International Journal of Research and Reports in Dentistry

4(4): 147-155, 2021; Article no.IJRRD.73766

Antibacterial Efficacy of *Allium Sativum* L. Extract As a Root Canal Irrigant in Pulpless Teeth with Infected Root Canal Systems

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Authors' contributions

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

Article Information

Editor(s):
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Reviewers:
(1) Maurice Barasa Silali, Maseno University, Kenya.
(2) Satish D. Ingale , Savitribai Phule Pune University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73766

Original Research Article

Received 02 August 2021
Accepted 10 October 2021
Published 12 October 2021

ABSTRACT

Aims: Numerous studies have shown that the raw garlic extract has bactericidal effect against many pathogenic bacteria, even some antibiotic-resistant strains. Considering the role of bacteria in the development of pulpal and periapical diseases, this study aimed to evaluate the antimicrobial efficacy of aqueous extract of garlic (*Allium Sativum* L.) as an endodontic irrigant.
Methodology: In this randomized clinical trial a total of 36 patients with an infected tooth were randomly assigned into two groups (intervention group and control group). Each patient should have a single-canal tooth with a pupless and infected root canal system and chronic apical

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periodontitis. In group I, canals were irrigated with *Allium Sativum* L. with 0.1mg/ml concentration (intervention group) and in group II canals were irrigated with 2.5% sodium hypochlorite (control group). Viable colony-forming units(CFU) were quantified before and 3 days after chemomechanical debridement in each group. The data were analyzed statistically using Mann-Whitney u test and Wilcoxon test. The significance level in the statistical analysis was considered to be 5% in both groups.

Results: The initial bacterial samples were positive in all 36 teeth. In both groups, the number of CFU counts for aerobic and anaerobic samples decreased significantly after intervention ($P < 0.001$). No significant differences were observed between garlic extract and 2.5% NaOCl in the reduction of CFU counts in both aerobic and anaerobic cultures.

Conclusion: There were no significant differences between the antibacterial efficacy of garlic extract and 2.5% NaOCl.

Keywords: Anti-bacterial agents; garlic; root canal irrigants; root canal therapy; sodium hypochlorite.

1. INTRODUCTION

Garlic (*Allium Sativum* L) has been used as a food or medicine with anti- infective properties for many years. Numerous experimental and clinical studies have shown that garlic extract has antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and immunomodulatory effects [1,2]. Antibacterial and anti-fungal effects of garlic are attributed to allicin (Diallyl thiosulfonate), which has the ability to inhibit bacterial cell growth [1,3].

Bacteria and their products are thought to be the main cause of pulp and periradicular diseases [4]. Therefore, the main goal of root canal treatment is to eliminate microorganisms from the root canal system and prevent its recontamination. The presence of anatomical complexities such as fins and isthmus has limited the mechanical debridement of the root canal system and made it necessary to use endodontic irrigants to maximize the removal of microorganisms [5]. The most widely used endodontic irrigant is 0.5%-6.0% sodium hypochlorite(NaOCl). It has a wide range of antibacterial activity and can dissolve pulp tissue remnant, but it has some undesirable characteristics and side effects such as tissue toxicity, unpleasant taste, allergic reaction, and hypochlorite accident [6]. Such potential side effects of synthetic irrigation solutions and the increase in antibiotic resistant bacterial species has led researchers to seek herbal alternatives with antimicrobial activity and biocompatibility [7,8].

Studies have shown that the raw garlic extract has bactericidal effect against many pathogenic bacteria, even some antibiotic-resistant strains [9,10] Previous in-vitro research have revealed that garlic extract has antibacterial and antifungal properties as an endodontic irrigant [5,9,11-13]. For clinical use of this natural substance, its efficacy and safety should also be considered in clinical conditions. It has been proven that garlic as a nutrient has no genotoxicity and mutagenicity and does not cause cytotoxicity for human gingival fibroblasts [14,15].

The caustic effect of endodontic irrigants can cause acute inflammation of periradicular tissues and flare-ups [16]. Flare-up is an acute manifestation of pulp and periapical pathosis after the onset or continuation of root canal treatment [17]. It has multifactorial etiology including microbial, chemical and/or mechanical injuries to the pulp or periapical tissues [18]. Therefore, in order to assess the efficacy of an endodontic irrigant in clinical situations, it is also necessary to investigate the occurrence of flare-up following the use of irrigant.

This randomized clinical trial evaluated the antibacterial effectiveness of aqueous garlic extract (AGE) as an irrigant during the preparation of infected teeth with apical periodontitis. The incidence of post-operative pain and swelling was also considered.

2. MATERIALS AND METHODS

Investigating the antimicrobial effect of garlic extract, Birring showed that garlic extract prevents the formation of E. faecalis's biofilm and also degrades it. In their study, antimicrobial effect of 70% garlic extract was equivalent to 5.25% sodium hypochlorite. They concluded that garlic extract could be used as a proper biocompatible endodontic herbal rinse to penetrate into dentinal tubules [13]. Kazemizadeh et al. in an in-vitro study used the

method of Well Agar Diffusion to compare the anti-bacterial effect of pure garlic extract (100%), garlic extract 80%, chlorhexidine 2%, sodium hypochlorite 5.25% and combined chlorhexidine 2% with pure garlic extract. They reported that the garlic extract was effective on Enterococcus Faecalis in both aerobic and anaerobic conditions nevertheless it had less efficacy than 5.25% sodium hypochlorite [19]. Jerin Jose showed in an in-vitro study that garlic extract as an endodontic irrigant has significant inhibitory effects against Enterococcus faecalis and Candida albicans when compared with 2.5% sodium hypochlorite [5]. According to Karkare and colleagues, there is no statistically significant difference in the antibacterial activity of diluted garlic extract, saturated garlic extract, and 5.25% NaOCl as root canal irrigant against Enterococcus faecalis [12]. Ambareen et al. compared extracts of ginger, garlic, aloe vera, neem, turmeric, and sodium hypochlorite as root canal irrigants against E.fecalis. There was no significant difference between the antimicrobial property of AGE and 2% sodium hypochlorite [11].

The study population consisted of patients referred to the Endodontic clinic of Mashhad Dental School. The inclusion criteria were adult patients having a single-canal tooth with pupless and infected root canal system confirmed by pulp tests (cold test and electric pulp tester) and clinical and radiographic evidence of chronic apical periodontitis (periapical radiolucency detectable in radiography). The exclusion criteria were teeth with calcified pulp chamber in periapical radiography, extensive destruction of the crown, teeth with previous endodontic treatment, vital pulp after exposure, patient with certain systemic conditions (including diabetes, any disease leading to immunosuppression and administration of immunosuppressive drugs) and patients who had received antibiotic therapy within a previous month. The study protocol and the informed consent format were approved by the Ethic Committee (IR.MUMS.DENTISTRY.REC.1397.003) of the Mashhad University of Medical Sciences. All included patients signed an informed consent after the explanation of the involved procedures and the possible risks.

This study was a single-blind randomized clinical trial (Trial Registration: IRCT20181103041534N1). An endodontic postgraduate student performed all procedures that was not possible to be unaware about the type of irrigant because of its recognizable odor. The microbiologist who performed the microbial examination of the specimens was blind.

2.1 Calculation of the Sample Size

The sample size calculation, based on an alpha error = 0.05 and a power of 0.95, indicated that 15 samples in each group would be required [20]. With the consideration of 20% dropout rate the final sample size was estimated to be 36 samples. The subjects were randomly distributed into two equal groups(n=18) according to the type of irrigant used. Randomization was obtained by NCSS software and block method.

2.2 Extraction of Garlic Filtrate Ware

After washing and peeling, 200g of Hamedan (province of Hamedan, Iran) fresh garlic cloves were crushed and then mixed with 200 mls of distilled water. The mixture was filtered through a double filter paper and centrifuged for 10mins at 5000rpm to precipitate garlic particles [21]. The extracted liquid was used at different concentrations to determine the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the garlic extract. A sterile syringe filter (0.22 µ) was used to sterilize the extract.

2.3 Susceptibility Test

The MIC and MBC of AGE against E.faecalis were determined using a broth microdilution susceptibility test method. According to the Test, the MIC and MBC of 0.1mg/ml AGE were both 6.25 µg/ml.

At the first visit, after administration of local anesthesia, the tooth crown was polished with pumice and isolated with rubber dam. The tooth surface and field of operation were cleaned using 5.25% NaOCl. After removing all caries, the second disinfection procedure of the operative field was performed before exposing the pulp chamber. Five percent sodium thiosulphate was used to deactivate any remnant NaOCl then the access cavity preparation was completed. Using a manual No.10 k-file (Mani Inc., Japan) the canal contents were debrided from the root canal walls by a gentle pull and push motion. If there were any anatomical variations in the root canal system such as extra canals or type IV root canal anatomy, the tooth was dismissed from the study.

The pretreatment samples were collected from the root canal under aseptic conditions as follow: Sterile distilled water was placed in the canal, taking care not to overfill. Two No. 20 sterile paper points (Meta Biomed Paper Point, Korea) were placed in the canal about 1mm short of the radiographic apex for 60s. Each of two paper points was transferred to a sterile microtube containing 2 ml of the brain heart infusion broth (Merck-Germany), one for aerobic culture and the other for anaerobic culture. The tubes were immediately sent to the microbiological laboratory for bacterial cultivation. The working length was estimated with an apex locator (Root ZX, J. Morita USA, Inc., Irvine, CA, USA) and confirmed by periapical radiographs. Then the canal was prepared chemomechanically by a single length technique with WaveOne reciprocating system (Dentsply Maillefer, Switzerland). Irrigation with either 2.5%NaOCl or 0.1mg/ml AGE was conducted throughout the procedure using a 5 ml syringe with a 27-gauge needle which was placed 1 mm shorter than the working length. During the root canal preparation, a total volume of 5 ml either irrigation solutions was used in each tooth. At the end of the root canal preparation, 5 ml of the same irrigant was used as the final rinse. The overall irrigation time for each tooth was limited to 5 minutes. After completion of the root canal preparation, the root canal was dried with sterile paper points, and the access cavity was temporarily sealed with glass ionomer (GC Corporation, Tokyo, Japan).

The patient was recalled after three days. After the injection of local anesthesia, placing the rubber dam, and disinfecting the tooth surface as in the first session, the canal was accessed again. The secondary specimens were collected using the same method described for the pretreatment samples. Then the root canal treatment was performed and completed using the standard operating protocol. The tooth was temporarily restored and the patient was referred for the permanent restoration.

At each session, for aerobic culturing, tubes containing the paper point and BHI broth medium were sent to the microbiological laboratory. The time between sample collection and the beginning of the culturing process should not exceed 1 hour. Under sterile conditions using a microbiology hood, 10µl of tube content was placed on 5% Columbia Sheep Blood Agar Plates (Merck-Germany) by a sampler. The agar plates were then placed in an incubator at 37°C for 24hours. After 24 hours, the bacterial load of each sample was measured as colony forming units(CFUs) using manual counting technique.

For anaerobic culture, the paper point was immediately inserted into a tube containing the BHI broth medium. Then the surface of the BHI medium was covered with mineral oil to create anaerobic conditions. According to the method described for aerobic samples, culturing was performed on 5% Columbia sheep blood agar plates. The culture plates were then placed in an anaerobic chamber containing Anaero Gas Pack and Anaero Indicator tablet. The chamber was kept in an incubator for 24 hours at 37°C, after which the CFU was counted.

The modified visual analog scale(VAS) was used to measure pre- and postoperative pain [22]. The VAS form was scaled from 0-9, and the patient was asked to mark his level of pain on that form at eight time-points: before the intervention, immediately after the first session, 6 hours,12 hours,24 hours, 2days and 3 days after the first session, and immediately after the second session. Data were collected and classified in 4 groups: no pain (0), mild, but not unpleasant pain [1-3], moderate, discomforting, but bearable pain [4-6], severe, unbearable pain [7-9].

The patient was asked to inform the practitioner in case of any severe pain or swelling in the area, to receive advice or medication(s). The patient was then recalled and the swelling was recorded by clinical examination in the form of presence or absence of the swelling. The patients were followed-up for 48h. In this study, none of the patients developed swelling or sever pain necessitating removal from the study.

The data were analyzed using SPSS software. The level for accepting statistical significance was set at $P < .05$. Shapiro–Wilk tests confirmed a non-normal distribution thus, the nonparametric Mann–Whitney U test was used to compare between the two irrigation groups.

3. RESULTS

A total of 36 subjects (18 per group) were included in the study. One of the cases in the NaOCl group was excluded from the study due to an increase in CFU counts in the second session, which revealed the probability of canal contamination between the sessions; finally, statistical analysis was done for 17 specimens in NaOCl group (control group) and 18 specimens in AEG group (intervention group).

Statistical analysis using Shapiro Wilk test demonstrated that the data were nonparametric. Both tested irrigants showed antibacterial activity. Table 1 summarizes the descriptive statistics of CFUs in the two groups. The rate of CFUs reduction after irrigation was significant in both groups (P<0.001). Using Mann-Whitney U test, the mean reduction in aerobes and anaerobes was not significantly different between intervention and control groups (P=0.0999 and P= 0.792, respectively).

All patients in both groups reported mild pain or no pain at all different time-points, except one patient in the NaOCl group who reported moderate preoperative pain, which lasted up to 6 hours after the first session. No significant difference was found for the mean of VAS score between two groups at each time-point. Pairwise comparison of time-points showed that in both groups the VAS score at 24, 48, and 72 h after the first session decreased significantly compared to the VAS score immediately after the first session. Pairwise comparison showed no significant difference between other time-points in any of the groups (Fig. 1).

None of the patients developed swelling.

4. DISCUSSION

To the best of our knowledge, this is the first randomized clinical trial evaluating the antibacterial efficacy of garlic extract as an endodontic irrigant. The rate of CFU reduction was significant for 2.5%NaOCl and 0.1mg/ml AGE, showing that both irrigants had antibacterial activity. There was no significant difference between the two irrigants in terms of CFU reduction.

Gopala-Krishnan showed that the proper contact time to reach the maximum antimicrobial efficacy of garlic extract in the root canal was 5min [9]. Some studies have shown that antimicrobial efficacy of 2.5%NaOCl with a contact time of 5 min is similar to 5.25% NaOCl with a contact time of 30s to 2min [23,24] therefore, in this study, the total irrigation time was considered 5 minutes.

The root canal sampling was performed in a similar method to Moller's works (microbiological root canal sampling technique) [25]. Although the culturing technique is reliable for the identification and counting of bacteria that persist after the antimicrobial treatment, part of the endodontic microbiota is not cultured in this technique. Modern molecular-based identification methods such as Polymerase Chain Reaction (PCR) have been introduced to identify microbial DNA instead of viable microorganisms. Yet the limitation of PCR is that it cannot differentiate between DNA of viable or dead bacteria. As a result, PCR is not applicable in the quantitative studies that tend to count the number of living bacteria in the canal [26].

In the current study, secondary samples were collected 3 days after the chemomechanical preparation of the root canal system. Studies have demonstrated that in many cases, immediately after the use of irrigant, the number of bacteria in the root canal is negligible that the bacterial culture doesn't show any bacterial growth [27]. Therefore, for an accurate evaluation of the irrigant's antibacterial efficacy, secondary sampling was performed at a later session. This method was called the "Golden Standard" and used in several studies [27,28].

In this study, none of the patients reported swelling or severe pain between or after the sessions. Only one case in the NaOCl group reported pain up to 6 hours after the operation, which may be due to his moderate pain level before the treatment. Several studies previously demonstrated that the presence of preoperative pain had significant effects on the severity of postoperative symptoms [29].

The findings of this study on antibacterial efficacy of *Allium Sativum* L. extract as a root canal irrigant are consistent with several in-vitro studies that demonstrated the antibacterial efficacy of garlic as an endodontic irrigant [9,11,13]. Birring *et al*. found that the antimicrobial efficacy of 70% concentration of garlic extract was equal to 5.25%NaOCl, which is consistent with our study results [13]. In a study by Ambareen, AGE showed to be a potent antimicrobial agent against E.fecalis, and there was no significant difference between the antimicrobial property of AGE and 2% NaOCl [11]. Kazemizadeh *et al*. reported that the garlic extract was effective on Enterococcus Faecalis in both aerobic and anaerobic conditions nevertheless it had less efficacy than 5.25% sodium hypochlorite [19]. The difference between their study results and ours may be due to using higher concentrations of NaOCl in Kazemizadeh's study. Another reason may be the different measuring techniques used in evaluating the irrigants' antimicrobial effect, which was growth inhibitory zones in Kazemizadeh's study and CFUs

counting in ours. In another study, Karkare and colleagues found no statistically significant difference in the antibacterial activity of diluted garlic extract, saturated garlic extract, and 5.25% NaOCl as root canal irrigant against Enterococcus faecalis. These results are consistent whit the present study, although they used hydroalcoholic extract of garlic for the preparation of saturated and diluted garlic extracts [12]. Jerin Jose showed in an in-vitro study that garlic extract as an endodontic irrigant has significant inhibitory effects against Enterococcus faecalis and Candida albicans. The zones of inhibition of bacterial growth attained by 2.5% NaOCl were greater than garlic extract for both microorganisms [5]. In contrast to the present study, 2.5% NaOCl had statistically significant superior antibacterial activity against the tested microorganisms. The difference may be due to the different methodologies used for evaluating antimicrobial efficacy. Another reason may be using garlic powder for preparing garlic extract in their study instead of fresh garlic cloves. Other investigations have shown several factors attributed to such discrepancies, including the use of different extraction methods, the use of garlic powder instead of fresh garlic cloves, or the use of garlic cloves originated from different geographical regions that cause different amounts of active ingredients in the extract [2,30].

One issue in the clinical application of garlic is the pungent odor of its sulfur compounds. Since these compounds are also responsible for the antimicrobial properties of garlic, they cannot be eliminated from the extract's composition [31]. Adding flavoring agents to the irrigant may help to make its smell more acceptable to the patients.

Regarding the antibacterial activity and biocompatibility of AEG, it may be considered as an alternative for NaOCl in root canal treatment. Other ideal requirements of root canal irrigants such as dissolving pulp tissue remnant, prevention of smear layer formation and being systemically nontoxic should also be investigated in future studies before the solution can be considered for clinical use. This study may provide a framework for further clinical trials in the future.

5. CONCLUSION

The results of this study showed that both 2.5% sodium hypochlorite and *Allium Sativum* L. extract as intra-canal irrigants significantly reduced the number of intra-canal bacteria in infected root canals. Although the antimicrobial efficacy of garlic extract was not significantly different from that of NaOCl, further studies are required to consider AGE as an alternative for sodium hypochlorite in root canal treatment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The study protocol and the informed consent format were approved by the Ethic Committee (IR.MUMS.DENTISTRY.REC.1397.003) of the Mashhad University of Medical Sciences. All included patients signed an informed consent after the explanation of the involved procedures and the possible risks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX 1

Table 1. Comparison of percentage decrease in bacterial count between two groups using Mann-Whitney U-test

Variable	Group	N	Mean±Std.	Median± interquartile range	Min.	Max.	Mean rank	Mann-Whitney U-test
Aerobic reduction	Garlic	18	86.58±13.14	90.59±18.25	58.33	99.95	20.8	Z=1.65
	NaOCl	17	78.26±15.37	81.11±31.71	58.33	99.98	15.1	P=0.099
Anaerobic reduction	Garlic	18	89.78±14.27	96.00±13.78	60.00	99.96	18.4	Z=0.26
	NaOCl	17	88.94±12.99	94.69±17.31	60.00	99.98	17.5	P=0.792

APPENDIX 2

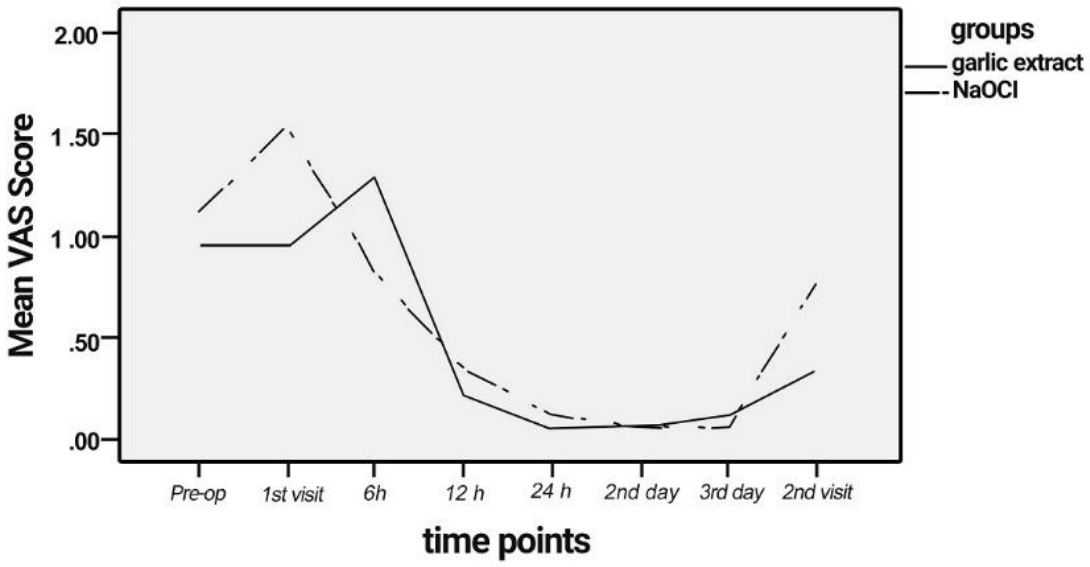

Fig. 1. Mean pain score at different time-points in both groups

Peer-review history:
The peer review history for this paper can be accessed here:
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