Comparative evaluation of antibacterial efficacy of *Allium sativum* extract, aqueous ozone, diode laser, and 3% sodium hypochlorite in root canal disinfection: An *in vivo* study

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**Abstract**

**Aim:** The present study aimed to individually evaluate and compare the aerobic and anaerobic antibacterial activity of *Allium sativum* extract, aqueous ozone, diode laser, and 3% sodium hypochlorite (NaOCl) as root canal irrigants.

**Materials and Methods:** Forty-eight patients were selected and randomly allocated to one of the four groups (n = 12 each) according to the irrigant to be used in each group. For each included tooth, the preirrigation and postirrigation (after irrigation with the test solution) samples were collected through sterile paper points and microbial culturing was done by swabbing on blood agar plates followed by incubation for aerobic and anaerobic bacteria.

**Statistical Analysis:** Manual colony-forming units counting were done, and statistical analysis was performed. Analysis of variance (one-way) followed by post hoc test was performed as a parametric test to compare the difference between the groups for both aerobic and anaerobic bacteria.

**Results:** All the groups showed a statistically significant reduction in bacteria (P < 0.05). However, between the groups, the maximum reduction was seen with 3% NaOCl followed by diode laser, *A. sativum* extract, and least by aqueous ozone.

**Conclusion:** *A. sativum* extract, aqueous ozone, diode laser, and 3% NaOCl showed significant antibacterial activity against aerobic and anaerobic bacteria.

**Keywords:** *Allium sativum*; aqueous ozone; diode laser; root canal irrigation; sodium hypochlorite

**INTRODUCTION**

Endodontic treatment outcome is highly dependent on efficient disinfection with the complete elimination of microorganisms and irritants from the root canal system and prevention of recontamination. Successful root canal treatment is a result of a combination of mechanical preparation by instrumentation and the use of disinfecting irrigating solutions. Therefore, root canal irrigation is essential, during and after instrumentation, to facilitate the proper removal of microorganisms, residual tissue, and debris from the root canal.[1,2] It is highly recommended that the endodontic irrigants should have broad antimicrobial activity, dissolve organic tissues, aid in proper debridement of the canal system, and be nontoxic to periapical tissues.[3]

Sodium hypochlorite (NaOCl) is considered the gold standard for root canal irrigation owing to its potent...
antibacterial action and ability to dissolve necrotic tissue. It is a highly effective antimicrobial agent, but it is known to be associated with disadvantages such as unpleasant flavor, cytotoxicity, and potential for irritation in periapical tissues, especially at high concentrations. Because of the undesirable effects of this irrigating agent, the use of alternate irrigating agents has increased in recent years.

One of the approaches to disinfecting the root canals is the use of high-power diode lasers. The bactericidal effect of high-power lasers can be attributed to their property of dose-dependent heat generation. Its efficacy against diverse microorganisms has already been verified in various studies. Another alternative that is currently being considered for disinfection of the root canal system is ozone therapy. The antimicrobial action of ozone is because of the lysis of dual bonds present on the cytoplasmic membranes of microorganisms. The use of aqueous ozone has advantages such as high potency, lack of mutagenicity, rapid antimicrobial effects, and ease of handling. It is highly biocompatible with the oral environment with no reported cytotoxicity.

However, with the constant increase in the antibiotic-resistant bacterial strains and to overcome side effects associated with synthetic irrigating agents, the use of natural plant extracts as irrigating solutions in endodontics has gained momentum as part of an evolving trend of natural remedies in dentistry. The herbal healing potential is an ancient belief, but it has garnered a lot of interest in recent years. The rise in the use of herbal is majorly due to its many advantages such as potent antimicrobial and antioxidant properties, safety, ease of availability, and cost-effectiveness. The literature has shown that Allium sativum (Garlic) has antimicrobial and therapeutic effects, suggestive of its potential to be used as a root canal irrigant. There are several in vitro studies that have confirmed the antimicrobial action of garlic on microorganisms, such as Enterococcus faecalis and Candida albicans, inhabiting the root canal system.

However, to the best of our knowledge, no in vivo study has been documented using garlic extract as a root canal irrigant.

The current study aimed to compare and evaluate the antibacterial efficacy of NaOCl, diode laser, Allium sativum extract, and aqueous ozone as endodontic irrigants in patients. The null hypothesis was that there is no difference between antibacterial effectiveness of these experimental disinfection techniques.

**MATERIALS AND METHODS**

This study was approved by an Institutional Ethical Committee under the protocol number MRDC/IEC/2018/10. Each participant of this study signed an informed consent form, to confirm their participation.

Sample size calculation was based on the assumptions as a minimum 80% power and 5% significance level (significant at 95% confidence level). Assuming variation in samples to be 25% of mean and relative error of experimental subjects to be 0.15, it was estimated that at least 11 experimental participants were needed in each of the groups. It was decided to keep the total sample size of 48, with 12 participants in each of the groups. Both males and females of the age group 18–55 years were included. All patients with carious pulp exposure on single-rooted, single canal teeth were included in the study. The inclusion criteria were established as: Carious exposure of pulp, teeth with mature apices, and teeth with a single, straight root canal. The exclusion criteria included teeth with periapical pathology, multiple roots, dilacerated root, immature apices, calcified canals, patients with a history of allergy to any of the materials being used, and patients who abstain from consuming garlic. These teeth were analyzed by preoperative radiographs followed by detailed medical and dental history recording of the patient.

The tooth and surrounding area were disinfected by swabbing with iodine tincture. Local anesthesia was administered, and isolation was done with a rubber dam followed by access opening of the root canal through sterile round bur (BR40: Mani, Inc., Tochigi, Japan). The root canal was accessed with a size 10K file (Mani, Inc., Tochigi, Japan). Debridement was done using sterile water. Working length was determined radiographically which was kept at 0.5-mm short of the apex. This was confirmed through an electronic apex locator. Then, the initial pretreatment root canal culture sample was taken with presterilized paper points. Two paper points were placed in the canal for 60 s and then transferred into two separate presterilized Eppendorf tubes of 2 ml brain–heart infusion broth (BHI broth) for aerobic culture and thioglycolate broth for anaerobic culture in each tube.

Cleaning and shaping of each root canal were done using Protaper gold rotary files (Dentsply Sirona, USA). Each patient was then randomly allotted to one of the four groups. Randomization was done using the sealed envelope technique wherein different envelopes mentioning the groups to be allotted were prepared and randomly picked by a person other than the operator, just before beginning the treatment. The entire patient selection criteria and distribution is summarized in a CONSORT flow chart (Figure 1). Each patient was blinded to the disinfection protocol being followed and randomly divided into four groups corresponding to the protocol: Group I – A. sativum extract (n = 12), Group II – aqueous ozone (n = 12), Group III – diode laser (n = 12), and Group IV – 3% NaOCl (n = 12).
Group I: 20 g garlic powder (prepared by crushing Pure Lasuna Tablets by Himalaya Wellness Pvt. Ltd.) was diluted in ethanol and distilled water in sterile conical flasks in a ratio of 1:1. The suspension of each flask was placed on a magnetic stirrer for 2–4 h to allow proper mixing. This was followed by incubation at 37°C for 24 h for the evaporation of ethanol. The supernatants were filtered using SEITZ Filtration Apparatus with a membrane filter of pore size 0.45 µm to obtain 100% garlic extract. The liquid extract was sterilized and microbial culturing was carried out on Sabourauds dextrose agar and blood agar to confirm the sterility of the extract. This sterile extract was deposited in the canals using side-vented irrigation needles (30 G, 25 mm, NeoEndo, Orikam Healthcare, Gurugram, Haryana, India) and irrigation was done for 30 min. Irrigation of the root canal was followed by sample collection through presterilized paper points placed in the root canal for 60 s and transferred to presterilized Eppendorf tubes of 2 ml BHI broth and thioglycolate broth in each. These tubes were transferred within 10 min for aerobic and anaerobic microbial culturing. A similar technique for acquiring samples from the canal was followed for all the test groups.

Group II: 5 ml of ozonated water was prepared from a commonly available domestic ozone generator (Prestige Engineering India Pvt Ltd, Noida, Uttar Pradesh, India). The prepared aqueous ozone was deposited into the root canals through a side-vented irrigation needle, and irrigation was done for 30 min followed by sample collection through presterilized paper points.

Group III: A diode laser (Epic X, Biolase Inc., USA) having a wavelength of 940 nm having an output power of 1.5W was used with a fiber optic tip of 200 µm diameter. The tip was inserted into each canal, kept 1 mm short of the working length and laser irradiation was performed with up and down movement of the tip, in three cycles of 5 s each with an interval of 10 s between each cycle. Sterile water was placed in the canal after irradiation, and sample collection was done.

Group IV: Irrigation was done with 20 ml of 3% NaOCl for 20 min. This was followed by flushing the canal with sterile water followed by sample collection through paper points.

After irrigation protocol in each group, the inoculation of each sample was done under a laminar air flow hood to avoid the contamination of the samples and the agar plates. Swabbing was done on blood agar plates followed by incubation. For the growth of aerobic bacteria, incubation was done at 37°C for 24 h and for the anaerobic bacterial growth, incubation was done using gas packs inside a McIntosh and Fieldes’ anaerobic jar to create the optimal environment for the growth of the bacteria.
growth of anaerobic bacteria and incubation at 37°C for 7 days. The microbial analysis was done by counting the colony-forming units (CFUs) through a digital colony counter. A formula was followed for the calculation of the number of colonies through the manual counting technique.\[6\]

Number of colonies in 2 ml (2000 µl) of broth = No. of CFUs × 2000 µl/10 µl

After manual calculation of CFUs, statistical analysis was carried out. Analysis of variance (one-way) followed by post hoc test was performed as a parametric test to compare the difference between the groups for both aerobic and anaerobic bacteria ($P < 0.05$ considered as statistically significant at 95% confidence level). The results were further analyzed by an evaluator who was blinded to the grouping protocol of the study.

**RESULTS**

The statistical software SPSS version 24.0 (SPSS Inc, Chicago, IL, USA) was used for the analysis. Mean CFU values of all the groups against aerobic and anaerobic bacteria, preirrigation, and postirrigation, with standard deviation were statistically significant [Tables 1 and 2]. According to the graphical representation, Group 4 showed maximum antimicrobial activity against aerobic and anaerobic bacteria followed by Group 3, Group 1, and finally Group 2 [Figure 2a and b].

The comparison between the differences of mean CFU’s for the four groups was statistically significant [Figure 2c]. The graphical representation denotes maximum antimicrobial activity by Group 4 (NaOCl) followed by Group 3 (diode laser), Group 1 (A. sativum extract), and finally Group 2 (aqueous ozone).

**DISCUSSION**

The success of the root canal treatment depends on the thorough elimination of the microbiota. Bacterial eradication is usually achieved by copious irrigation and disinfection of the entire root canal system.\[6\]

Infections in the root canals are polymicrobial in nature and a combination of aerobic and anaerobic bacteria are responsible for pulpal infections.\[14\] In the present study, the irrigating agents were tested on their overall antibacterial efficacy against aerobic and anaerobic microorganisms in single straight canals so that the disinfection potential of the agents can be tested with minimal procedural errors.

*Allium sativum* is one of the most extensively studied plants in use since ancient times due to its antibacterial, antifungal, and antiviral properties. The extract exhibits a wide range of inhibitory effect on the growth of various bacteria due to the presence of thiosulfurates which include diallyl sulfide, diallyl disulfide, and allyl methyl sulfide, which disrupt the cell membrane of the bacteria and block their enzymatic pathways.\[12,13,15,16\] The results of the current study showed statistically significant activity against both aerobic and anaerobic bacterial growth ($P < 0.01$). Up to the available data, the use of AS extract in endodontic treatment is limited. Thus, the current, in vivo study was conducted and the extract to be used as an irrigant was prepared from powdered Himalaya tablets that have been established as a safe product for human use in various medical studies to ensure the safe application and minimize the chances of adverse reactions.\[17,18\]

The bactericidal effect of diode laser could be due to its greater penetration depth (1000 µm into dentinal tubules).\[19\] In addition, diode laser causes thermal photodisruptive action in inaccessible areas of dentin, overall resulting in an enhanced bactericidal effect. In the present study, the antibacterial action was significantly high against both aerobic and anaerobic bacteria. A recent in vivo study by Sonarkar et al. analyzed the antibacterial action of a 808 nm diode laser with a 0.8W output power, which was unable to show significant results against anaerobic bacteria, and this was attributed to failure to penetrate the cell wall structure of anaerobic bacteria due to low output power.\[6\] Another in vivo study by Tilakchand et al. showed a significant decrease in anaerobic bacteria count by a 940 nm diode laser at different output powers.\[19\]

In the current study, 1.5W power of a 940 nm diode laser showed significant results against anaerobic and aerobic bacterial growth.

**Table 1: Mean colony-forming units ($\times 10^6$) of aerobic bacteria in preirrigation and postirrigation samples of the 4 groups**

| Group | $n$ | Preirrigation Mean | Preirrigation SD | Postirrigation Mean | Postirrigation SD | $P$   |
|-------|-----|-------------------|-----------------|--------------------|------------------|------|
| Group 1: AS extract | 12 | 1034.16 | 94.11 | 611.16 | 117.97 | <0.001 |
| Group 2: Aqueous ozone | 12 | 1059.00 | 109.86 | 943.33 | 101.13 | <0.001 |
| Group 3: Diode laser | 12 | 1051.33 | 64.68 | 503.50 | 65.15 | <0.001 |
| Group 4: NaOCl | 12 | 1033.33 | 55.78 | 348.41 | 36.53 | <0.001 |

SD: Standard deviation, AS: Allium sativum, NaOCl: Sodium hypochlorite

**Table 2: Mean colony forming units ($\times 10^6$) of anaerobic bacteria in preirrigation and postirrigation samples of the 4 groups**

| Group | $n$ | Preirrigation Mean | Preirrigation SD | Postirrigation Mean | Postirrigation SD | $P$   |
|-------|-----|-------------------|-----------------|--------------------|------------------|------|
| Group 1: AS extract | 12 | 1030.50 | 118.19 | 720.83 | 90.66 | <0.001 |
| Group 2: Aqueous ozone | 12 | 1022.33 | 99.72 | 941.50 | 96.41 | <0.001 |
| Group 3: Diode laser | 12 | 1051.33 | 64.68 | 506.00 | 45.57 | <0.001 |
| Group 4: NaOCl | 12 | 1060.50 | 43.25 | 351.16 | 36.72 | <0.001 |

SD: Standard deviation, AS: Allium sativum, NaOCl: Sodium hypochlorite

The present study evaluated the antibacterial properties of garlic, diode laser, and ozone against preirrigated and postirrigated samples of root canals.
Ozonated water usage for endodontic disinfection has been recommended by in vitro and in vivo studies that have successfully shown antibacterial activity of aqueous ozone. Nagayoshi et al. concluded that ozonated water had almost equal antimicrobial effectiveness as 2.5% NaOCl for endodontic irrigation. They also showed that a lower grade of toxicity against bacterial cells. On the other hand, Hems et al. evaluating the capability of ozone to terminate an E. faecalis strain confirmed that its antibacterial effectiveness was not comparable to NaOCl. Similar results were found in the present study, as ozonated water showed significant antibacterial activity against aerobic and anaerobic bacteria; however, the results were not comparable to NaOCl.

NaOCl is considered the gold standard for endodontic irrigation. NaOCl concentrations ranging from 0.5% to 5.25% are present, but there has been no agreement on the optimal concentration for endodontic usage. According to Siqueira et al., instrumentation and irrigation by using 2.5% NaOCl provided a decrease of 99.9% in the count of viable bacteria in the root canal. In the current study, 3% NaOCl was used and showed significant antibacterial action against both anaerobic and aerobic bacteria.

In our study, while all four groups significantly decreased the viable bacteria from the root canals, but when comparisons were drawn between the groups, maximum antibacterial activity was exhibited by NaOCl followed by diode laser, A. sativum extract, and finally, ozonated water. Randomization was followed for allocation of patients to each group along with blinding of the patient to the disinfection protocol being applied; however, a limitation of the study was that blinding of the operator to the laser group was not possible.

A. sativum showed substantial antibacterial action without any adverse effect to the patients, it can be considered as an endodontic irrigant. However, the study had a limited sample size because of which the results cannot be definitely correlated with clinical success and more such in vivo studies are required to evaluate the effectiveness of garlic as an endodontic irrigant.

**CONCLUSION**

Within the limitations of this experimental study, it can be concluded that A. sativum, 3% NaOCl, diode laser, and aqueous ozone show considerable antibacterial efficacy against anaerobic and aerobic microorganisms.

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**Conflicts of interest**
There are no conflicts of interest.

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