



Efficacy of Ozonized Sunflower Oil with Tea Tree Oil as Desensitizing Agents in Dental Bleaching: Randomized and Double-Blind Clinical Trial

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Dental bleaching is one of the most commonly prescribed procedures in aesthetic dentistry due to its effectiveness and minimally invasive nature. However, a recurrent and challenging side effect is dentin hypersensitivity after the procedure.

Aim: This study aimed to evaluate whether the use of ozonized oil combined with tea tree oil is effective in controlling post-bleaching dentin sensitivity.

Methods: A randomized, double-blind, split-mouth clinical trial was conducted. Participants were divided into two experimental groups (n=29) according to the desensitization protocol: control group: potassium nitrate and sodium fluoride; test group: ozonized sunflower oil combined with tea

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tree oil. Both groups were applied before dental bleaching with 35% hydrogen peroxide. The obtained data were subjected to statistical analysis using a non-parametric Friedman repeated measures ANOVA test ($p < 0.05$), followed by the Durbin-Conover post hoc test ($p < 0.05$).

Results: In the assessment of sensitivity level, there were no statistically significant differences between the test group and the control group. A higher level of sensitivity was observed for the lower arch after one hour of bleaching agent removal in both groups, and the color change was statistically equal between the two groups.

Conclusion: The study concluded that the test group paste was as effective in reducing dental sensitivity caused by dental bleaching as the control group. Additionally, neither of the groups affected the bleaching capacity of hydrogen peroxide.

Keywords: Ozone; dentin sensitivity; tooth bleaching; ozonized sunflower oil; tea tree oil.

1. INTRODUCTION

Dental bleaching is an aesthetic dental procedure that reflects the contemporary culture of beauty appreciation. Its popularity stems from its ability to promote tooth whitening in a highly conservative manner [1].

Dental discoloration can be caused by a variety of factors, including intrinsic and extrinsic pigments. Intrinsic pigments are present within the tooth, resulting from natural aging or the use of certain antibiotics, such as tetracycline. On the other hand, extrinsic pigments result from dietary habits, such as frequent consumption of coffee, tea, dark sodas, and red wine, which can stain the tooth surface over time. Additionally, dental trauma and cavities can also contribute to dental discoloration [2,3].

In the dental bleaching process, hydrogen peroxide plays an important role. It acts through an oxidation-reduction reaction, breaking down the organic molecules responsible for dental pigmentation [3]. This reaction reduces the saturation of the teeth, resulting in a visible whitening effect [2]. Therefore, hydrogen peroxide is capable of combating both intrinsic and extrinsic pigments, contributing to effective teeth whitening. However, the possibility of dentin sensitivity is the main drawback of this technique [1]. The dentin sensitivity resulting from bleaching may indicate an inflammatory response in the dental pulp. Although dentin is not innervated, it has permeable dentinal tubules that house fluids. The hydrodynamic hypothesis suggests that under external stimuli, these fluids move within the tubules, activating pulp nociceptors and causing brief and acute pain [3]. For some authors, bleaching sensitivity results from the formation of oxygen bubbles in the dentinal tubules during the application of hydrogen peroxide. The release of this gas

triggers the movement of dentinal fluid, activating the interdental nerves [4].

To minimize patient discomfort, systemic agents such as analgesics and anti-inflammatories, and/or topical desensitizing agents can be used. These topical agents can have a neural action, reducing the excitability of receptors, as is the case with potassium-based products. On the other hand, obliterating agents react with dentin components, precipitating crystals that block dentinal tubules [2]. The most commonly used agents in this category are based on sodium fluoride, calcium, GLUMA, and toothpaste, among others. However, none of these products are completely effective in controlling dentin sensitivity [5].

Currently, the use of ozone in the treatment of dentin sensitivity is being investigated. Ozone (O_3) is a natural gas composed of three oxygen atoms and has been used in various pathological conditions due to its oxidation, circulatory stimulation, and analgesic properties, among others. In the dental field, ozone is indicated for the control of dentin sensitivity and can be used in different forms, such as diluted in water, in the form of gas, or incorporated into oils [6].

In the control of dentin sensitivity, a widely used approach is the dilution of ozone in vegetable oils, which provides greater safety in therapy. Furthermore, another promising alternative has been the combination of different oils for the treatment of various pathological conditions, as natural products have been a primary source of medicines throughout the centuries [7]. For example, tea tree oil, also known as melaleuca oil, demonstrates anti-inflammatory and antioxidant effects, making it relevant in this context. Therefore, the anti-inflammatory action of tea tree oil is being explored as a preventive

measure to avoid pulpal inflammatory response during the whitening treatment. The water-soluble components present in tea tree oil have the ability to suppress the production of pro-inflammatory mediators, thus demonstrating its anti-inflammatory effect on dental structure. This property of tea tree oil may contribute to minimizing dentin sensitivity and promoting a more comfortable and safe whitening process [8]. The ozonized sunflower oil has been found to enhance the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase. This effect helps prepare the body to combat pathophysiological and injurious conditions caused by hydrogen peroxide. The beneficial effects of ozone are often attributed to its potent oxidation potential, particularly on calcium-covered surfaces, which allows for the occlusion of dentinal tubules [9].

Based on the limited availability of randomized clinical trials studying the combined use of ozonized sunflower oil, ozonized toothpaste, and tea tree oil in the treatment of post-whitening dental pain, a paste was prepared using equal proportions of these ingredients, the hypothesis of this study is that the combination of tea tree oil and ozonized sunflower oil will be as effective as the control group in reducing dentin sensitivity, a common adverse reaction to dental whitening treatment.

2. METHODS

2.1 Clinical Study Design, Randomization, Allocation, and Recruitment

This was a randomized, prospective, double-blind, split-mouth study, in which the patient and evaluator were blinded to the distribution of the groups. This controlled clinical trial had an equal allocation rate to the groups. Simple randomization was performed using an open-access online system (www.sealedenvelope.com) by a third person not involved in the implementation and evaluation steps.

The distribution of the group to be assigned for the first time was recorded sequentially on numbered cards and placed in sealed envelopes. The information contained in the envelope determined the treatment to be assigned to the upper right arch, while the other arch received the alternative treatment. Once the participant was fit for the procedure and all evaluations were completed, the allocation assignment was

revealed when opening the envelope immediately after implementation.

The recruitment of patients was carried out through disseminating research on the social network: Instagram. All participants were informed about the nature and objectives of the study. The study was conducted from October 2022 to May 2023, at the Dentistry Clinics of the Local University.

This clinical trial evaluated the following variables: I- the intensity of sensitivity at different times in the same group; II- the intensity of sensitivity at different times in different groups; III- global sensitivity (GS) (sum of sensitivity throughout treatment, up to 48 hours); IV- worst pain (WP) and V- Bleaching effectiveness.

2.2 Eligibility Criteria

Based on pre-established criteria, 40 volunteer patients were selected. General and oral health and aged between 18 and 50 years and had at least six caries-free upper anterior teeth, restorations, or endodontic treatment, with canine tone A2 or darker, according to the vita color scale (VITA ClassicalShade, VITA Zahnfabrik, BadSäckingen, Germany). "Otherwise, participants with cognitive difficulties, that is, patients who did not understand the correct way of filling out forms for the registration of dental sensitivity were not included in the study, just as patients with orthodontic appliances, dental prostheses, and severe internal tooth discoloration, such as tetracycline, fluorosis or pulped teeth stains were not included in the study. Pregnant and lactating women, patients with bruxism or any pathology that could cause tenderness, such as recession, dentin exposure, visible clefts in the teeth, and patients who use anti-inflammatory drugs or analgesics" [10].

2.3 Sample Size Calculation

The sample calculation was performed based on probability distributions of the t-test family (Wilcoxon and Mann-Whitney tests for comparison of two groups). The effect size used of 0.50, error type 1(α) of 0.05, and power of analysis (error β) of 0.90 resulted in a total of 26 volunteers per group. For convenience, due to the possible dropout of patients, 30 patients were selected per group [11,12]. The sample calculation was performed in the GPower program, version 3.1.9.2 - University of Düsseldorf.

2.4 Study Intervention

After the insertion of a lip reformer (Arcflex, FGM Dental Products, Joinville, Brazil), a light-curing gingival barrier (Top Dam Angelus Dam, FGM Dental Products, Joinville, Brazil), was placed in the gingival tissue of the teeth to be bleached (from the second left premolar to the second right premolar of the upper arch). The gingival barrier was light curing using a light curing machine with a power of 1250 mW/cm² (Emitter NOW, Schuster Dental Equipment, Santa Maria, Brazil) according to the manufacturer's recommendations. After that, the right and left sides of the dental arch were separated with a Mylar matrix (Superdent, United States). In hemiarch, the process of the test group (GT) was performed. It involved the application of a paste that combined 5ml of ozonized sunflower oil (Philozon, Balneário Camburiú, Santa Catarina, Brazil), 5 drops of tea tree oil (Biovitare, São José do Rio Preto – SP, Brazil), and 5mL of ozonized toothpaste (Philozon, Balneário Camburiú, Santa Catarina, Brazil). The components were accurately weighed and mixed until a homogeneous paste was obtained. The paste was applied for 2 minutes using a low-speed rubber cup, followed by an additional 5 minutes of application solely on the dental surface according to the study by Veena et al., 2020¹⁵. In the other hemiarch, the control group (CG), a desensitizing gel based on potassium nitrate (Desensibilize KF, FGM Dental Products, Joinville, Brazil) was applied for 10 minutes, and subsequent removal with water for one minute. Then, both arches were bleached with 35% hydrogen peroxide gel manipulated with carbopol (Chamomilla Pharmacy, Cascavel, Paraná, Brazil), for 45 minutes and removed with a saliva ejector, gauze, and rinse with water for 1 minute. After seven days, all participating patients were reassessed. The composition of the materials used in the study is described in Table 1.

2.5 Tooth Sensitivity Evaluation

“Each patient received a form to evaluate the sensitivity experienced by them. This data collection instrument form for dental sensitivity registration every 5 minutes during the action of the bleaching gel, after 1 hour, 24 hours, and 48 hours after bleaching treatment. Patients were instructed in detail on how to record their most intense pain experience each day, in each hemiarch (right and left), based on the visual analogue scale (VAS)” [13].

In addition, messages were sent daily to all research participants via WhatsApp Messenger, version 17.2.443 (WhatsApp Messenger, Social Networks. Facebook Inc., Menlo Park, CA, USA), informing them about completing the form, to ensure that the pain level was assessed correctly each day. All were instructed not to use any analgesic medication; if they did, they should notify the person responsible for the treatment. At the end of the treatment, the form was delivered by the patient to the researcher in charge.

If the participant scored 0 (without sensitivity) in all time evaluations of both bleaching sessions, this participant was considered insensitive to the whitening protocol. In all other circumstances, participants were considered to have whitening-induced dental sensitivity (Fig. 1).

2.6 Bleaching Effectiveness

The color assessments were performed during the bleaching treatment, using the upper central incisors as a reference. An operator, previously calibrated, conducted these using the visual comparison method with the aid of the Vitapan Classical color scale (Vita, Bad Säckingen, Germany). Before the initial application of the bleaching gel and seven days after treatment completion, color assessments were performed. The shade guide was mounted in a sequence of increasing luminosity, from the most luminous shade (B1) to the least luminous (C4). Each shade received a score in this sequence: B1, score 1; A1, score 2; and so on, with A3 being a score of 9. The scores are shown in Table 2.

The bleaching effectiveness (ΔB) was calculated by the difference between the color assessment initial (ΔI) and the color assessment final (ΔF), in each patient, according to the following formula:

$$\Delta B = (\Delta I) - (\Delta F)$$

2.7 Statistical Analysis

The results were tabulated and subjected to statistical analysis using JAMOVI software, version 1.2.24. For the analysis of data related to the degree of sensitivity of patients in the intragroup evaluation, the Friedman repeated measures ANOVA test was performed, followed by the Durbin-Conover post hoc test ($p < 0.05$). On the other hand, for the intergroup analysis, comparing the same time interval, the Wilcoxon test was performed ($p < 0.05$).

Table 1. Composition of the materials used

Material	Manufacturer	Composition
Hydrogen Peroxide	Chamomilla Pharmacy	Hydrogen Peroxide 35% with Carbopol
Top Dam	FGM/ Dental Products, Joinville, Brazil	HEMA, Urethane Dimethacrylate Monomer, Inert fillers, Pigments, and Photoinitiators
Desensitize KF	Philozon// Balneário Camburiu, Santa Catarina, Brazil	5% Potassium Nitrate with 2% Sodium Fluoride
Ozonized Sunflower Oil	Philozon// Balneário Camburiu, Santa Catarina, Brazil	Ozonized Sunflower Seed Oil
Tea Tree Oil	Biovitarte Compounding Pharmacy, São José do Rio Preto - SP, Brazil.	A-pinene (2.5%), B-pinene (1%), myrcene (1%), A-terpinene (8%), G-terpinene (18%), p-cymene (3.9%), limonene (2%), terpinolene (3%), p-phellandrene, sabinene hydrates (trans and cis), and trans-piperitol
Ozonized sunflower oil	Philozon// Balneário Camburiu, Santa Catarina, Brazil	Ozonized Olive Oil; Tea Tree Oil; Calendula Extract; Grapefruit; Tahiti Lemon; Xylitol

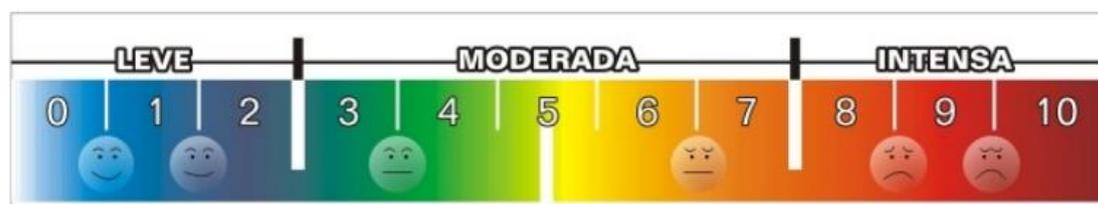


Fig. 1. Visual analog scale (VAS), from Wiggers et al., 2023

Table 2. Scores for color assessment

B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3,5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

3. RESULTS

During the period between October 2022 and May 2023, 40 patients were examined. After applying the eligibility and exclusion criteria, 30 patients were included as per the CONSORT (Consolidated Standards of Reporting Trials) flowchart, as depicted in Fig. 2.

The statistical analysis was conducted by a blinded researcher who did not know which treatment protocol had been applied to each experimental group. The obtained results were subjected to statistical analysis using the non-parametric Friedman repeated measures ANOVA test ($p < 0.05$), followed by the Durbin-Conover post hoc test ($p < 0.05$).

It can be observed that the use of OM did not result in statistically significant differences when compared to the NPFS desensitizer. The assessment of sensitivity level for the upper

quadrant revealed no statistically significant difference among the evaluated time points in the intragroup analysis, except for a statistically significant difference at the 1 hour. As for the intergroup analysis, there was no statistically significant difference among the groups at all reassessment time points. The data are presented in Table 3.

For the lower quadrant, a similarity was observed between the initial evaluation periods and the 24 and 48-hour reevaluations. However, the highest sensitivity was observed at the 1-hour time point, both in the test and control groups. Regarding intergroup analysis, there were no statistically significant differences between the groups at all reevaluation times. The data are presented in Table 4.

For the evaluation of whitening degree in the intragroup analysis, both tested groups showed statistically significant differences between the

initial and final color, indicating a reduction in color saturation for both groups (Table 5). However, when comparing the difference between the initial and final color achieved after the whitening procedure, there was no statistically significant difference between the groups (Table 6).

4. DISCUSSION

The hypothesis that the combination of ozonized tea tree and sunflower oils would be as effective as the control group in reducing dentin sensitivity was confirmed in the present study.

The main objective of this study was to evaluate dental sensitivity during and after an in-office tooth whitening procedure using 35% hydrogen peroxide as the bleaching agent. In the control group, a 5% potassium nitrate with 2% sodium fluoride desensitizing gel (NPFs) was used. This gel is the most commonly used control in the literature but still presents contradictory results. In a study conducted by Rezende et al. [14], evaluating the effectiveness of 10% sodium fluoride as a desensitizing agent, no significant reduction in post-bleaching dentin sensitivity was observed. However, in a previous study by Tay et al. [12], using 5% potassium nitrate and 2% sodium fluoride, a positive effect on reducing dentin sensitivity was found. These findings are in line with a recent systematic review conducted by Krishnakumar et al. [4], which compared different desensitizing agents and concluded that NPFs was the most effective agent in controlling sensitivity immediately after the procedure and in the first 24 hours.

In this research, no significant differences were found between the control group and the test group. This highlights the ability of the association of oils (OM) to reduce dental sensitivity after bleaching, as the test group showed similar results to NPFs, which is the most commonly used in the literature [2]. Ozonized oil was selected due to its advantage of allowing prolonged contact of ozone with the dental surface, as its half-life is significantly longer [15]. In a randomized clinical trial, Wiggers et al. [11] found a significant reduction in post-bleaching sensitivity using ozonized sunflower oil associated with glutaraldehyde. Possibly, the authors found a more pronounced desensitizing effect in the group treated with ozonized sunflower oil in combination with glutaraldehyde. This could be attributed to the obliterative action of glutaraldehyde, which enhances the desensitization properties of the ozonized sunflower oil. Supporting the efficacy of this oil, Veena H. R. et al. [15] reported that it can contribute to the opening of dentinal tubules, providing an anti-inflammatory action in dental pulp [16]. Similar results to the present study, regarding the reduction of sensitivity when combining ozone with dental bleaching, were found by Al-Omiri et al.3 (2018). These findings can be justified by the action of ozone in enhancing the activity of antioxidant enzymes, thus preparing the host for pathophysiological conditions mediated by hydrogen peroxide. Initially, ozone causes a reduction in cell viability as transient oxidative stress, but later it increases the potential for a cellular response, stimulating ATP production and reducing the inflammatory process [15].

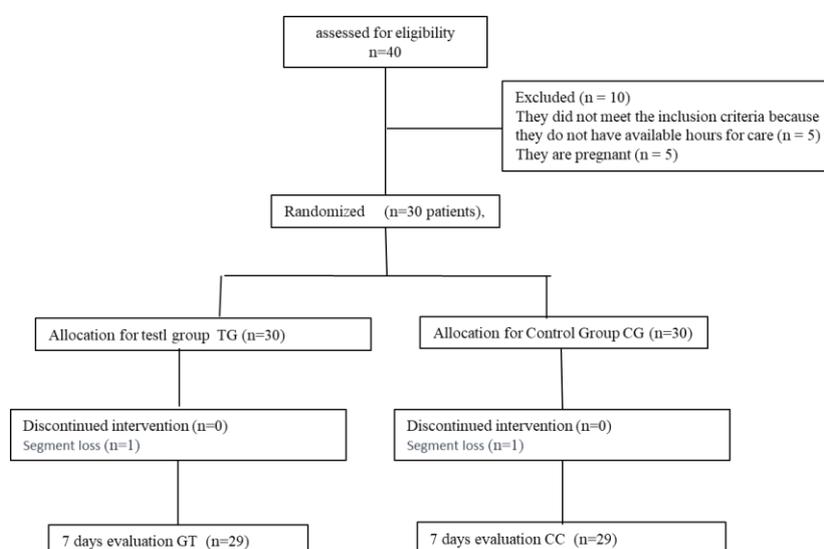


Fig. 2. Distribution and dynamics of the groups

Table 3. Degree of sensitivity during the execution of the whitening protocol using desensitizing agents in Group T and Group C at different evaluation times for the upper arch

Group	Reassessment period											
	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	1 hr	24 hr	48 hr
Test	0.21 ± 0.48 ABCa	0.28 ± 0.58 ABDa	0.35 ± 0.62 Da	0.37 ± 0.60 Da	0.511 ± 1.03 Da	0.40 ± 0.88 Da	0.46 ± 0.92 Da	0.48 ± 0.98 Da	0.46 ± 0.97 Da	0.87 ± 1.05 Ea	0.06 ± 0.22 Ba	0 ± 0 Ca
Control	0.34 ± 0.69 AEFa	0.36 ± 0.47ABEa	0.36 ± 0.62 ABEa	0.53 ± 0.81 ABCa	0.5 ± 0.73 ABCa	0.40 ± 0.67 ABCEa	0.45 ± 0.69 ABCEa	0.54 ± 0.80 BCa	0.57 ± 0.86 CDa	1.04 ± 1.26 Da	0.23 ± 0.59 EGa	0.05 ± 0.20 FGa

*Different uppercase letters in the row indicate significant differences with $p < 0.05$ in the intragroup analysis using Friedman's repeated measures ANOVA test. Different lowercase letters in the column indicate significant differences with $p < 0.05$ in the intergroup analysis using the Wilcoxon test

Table 4. Degree of sensitivity during the execution of the bleaching protocol using desensitizers from the test group and control group at different evaluation times for the lower arch

Group	Reassessment period											
	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	1 hr	24 hr	48 hr
Test	0.18 ± 0.54ACa	0.29 ± 0.70ABCa	0.35 ± 0.73 ABCa	0.58 ± 0.99 BCDa	0.82 ± 1.13DEa	0.75 ± 1.18DFa	0.91 ± 1.21 EFa	0.92 ± 1.16 EFa	1.05 ± 1.25Fa	1.68 ± 1.00 Ga	0.31 ± 0.53 Ca	0.04 ± 0.19Aa
Control	0.22 ± 0.57AHa	0.39 ± 0.72ABHa	0.37 ± 0.75 ACHa	0.58 ± 0.92 BCDa	0.70 ± 1.05DEa	0.71 ± 1.03Ea	0.85 ± 1.06 Ea	1.07 ± 1.17Fa	1.08 ± 1.26Ea	1.96 ± 1.21 Ga	0.38 ± 0.71Aa	0.04 ± 0.19Ha

*Different uppercase letters in the row indicate significant differences with $p < 0.05$ in the intragroup analysis using the Friedman repeated measures ANOVA test. Different lowercase letters in the column indicate significant differences with $p < 0.05$ in the intergroup analysis using the Wilcoxon test

Table 5. Mean values and standard deviation of color score for the control and test groups

	Control (NPFS)	Test (OM)
Initial	8.76 ± 2.49 a	8.76 ± 2.49 a
Final	3.14 ± 1.51 b	3.14 ± 1.51 b

**In the column, different lowercase letters indicate statistically significant differences by the Wilcoxon test ($p < 0.05$)*

This finding is corroborated by the study of Holanda A.J.S. in 2020, which, in their literature review, mentions the anti-inflammatory and analgesic properties of local ozone application, countering the hypothesis that hydrogen peroxide generates a reversible inflammatory process in dental pulp. Additionally, ozone has analgesic properties that can prevent the activation of nerve branches present in dentin [10]. However, more studies are needed to confirm its effectiveness in the dental pulp and clarify its mechanism of action. Considering the anti-inflammatory, healing, analgesic, and antimicrobial properties of ozonized sunflower oil [17,18], its wide application in dentistry becomes evident. This characteristic provides a biological, practical, and economical advantage, as it allows obtaining a product with a broader spectrum of action compared to NPFS.

Table 6. Mean values and standard deviation of the difference between initial and final color for groups X and Y

Control	Test
5.62 ± 1.76 a	5.62 ± 1.76 a

**In the row, different lowercase letters indicate statistically significant differences according to the Mann-Whitney test ($p < 0.05$)*

The objective of this study was to evaluate the association of ozonized sunflower oil with tea tree oil. Tea tree oil is known for its antimicrobial action, but there is also evidence of its anti-inflammatory activity, as its components can inhibit the production of inflammatory mediators [19]. In the context of this study, the focus was on the anti-inflammatory action of tea tree oil in relation to the possible inflammatory reaction in dental pulp caused by the bleaching procedure, and the results indicated a positive effect. Supporting this finding, the research by Stea S. et al. [20] addressed terpinene-4-ol, the main component of tea tree oil, which demonstrated the suppression of inflammatory mediators' production and reduction of inflammation.

However, there is a scarcity of studies investigating the use of tea tree oil as a desensitizing agent in dental bleaching, and more research is needed to understand its efficacy and mechanism of action. Additionally, further studies are required to determine the capacity of each oil (ozonized sunflower oil vs. tea tree oil) as a desensitizing agent.

The bleaching capability of 35% hydrogen peroxide was evident, as all patients showed a reduction in color saturation. Furthermore, none of the protocols used negatively influenced the initial color stability after the 7-day evaluation period. In this regard, both the NPFS and OM groups achieved lighter shades, and there were no significant differences between them. However, to gain a more comprehensive and thorough understanding, it is highly recommended to conduct additional studies that track color stability over longer periods. This would allow for a more precise evaluation of the durability of the obtained results and provide valuable insights into the longevity of dental bleaching.

Regarding future prospects, this study opens up avenues for further investigation into the combination of ozonized sunflower oil and tea tree oil as desensitizing agents in dental bleaching. The limited existing literature on this topic indicates a need for more comprehensive research to elucidate their effectiveness, safety, and mechanisms of action. Investigating the long-term stability of the results and exploring additional potential benefits of these oils in dental procedures would contribute to the advancement of knowledge in this field.

5. CONCLUSION

Despite the limitations of this study, it can be concluded that the combination of ozonized sunflower oil with tea tree oil has a positive effect as a desensitizing agent in dental bleaching. Furthermore, it was observed that the bleaching gel made with 35% hydrogen peroxide is effective for tooth whitening, and it is important to note that none of the desensitizing agents interfered with the color change during dental bleaching.

CONSENT AND ETHICAL APPROVAL

All patients who met the selection criteria were informed about the objectives, procedures, risks, and benefits of the study and expressed their consent to participate by signing the Free and

Informed Consent Form. The experimental design followed the statement CONSORT and was registered with the Brazilian Registry of Clinical Trials (RBR-88kny27). The study protocol was reviewed and accepted by the Local Ethics Committee on Investigations Involving Human Beings (64011722.0.0000. 0107).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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