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Main findings from the use of ozone therapy alone or combined with conventional treatments in root canal treatment: a systematic review

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Abstract

Introduction: The positive effects of ozone on biological properties include antimicrobial, immunostimulating, and biosynthetic impacts used in the treatment and maintenance of good oral hygiene. Ozone results in alteration in the metabolism of cells by raising the partial pressure of oxygen in tissues which improves the transporting capacity of oxygen in the blood. Ozone causes more blood supply to the ischemic zones due to surgical interventions like tooth extractions and implant placement. In this context of decontamination of root and periapical canals, ozone has emerged as an important sanitizer. **Objective:** It was to develop a systematic review of the literature to list the main findings of the use of ozone therapy alone or combined with conventional treatments in the treatment of root canals. **Methods:** The PRISMA Platform systematic review rules were followed. The search was carried out from March to June 2024 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** A total of 122 articles were found, 35 articles were evaluated in full and 11 were included and developed in the present systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 25 studies with a high risk of bias and 22 studies that did not meet GRADE and AMSTAR-2. Most studies did not show homogeneity in their results, with X^2=88.5%<50%. **Results and Conclusion:** It is concluded that ultrasonic and sonic ozone activation resulted in less pain in patients undergoing single-session endodontics compared to no ozone treatment. Ozonated olive oil with zinc oxide and olive oil paste with zinc oxide demonstrated good clinical and radiographic success for pulpectomy of primary teeth. Furthermore, low-intensity laser and ozone therapy are useful methods for postoperative pain in vital symptomatic teeth, but they are not superior to each other.

Keywords: Endodontic treatment. Ozone therapy. Conventional treatment. Root canal. Microorganisms.

Introduction

Ozone therapy is an alternative medical treatment that increases oxygen content by applying ozone to the body's surfaces. The positive effects of ozone on biological properties include antimicrobial, immunostimulating, and biosynthetic impacts used in the treatment and maintenance of good oral hygiene [1].

In this context, ozone leads to the destruction of organisms by primary damage to the cytoplasmic membrane of cells as a consequence of ozonolysis and second changes in the intracellular contents due to secondary oxidant effect that leads to oxidation of protein loss of organelle function. Ozone acts as a strong antioxidant leading disinfecting effect by breaking the cell membrane of the microorganism. Ozone leaves no toxic byproducts. Due to the ability of ozone, cells are not damaged and the action remains non-specific and selective to microbial cells. All vital functions of bacteria are halted after a few seconds of ozone application. Gram-positive bacteria show more sensitivity to ozone compared to gram-negative organisms. Ozone disrupts bacterial cells, leading to the removal of acidogenic bacteria that commonly cause dental caries [2,3].

The immune-competent cell proliferation and immunoglobulin synthesis are stimulated by the influence of ozone on the cellular and humoral immune system. The function of macrophages is activated due to which the sensitivity of microorganisms to phagocytosis is increased which leads to the production of cytokines as a consequence other immune cells are activated [2].

Ozone results in alteration in the metabolism of cells by raising the partial pressure of oxygen in tissues which improves the transporting capacity of oxygen in the blood. Ozone when given multiple times in low doses activates enzymes such as dehydrogenase, superoxide dismutases, glutathione peroxidases, and catalases [4]. Ozone enables the activation of protein synthesis in cells. It helps to increase the ribosome and mitochondria that cause regeneration of tissues by increasing the functional activity. Ozone secretes vasodilators, such as nitric oxide, that cause dilation of arteries and veins. Nitrous oxide is used as anesthesia. Ozone causes tooth mineralization by acting on its organic substances. It enables the diffusion of calcium and phosphorus ions to the inner surface of a decayed tooth by opening of the dentinal tubules [4].

Also, interleukins, prostaglandins, and leukotrienes are the proteins synthesized by ozone that help in cell growth and differentiation in the reduction of inflammation and wound healing [5-10]. Ozone application initiates early healing of wounds by improving the properties of erythrocytes and facilitating oxygen release in the tissues. Ozone causes more blood supply to the ischemic zones due to surgical interventions like tooth extractions and implant placement [11].

In this context of decontamination of root and periapical canals, ozone has emerged as an important sanitizer. Ozone is a natural gas and a very strong and selective oxidant. Ozone therapy is based on the assumption that ozone (O_3) rapidly dissociates into water and releases a reactive form of oxygen that can oxidize cells, thus having antimicrobial efficacy without inducing drug resistance. Ozone acts on glycolipids, glycoproteins, or certain amino acids, which are present in the cytoplasmic membrane of microorganisms [12-14]. The oxidation process of these unsaturated lipids and proteins generates a quantitative conversion of the olefinic bonds present to reactive species (ozonide) of lipid oxidation products [15]. Ozonides signal and trigger metabolic changes that produce microbicidal effects [13,15].

Furthermore, ozone therapy is being tested as an alternative or joint agent to NaOCl. However, some authors have demonstrated that ozone therapy has similar results compared to NaOCl in reducing various bacterial species, while others have reported less efficacy. Also, the action of ozone directly and indirectly modulates the relationship between the patient's immune system, thus improving the body's response to the etiological agent [15-19].

Given this, the present study developed a systematic review of the literature to list the main findings of the use of ozone therapy alone or combined with conventional treatments in the treatment of root canals.

Methods

Study Design

The present study followed the international systematic review model, following the rules of PRISMA [20] (preferred reporting items for systematic reviews and meta-analysis). Available at: http://www.prisma-statement.org/?AspxAutoDetectCookieSupport=1. Accessed on: 04/16/2024. The methodological quality standards of AMSTAR-2 (Assessing the methodological quality of systematic reviews) were also followed. Available at: https://amstar.ca/. Accessed on: 04/16/2024.

Data Sources and Research Strategy

The literary search process was carried out from March to June 2024 and was developed based on Scopus, PubMed, Lilacs, Ebsco, Scielo, and Google Scholar, covering scientific articles from various eras to the present. The descriptors (MeSH Terms) were used: *"Endodontic treatment. Ozone therapy. Conventional treatment. Root canal. Microorganisms"*, and using the Boolean "and" between the MeSH terms and "or" between historical discoveries.

Study Quality and Risk of Bias

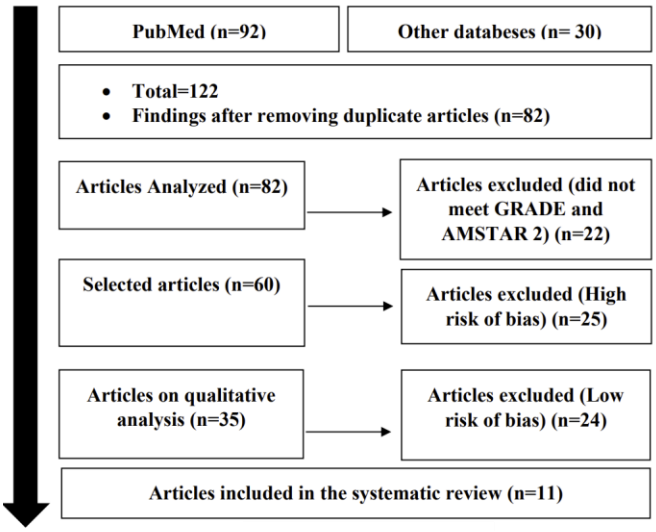
Quality was classified as high, moderate, low, or very low in terms of risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident emphasis was on systematic review articles or meta-analyses of randomized clinical trials, followed by randomized clinical trials. The low quality of evidence was attributed to case reports, editorials, and brief communications, according to the GRADE instrument [21]. The risk of bias was analyzed according to the Cochrane instrument [22] by analyzing the Funnel Plot graph (Sample size versus Effect size), using the Cohen test (d).

Results and Discussion

Summary of Findings

A total of 122 articles were found that were subjected to eligibility analysis, with 11 final studies being selected to compose the results of this systematic review. The studies listed were of medium to high quality (Figure 1), considering the level of scientific evidence of studies such as meta-analysis, consensus, randomized clinical, prospective, and observational. The biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies showed homogeneity in their results, with X^2=88.5%<50%. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 25 studies with a high risk of bias and 22 studies that did not meet GRADE and AMSTAR-2.

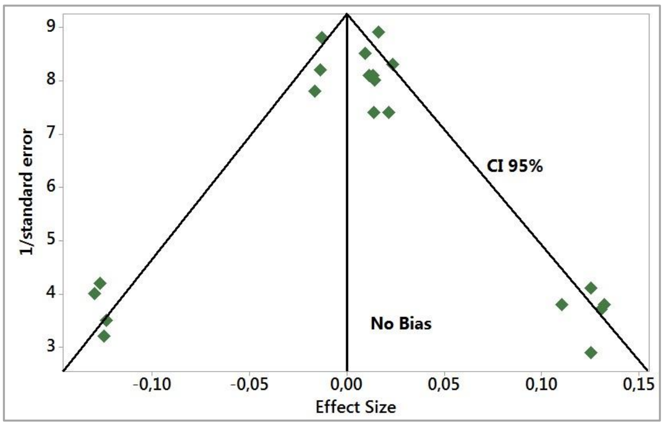
Figure 1. Articles eligibility process.



Source: Own authorship.

Figure 2 presents the results of the risk of bias of the studies using the Funnel Plot, showing the calculation of the Effect Size (Magnitude of the difference) using the Cohen Test (d). Precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph had a symmetrical behavior, not suggesting a significant risk of bias, both between studies with a small sample size (lower precision) that are shown at the bottom of the graph and in studies with a large sample size that are presented at the top.

Figure 2. The symmetric funnel plot suggests no risk of bias among the small sample size studies that are shown at the bottom of the graph. High confidence and high recommendation studies are shown above the graph (n=11 studies).



Source: Own authorship.

Major Clinical Findings

One study compared the effect of different ozone application techniques on the prevalence of post-endodontic pain in patients undergoing endodontic treatment in a single session. A total of 108 patients with necrotic pulp in single-rooted teeth and apical periodontitis participated in the study. A standard single-visit endodontic protocol was followed with 5.25% sodium hypochlorite and nickel-titanium rotary files. After modeling and cleaning, patients were randomly allocated into the following groups: group 1 (n=21), ozone treatment without activation (NA); group 2 (n=22), ozone treatment with manual dynamic activation (MDA); group 3, (n=21), ozone treatment with passive ultrasonic activation (PUA); group 4 (n=23), ozone treatment with sonic activation (SA); and group 5 (n=21), without ozone treatment (control group). Patients' discomfort levels were recorded at 6 different time intervals using the visual analog scale (VAS). VAS scores were higher for the control > NA > MDA > SA > PUA groups. A statistically significant reduction in VAS scores was observed in the PUA and SA groups compared to the NA, control, and MDA groups. Temporal comparison showed a highly significant decline in VAS scores across all time intervals [23].

The authors Meire et al. (2023) [24] analyzed the available evidence on the effectiveness of adjuvant therapy for the treatment of apical periodontitis (AP) in permanent teeth, according to a population, intervention, comparison, outcome, time and design structure study formulated a priori by the European Society of Endodontology. A total of 14 studies (13 RCTs and one retrospective cohort) met the inclusion criteria for this review. They evaluated different types of adjuvant therapy such as antimicrobial photodynamic therapy (aPDT; three studies), diode laser channel irradiation (3), Nd: YAG laser channel irradiation (2), Er; Cr laser channel irradiation: YSGG (1), ozone therapy (2), and ultrasound-activated irrigation (UAI) (4). Radiographic healing was reported in seven studies, but meta-analysis was only possible for UAI (two studies), showing no statistically significant difference in healing after 12 months. Pain after 7 days was reported in seven studies. The meta-analysis of three studies that used aPDT and two studies that used diode laser irradiation showed no significant difference in the prevalence of pain after 7 days between control and adjuvant therapy.

Furthermore, the authors Sağlam H, Aladağ (2023) [25], through a randomized placebo-controlled clinical study, compared the effect of ozone and low-intensity laser therapy (LLLT) on postoperative pain after treatment of root canal in symptomatic apical periodontitis in vital teeth. A total of 80 patients were divided into four groups using a web program as follows: LLLT placebo (simulation of laser therapy), LLLT, Ozone placebo (simulation of irrigation with ozonized water) and Ozone. Postoperative pain levels for 7 days after treatment and percussion pain levels on the 7th day were recorded on the visual analog scale. Regression analysis demonstrated that the most effective variables are "group" and "jaw". Pain in the lower jaw is higher than in the upper jaw. There was a difference between the groups regarding postoperative pain on days 1, 2, and 3; however, there was no significant difference on other days. The LLLT and ozone groups had less postoperative pain and percussion pain.

Furthermore, a randomized clinical study evaluated the clinical and radiographic performance of ozonated olive oil with zinc oxide as a primary filling material for root canals. A total of 90 non-vital primary molars in children aged 4 to 8 years were allocated into three groups in which the root canals were filled with ozonized olive oil with zinc oxide, olive oil with zinc oxide, and eugenol with zinc oxide (ZOE) of according to each group after pulpectomy procedure. Clinical and radiographic evaluations were performed at the 3-, 6- and 12-month follow-up periods. All study groups showed significant improvement in clinical signs and symptoms during the follow-up periods. The ozonized olive oil group revealed a significant increase in furcation radiodensity and a decrease in periodontal ligament space at the 3-, 6-, and 12-month follow-up intervals compared to other groups [26].

Despite significantly reducing bacterial levels, ozone when used alone is not capable of producing results similar to NaOCl. Ozone has shown results comparable to NaOCl solution in vitro studies with higher concentrations or periods of use, especially when associated with PUI, NaOCl or chlorhexidine gluconate. Furthermore, studies indicate that ozone is associated with smaller bacterial load reductions than NaOCl [12,16,19,23].

A systematic review study revealed that ozone's antimicrobial effect is strongly associated with the application protocol used such as dose, and time, in addition to the correlation with the use of complementary disinfection sources. Furthermore, ozone has different antimicrobial effects according to groups of bacteria (Gram-positive and Gramnegative). Since the structure of Gram-negative bacteria contains lipopolysaccharides (LPS) and phospholipids in the membrane, this group appears more susceptible to ozone [27].

In this aspect, higher concentrations and longer periods of ozone application allow for better disinfection results. Furthermore, better results are also found when using ultrasound, NaOCl, or chlorhexidine associated with ozone therapy. In addition, a study analyzed whether irrigation with sodium hypochlorite, chlorhexidine, and ozone gas, alone or in combination, was effective against *Enterococcus faecalis* and *Candida albicans*. A total of 220 recently extracted unimodal teeth were inoculated with Candida albicans and *Enterococcus faecalis*. The formulations tested were 1, 3, and 5% sodium hypochlorite, 0.2% and 2% chlorhexidine, and ozone gas applied for different periods. The combination of 5% sodium hypochlorite and 2% chlorhexidine, with gaseous ozone, was also evaluated. Sodium hypochlorite, chlorhexidine, and gaseous ozone alone were ineffective in eliminating microorganisms. The association of 2% chlorhexidine followed by ozone gas for 24 seconds promoted the complete elimination of *Candida albicans* and *Enterococcus faecalis* [28].

A randomized study looked at the effectiveness of ozone or NaOCl/Chlorhexidine disinfection protocol compared to root canal treatment of apical periodontitis. A total of 60 permanent teeth were randomly allocated. Ozone gas (32 g m^{-3}) or NaOCl (3%) was applied, followed by a 1-week interval dressing ($Ca(OH)_2$). There were no significant differences between the success rates between the ozone and NaOCl groups after 6/12 months. The most commonly found bacterial genera were *Streptococcus spp.*, *Parvimonas spp.* and *Prevotella spp.* Therefore, the ozone gas and NaOCl/chlorhexidine gluconate protocols used here showed no difference in bacterial reduction in the sampled areas of the root canals [18].

Finally, a study evaluated the effectiveness of irrigating periodontal pockets with ozonated water and 0.2% chlorhexidine gluconate as adjuvants to scaling and root planing in the management of chronic periodontitis. A total of 20 patients aged 30-60 years, with chronic periodontitis, were included. Irrigation was performed after 2 weeks of scaling and root planing on the same day with ozonized water and 0.2% chlorhexidine gluconate for two and a half minutes. Both groups showed improvement in clinical parameters. When the comparison was made between the two groups, the ozonated water showed a slightly better improvement than the chlorhexidine group. Therefore, subgingival irrigation with ozonized water is beneficial compared to conventional therapeutic modalities. Ozonated water restricts the formation of dental plaque and reduces the number of subgingival pathogens, thus treating periodontal diseases [29].

Limitations

There is moderate evidence to provide important preliminary information about ozone therapy. Regarding microbial load reduction for patients undergoing root canal treatment, ozone therapy has inferior results when compared to conventional chemomechanical techniques using NaOCl.

Conclusion

It is concluded that ultrasonic and sonic ozone activation resulted in less pain in patients undergoing single-session endodontics compared to no ozone treatment. Ozonated olive oil with zinc oxide and olive oil paste with zinc oxide demonstrated good clinical and radiographic success for pulpectomy of primary teeth. Furthermore, low-intensity laser and ozone therapy are useful methods for postoperative pain in vital symptomatic teeth, but they are not superior to each other.

CRediT

Author contributions: **Conceptualization -** Camila Oliveira Araujo, Artur Felipe Lima de Macêdo, Arnaldo Sant'Anna Junior; **Data curation -** Camila Oliveira Araujo, Artur Felipe Lima de Macêdo; **Formal Analysis -** Camila Oliveira Araujo, Artur Felipe Lima de Macêdo, Arnaldo Sant'Anna Junior; **Investigation -** Camila Oliveira Araujo, Artur Felipe Lima de Macêdo; **Methodology -** Camila Oliveira Araujo; **Project administration-** Artur Felipe Lima de Macêdo; **Supervision -** Arnaldo Sant'Anna Junior; **Writing - original draft -** Camila Oliveira Araujo, Artur Felipe Lima de Macêdo, Arnaldo Sant'Anna Junior; **Writing-review & editing –** Camila Oliveira Araujo, Artur Felipe Lima de Macêdo, Arnaldo Sant'Anna Junior.

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Ethical Approval

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Data Sharing Statement

No additional data are available.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate@.

Peer Review Process

It was performed.

References

1. Saini R, Saini S, Sharma S. Periodontal disease linked to cardiovascular disease. J Cardiovasc Dis Res. 2010;1:161–162.
2. Kumar A, Bhagawati S, Tyagi P, Kumar P. Current interpretations and scientific rationale of the ozone usage in dentistry: A systematic review of literature. Eur J Gen Dent. 2014;3:175–180.
3. Moore G, Griffith C, Peters A. Bactericidal properties of ozone and its potential application as a terminal disinfectant. J Food Prot. 2000;63:1100–1106.
4. Lynch E. Leczenie próchnicy z wykorzystaniem systemu HealOzon. eDentico. 2004;134:3.
5. Seidler V, Linetskiy I, Hubálková H, Stanková H, Smucler R, Mazánek J. Ozone and its usage in general medicine and dentistry. A review article. Prague Med Rep. 2008;109:5–13.
6. Makeeva MK, Daurova FY, Byakova SF, Turkina AY. Treatment of an Endo-Perio Lesion with Ozone Gas in a Patient with Aggressive Periodontitis: A Clinical Case Report and Literature Review. Clin Cosmet Investig Dent. 2020 Oct 28;12:447-464. doi:

10.2147/CCIDE.S267933.
7. Sen S, Sen S. Ozone therapy a new vista in dentistry: integrated review. Med Gas Res. 2020 Oct-Dec;10(4):189-192. doi: 10.4103/2045-9912.304226.
8. A SR, Reddy N, Dinapadu S, Reddy M, Pasari S. Role of ozone therapy in minimal intervention dentistry and endodontics - a review. J Int Oral Health. 2013 Jun;5(3):102-8. Epub 2013 Jun 23. PMID: 24155611; PMCID: PMC3769872.
9. Halbauer K, Prskalo K, Janković B, Tarle Z, Pandurić V, Kalenić S. Efficacy of ozone on microorganisms in the tooth root canal. Coll Antropol. 2013 Mar;37(1):101-7. PMID: 23697257.
10. Vincent JL, Lartigau G. A propos de l'ozone [Ozone]. Rev Fr Odontostomatol. 1971 Aug-Sep;18(7):839-58. French. PMID: 4945222.
11. Brignardello-Petersen R. Insufficient evidence regarding the effects of ozone when used as a disinfectant in root canal therapy. J Am Dent Assoc. 2020 May;151(5):e43. doi: 10.1016/j.adaj.2019.12.007. Epub 2020 Mar 24. PMID: 32220347.
12. Boch T, Tennert C, Vach K, Al-Ahmad A, Hellwig E, Polydorou O (2016) Effect of gaseous ozone on Enterococcus faecalis biofilm-an in vitro study. Clinical Oral Investigations, 20, 1733–9.
13. Case PD, Bird PS, Kahler WA, George R, Walsh LJ (2012) Treatment of root canal biofilms of Enterococcus faecalis with ozone gas and passive ultrasound activation. Journal of Endodontics 38, 523–6.
14. Rojas-Valencia MN (2011) Research on ozone application as disinfectant and action mechanisms on wastewater microorganisms. In: Mendez-Vilas A, ed. Science Against Microbial Pathogens: Communicating Current Research and Technological Advances. Badajoz: Formatex, pp. 263–71.
15. Junior OO, Lages GC (2012) Ozone therapy for lumbosciatic pain. Revista Dor 13, 261–70.
16. Hubbezoglu I, Zan R, Tunc T, Sumer Z (2014) Antibacterial efficacy of aqueous ozone in root canals infected by Enterococcus faecalis. Jundishapur Journal of Microbiology 7, e11411.
17. Huth KC, Quirling M, Maier S, et al. (2009) Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. International Endodontic Journal, 42, 3-13.
18. Kist S, Kollmuss M, Jung J, Schubert S, Hickel R, Huth KC. Comparison of ozone gas and sodium hypochlorite/chlorhexidine two-visit disinfection protocols in treating apical periodontitis: a randomized controlled clinical trial. Clinical Oral Investigations. 2017, 21, 995-1005.
19. Kaya BU, Kececi AD, Guldas HE, et al. Efficacy of endodontic applications of ozone and low-temperature atmospheric pressure plasma on root canals infected with Enterococcus faecalis. Letters in Applied Microbiology. 2014, 58, 8-15.
20. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; 372 doi: https://doi.org/10.1136/bmj.n71
21. H Balshem H, Grade guidelines: 3 ratng the quality of evidence. Journal of Clinical Epidemiology, Maryland Heights, 64 (4) (2011) 401-406.
22. Higgins, S Green, Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration; 2011.
23. Sinha N, Asthana G, Parmar G, Langaliya A, Shah J, Kumbhar A, Singh B. Evaluation of Ozone Therapy in Endodontic Treatment of Teeth with Necrotic Pulp and Apical Periodontitis: A Randomized Clinical Trial. J Endod. 2021 Dec;47(12):1820-1828. doi: 10.1016/j.joen.2021.09.006.
24. Meire MA, Bronzato JD, Bomfim RA, Gomes BPFA. Effectiveness of adjunct therapy for the treatment of apical periodontitis: A systematic review and meta-analysis. Int Endod J. 2023 Oct;56 Suppl 3:455-474. doi: 10.1111/iej.13838.
25. Sağlam H, Aladağ H. Comparison of ıntracanal ozone and low-level laser therapy on postoperative pain in vital teeth with symptomatic apical periodontitis:placebocontrolled randomize trial. Lasers Med Sci. 2023 Sep 30;38(1):227. doi: 10.1007/s10103-023-03881-4.
26. El-Desouky SS, Omer SMM, Ghouraba RF, Latif RMAA, Kabbash IA, Hadwa SM. Zinc oxide-ozonated olive oil as a new root canal filling material in primary molars: a clinical randomized controlled trial. Clin Oral Investig. 2023 Dec;27(12):7395-7405. doi: 10.1007/s00784-023-05329-z.
27. Silva EJNL, Prado MC, Soares DN, Hecksher F, Martins JNR, Fidalgo TKS. The effect of ozone therapy in root canal disinfection: a systematic review. Int Endod J. 2020 Mar;53(3):317-332. doi: 10.1111/iej.13229.
28. Noites R, Pina-Vaz C, Rocha R, Carvalho MF,

Goncalves A, Pina-Vaz I (2014) Synergistic antimicrobial action of chlorhexidine and ozone in endodontic treatment. BioMed Research International 2014, 592423.

29. Kaur A, Bhavikatti SK, Das SS, Khanna S, Jain M, Kaur A. Efficacy of Ozonised Water and 0.2% Chlorhexidine Gluconate in the Management of Chronic Periodontitis when Used as an Irrigant in Conjugation with Phase I Therapy. J Contemp Dent Pract. 2019 Mar 1;20(3):318-323.