1.59). These groups showed significantly greatercolor enhancement compared to the H_2O_2 (7.65 ± 2.21), H_2O_2 + Laser (7.70 ± 1.98), and H_2O_2 + 5% calcium oxalate + Laser (7.73 ± 2.15) groups. After the second gel application, similar results were found. The ΔE values in teeth treated with H_2O_2 + 1% calcium oxalate (9.79 ± 1.95) and H_2O_2 + 1% calcium oxalate + laser (9.55 ± 1.48) were significantly higher than those seen in the H_2O_2 (7.54 ± 2.77), H_2O_2 + Laser (7.59 ± 0.94), and H_2O_2 + 5% calcium oxalate + Laser (7.34 ± 2.51) groups. These findings indicate that adding 1% calcium oxalate into H_2O_2 significantly enhanced the MTA-induced discoloration, but diode laser irradiation was ineffective in enhancing the whitening process.

The superior performance observed in the 1% calcium oxalate groups can be attributed to the ability of oxalate ions to chelate metal cations. Oxalate is a potent chelating agent commonly used in industries such as ceramics and paper bleaching to remove metal contaminants [11, 12, 35]. There is also evidence regarding the bleaching effect of oxalate in dental literature. A study on tooth-whitening gels made with natural fruit juices reported minor amounts of oxalic acid in their composition [36], and some tooth-whitening chewing gums contain organic acids like oxalic acid [9].

White MTA was used in this study as it causes less tooth discoloration than grey MTA. According to Chang et al. [37], the iron content of grey MTA (23229.16 ± 349.6 mg/kg) is approximately twenty times greater than that of white MTA (1108.33 ± 100.23 mg/kg). Asgary et al. [38] also found that the Al_2O_3, MgO, and FeO percentages in white MTA are 1.92%, 1.35%, and 0.4%. On the other hand, grey MTA contains 4.26%, 3.10%, and 4.39% of Al_2O_3, MgO and FeO. Although at a lower rate, iron is still present in the white MTA and may contribute to tooth discoloration. Furthermore, white MTA contains arsenic, bismuth, cadmium, chromium, copper, and iron at levels of 3.75, 89520.25, 0.23, 4.53, 2.09, and 1108.33 mg/kg, respectively [37]. Considering the high amount of metal oxides in white MTA, the chelating effect of calcium oxalate contributes to the superior bleaching effect observed in the H_2O_2 + 1% calcium oxalate groups.

In the present study, the 1% calcium oxalate concentration performed better than the 5% concentration. At the lower concentration, calcium oxalate may penetrate deeper into the dentinal tubules to address the stains. At

a higher concentration, there might be a risk of calcium oxalate particle agglomeration, leading to surface saturation and creating a protective layer that prevents bleaching agent penetration. Other studies have also used lower concentrations of oxalate in bleaching treatments. For instance, Panahandeh et al. [9] and Oliveira Barros et al. [14] used 0.24 M oxalic acid (equivalent to approximately 2.16% by weight) and 1.5% potassium oxalate, to enhance bleaching efficacy and reduce post-bleaching sensitivity, respectively.

In this study, the calcium salt of oxalic acid was used. Several studies demonstrated that bleaching agents cause adverse effects such as surface alterations and reductions in the calcium and fluoride content of tooth structure (8). Adding calcium to bleaching agents may increase calcium incorporation into tooth structure, thus increasing resistance to demineralization. Alexandrino et al. [39] found that a 35% H_2O_2 gel with 2% calcium prevented changes in enamel microhardness without compromising the bleaching efficacy. This issue is critical for internal bleaching as the gel remains within the pulp chamber for several days.

The outcomes of this study agree with those of some previous studies [9, 13]. Lo Giudice et al. [13] used a combination of oxalic acid and a bleaching gel and found that activation of the bleaching gel with LED lamp enhanced the whitening procedure compared to the control group. However, their study did not have a control group without oxalic acid addition. Panahandeh et al. [9] studied the effect of pretreatment with 0.24 M oxalic acid and 5.25% sodium hypochlorite before in-office bleaching with 37.5% H_2O_2 gel to treat tea-stained teeth. They found that applying oxalic acid for 5 min, followed by NaOCl application for 1 min, showed superior results compared to the bleaching gel alone, whereas NaOCl and oxalic acid alone were not significantly different from the control group. However, their study focused on tea-stained teeth, whereas the source of discoloration in the present study was MTA. Furthermore, there were differences in the bleaching method (in-office versus internal bleaching) and H_2O_2 concentrations (37.5% versus 15%) between the two studies.

In the present study, diode laser irradiation did not significantly affect tooth color changes in the study groups. The color change values in groups 5 to 8, which underwent laser exposure, were somewhat lower than the respective control groups, but the differences were not significant. Sağlam et al. [20] also found no significant difference between the diode laser-assisted group (30 s) and control group regarding the efficacy of intracoronal bleaching. Although in the present study, laser irradiation was performed three times for 30 s each, extending the duration of irradiation was ineffective for enhancing the bleaching effects. Saeedi et al. [40] reported that laser-assisted in-office bleaching at wavelengths of 810, 940, and 980 nm achieved similar efficacy to conventional bleaching, but in a shorter period. In contrast, several studies have indicated that laser irradiation accelerates the release of free radicals from bleaching agents during in-office bleaching procedures [16, 17, 41, 42]. Papadopoulou et al. [24] found that diode laser application (445 nm) significantly enhanced the results of in-office bleaching, although the effect depended on the laser power, duration of irradiation, and measurement time after whitening treatments. It appears that the accelerating effect of laser on chemical reactions is more critical for in-office procedures where the gel remains on the tooth surface for a short period (less than one hour). This effect may not be perceivable in the internal bleaching process because the whitening agent remains within the tooth for several days, and there is enough time for chemical reactions. Laser irradiation may also cause binding and agglomeration of calcium oxalate particles, leading to surface saturation and prevention of oxalate penetration into the dentin structure. The difference in the structure of dentin versus enamel may also play a role in the different results obtained between laser-assisted internal bleaching and laser-assisted in-office bleaching. Regarding the efficacy of different lasers, some studies have shown that diode lasers have similar performance to LED [43, 44] and Er, Cr: YSGG laser [45] in tooth bleaching. Another study demonstrated that internal bleaching with sodium perborate activated by either diode laser or LED caused a comparable whitening efficacy [46]. In contrast, Zhang et al. [47] revealed that laser-assisted bleaching using potassium-titanyl-phosphate (KTP) laser was more effective than LED and diode laser at providing brighter teeth according to $\Delta L*$ value, although ΔE was comparable between the groups.

The present results indicated that adding 1% calcium oxalate to the bleaching gel improves the whitening of discolored teeth and may be recommended in clinical settings. However, safety should be regarded when adding oxalate to bleaching agents. High concentrations of oxalates in direct contact with human tissue may pose potential health risks, including calcium loss, skin irritation, electrolyte imbalances, changes in breast cells to tumor cells, kidney stones, and neural damage [48]. The systemic toxicity threshold of oxalic acid for humans is approximately 4–5 g [48]. However, a very low concentration of calcium oxalate, such as 1%, applied within the pulp chamber at a distance from the periodontal tissue, followed by thorough rinsing, is unlikely to pose a health risk.

This study had some limitations, primarily due to its in vitro design. While iron and bismuth oxide are considered major contributors to MTA-induced discoloration, erythrocyte infiltration into the porosities of unset

MTA may also aggravate staining [49]. The cytotoxicity of calcium oxalate was not assessed in this study, which is considered a limitation. Future research must explore the surface characteristics and bonding of coronal restorations to dentin treated with calcium oxalate-containing bleaching agents. Further studies are also suggested to investigate the impact of bleaching agents containing 1% calcium oxalate on discoloration caused by other types of calcium silicate-based cement. Additional research is required to investigate the potential cytotoxic effects of calcium oxalate at different concentrations on dental tissues. Furthermore, it is necessary to evaluate the efficacy of other lasers such as KTP, for activating experimental gels used in internal bleaching.

Conclusions

Under the conditions used in this study:

1. White MTA caused noticeable coronal discoloration in all samples.
2. After the first and second gel applications, ΔE values were significantly higher in Groups 2 (H_2O_2 + 1% calcium oxalate) and 6 (H_2O_2 + 1% calcium oxalate + laser) than in Groups 1 (H_2O_2), 5 (H_2O_2 + laser), and 8 (H_2O_2 + 5% calcium oxalate + laser) ($P < 0.05$).
3. Incorporating 1% calcium oxalate into a 15% H_2O_2 gel can enhance the whitening of teeth discolored by white MTA. This method can be considered as a suitable alternative to using high concentrations of hydrogen peroxide, providing desirable tooth whitening with minimal side effects in MTA-induced discolorations.
4. Neither higher concentrations (3% and 5%) nor the activation of the bleaching agent with a diode laser significantly improved MTA-induced discoloration, possibly due to surface saturation and, thus, lower penetration of the bleaching agent into the dentin structure.

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Author contributions
S.M., F.A., and N.R. contributed substantially to the study's concept and design, supervised the study, and proofread the manuscript. S.Z.J., A.S., and M.H. conducted the study, collected and analyzed the data, and prepared the first draft of the manuscript. All authors have approved the submitted manuscript.

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Data availability
The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All methods were carried out under relevant guidelines and regulations. The protocol of the current study was approved by the ethics committee of Mashhad University of Medical Sciences, Iran (IR.MUMS.DENTISTRY.REC.1403.017). The bovine teeth were obtained from a slaughterhouse, which was the regular slaughtering of cattle for human food consumption.

Consent for publication
Bovine teeth were used in this study, and the publication consent was irrelevant.

Competing interests
The authors declare no competing interests.

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