Original Article

Comparing the efficacy of herbal irrigants using laser activation in removing endodontic pathogens: An *in vitro* analysis

Arvind Kumar Alexander, Benin Paulaian, Shunmuga Priya Tamilarasan, Nagaraj NJ, Beautlin JS, Mohan Kumar RS[1]

Department of Conservative Dentistry and Endodontics, Rajas Dental College and Hospital, Tirunelveli, [1]Priyadarshini Dental College and Hospital, Thiruvallur, Tamil Nadu, India

Abstract

Context: The removal of harmful endodontic pathogens involves the mechanical enlargement of the root canal space along with the use of irrigants to improve debridement and disinfection. Although this is effective in most of the cases, failures still do occur due to the microorganisms which remain inside the root canal system.

Aim: This research aims to assess the antibacterial efficacy of *Azadirachta indica* and *Morinda citrifolia*, comparing them with 3% sodium hypochlorite (NaOCl), while also examining the impact of laser activation on these irrigants.

Methodology: Sixty-four single-rooted teeth with single canal were selected and decoronated to a standard length of 16 mm. All the samples were cleaned of debris and autoclaved. Two samples were selected randomly to check the complete disinfection, and the rest were inoculated with *Enterococcus faecalis* and *Streptococcus mutans*. Biofilm formation was checked in two randomly selected samples after 7 days. The remaining samples were then divided into three groups, namely A, B, and C. Then, each group was subdivided into two subgroups (A1, A2, B1, B2, C1, and C2). Group A was irrigated with 3% NaOCl solution. Group B was irrigated with *A. indica* extract, and Group C was irrigated with *M. citrifolia* extract. The subgroups A2, B2, and C2 were activated with Biolase diode laser, and colony-forming units (CFUs) were counted for all samples.

Statistical Analysis: Statistical analysis was done using analysis of variance and paired *t*-test.

Results: The mean and standard deviation of CFUs in all the groups before and after laser activation denote no significant difference.

Conclusion: The antibacterial activity of *A. indica* and *M. citrifolia* was comparable with 3% NaOCl. Hence, they can be used as an alternative to the most commonly used chemical root canal irrigants. Laser activation can be used as an adjuvant in eradication of microbes from the root canal system.

Keywords: Antibacterial effectiveness; *Azadirachta indica*; herbal irrigants; laser activation; Morinda citrifolia; root canal irrigants

INTRODUCTION

Root canal therapy entails the mechanical enlargement of the root canals, complemented by the use of various irrigants to enhance cleaning and disinfection. While this method is usually effective, there are instances of occasional treatment failures.[1,2] Because of the complex three-dimensional nature of the root canal system, mechanical instruments alone cannot completely prepare the canal surface.[3] In areas where instrumentation is insufficient, irrigation plays a crucial role in eliminating both biofilm and debris. Commonly used root canal irrigants are 2% chlorhexidine (CHX) and 5.25% sodium

Address for correspondence:
Dr. Shunmuga Priya Tamilarasan,
1A/1B, Bryant Nagar, 5th Street – Middle (Near Kamaraj Mahal), Tuticorine - 628 008, Tamil Nadu, India.
E-mail: 2mdentalcare21@gmail.com

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hypochlorite (NaOCl). Both are known for their effectiveness. Ideal root canal irrigants should have a pleasant taste and odor, be nontoxic, and be biocompatible. However, CHX has some disadvantages, including an unpleasant taste and smell. Although it provides a long-lasting antibacterial effect, it can cause tooth discoloration and has limited tissue-dissolving capabilities. Moreover, NaOCl can also irritate periapical tissue, cause allergic reactions, be toxic to tissues, stain instruments, and have an unpleasant taste and odor. As antibiotic-resistant strains become more prevalent, and the adverse effects of chemical irrigants are noted, there is increasing interest in alternative herbal remedies.

For over 2000 years, Polynesians have utilized the juice of *Morinda citrifolia*, or noni, in traditional healing practices. Noni is known for its wide range of therapeutic properties, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing effects.[4-6] Acetone extracts of *M. citrifolia* have also displayed antimicrobial properties.[4,7] *A. indica*, or neem, belongs to the mahogany family (Meliaceae*)* and is known for its antiviral, antifungal, antibacterial, and anticancer properties. It has been effectively used to treat dental plaque and gingivitis, making it a powerful agent for root canal irrigation.[8-10]

Even with the most effective root canal irrigants, proper delivery and penetration of the antimicrobial solutions into the three-dimensional microstructure is crucial for achieving efficient debridement and disinfection of the canal space.[11,12] Traditional root canal irrigation using a syringe and needle combination frequently fails to accomplish this effectively.[13,14] A small but significant amount of bacteria can linger in the root canals following conventional irrigation, even after apical enlargement.[15] Irrigant activation techniques have been proposed to enhance the distribution, flow, and disinfection of irrigants within the canal system, thereby improving the effectiveness of irrigation.[16] Lasers have been considered a promising adjunct to improve the effectiveness of endodontic treatment.[17]

Although the effects of laser activation have been confirmed for conventional chemical root canal irrigants,[18] its influence on herbal irrigants has not yet been explored. Hence, this research aims to assess the antibacterial efficacy of *Azadirachta indica* and *M. citrifolia*, comparing them with 3% NaOCl, and also examine the impact of laser activation on these irrigants.

METHODOLOGY

Dual-species biofilm

The bacterial species utilized in our study were *Enterococcus faecalis* (MTCC497) and *Streptococcus mutans* (MTCC6845). These bacteria were sourced from a stock culture, streaked onto nutrient agar plates, and incubated for 24 h at 37°C. The cultures were preserved on nutrient agar at 4°C and subcultured regularly. Before each experiment, single colonies were inoculated into nutrient broth and grown overnight at 37°C. The cell suspensions were adjusted spectrophotometrically to an optical density of 600 nm to ensure uniform bacterial colony-forming units (CFUs) for each experiment.

Source of data

The study included 64 uniradicular permanent teeth with a single root canal, which were extracted for periodontal or orthodontic reasons. Multirooted teeth, teeth with root caries, and those that had previously undergone endodontic treatment were excluded.

Sample preparation

All extracted uniradicular teeth were immersed in a 5.2% NaOCl solution for 30 min to remove organic residues, then stored in saline solution. The crowns were sectioned at the cementoenamel junction using a high-speed diamond disk to establish a standardized root canal length of 16 mm. A size 10 K-file (Dentsply Maillefer) was introduced into each canal until it reached the apical foramen, and the working length was determined by subtracting 0.5 mm from this measurement. The teeth were instrumented to the working length using a rotary file (ProTaper System; Dentsply Tulsa Dental, Tulsa, OK) up to size F3. During the instrumentation process, the root canals were irrigated with 2 mL of 3% NaOCl between each file, followed by a final rinse with sterile saline solution to eliminate any NaOCl residue. The root canals were dried with sterile paper points (Dentsply Tulsa Dental). The teeth were then air-dried overnight at room temperature, and the apical foramen was sealed externally with two coats of nail varnish to ensure waterproofing. Finally, the teeth were sterilized in an autoclave at 121°C for 15 min under 15 lbs of pressure. Two samples were then selected randomly to check for complete disinfection. The remaining samples were inoculated with *E. faecalis and S. mutans*.

Each sterilized root canal was inoculated with a 1:1 mixture of the two cell suspensions, filling the canals to the entrance level. The inoculated specimens were incubated at 37°C with 100% humidity for 2 weeks. After this period, the selected samples were randomly divided into three equal groups. Then, 10 teeth from each group were disinfected as follows:

- Group A1: The root canals were irrigated with 5 mL of 3% NaOCl for 60 s using a 5-mL syringe and a 30G double-side vented needle, positioned 2 mm short of the working length
- Group A2: The root canals were rinsed with 5 mL of 3% NaOCl for 60 s, followed by activation with a diode laser operating at a wavelength of 940 nm and a frequency

of 50–60 Hz. This laser can deliver energy in either pulsed or continuous wave mode, with a maximum output power of 7W, using a 200-µm plain-ended fiber designed for endodontic applications (Epic X, Biolase, San Clemente, CA, USA). The continuous wave mode was employed in this study[19]

- Group B1: The root canals were irrigated with 5 mL of *A. indica* for 60 s using a 5-mL syringe and a 30G double-side vented needle, placed 2 mm short of the working length
- Group B2: The root canals were rinsed with 5 mL of *A. indica* for 60 s, followed by activation with the diode laser as previously described
- Group C1: The root canals were irrigated with 5 mL of *M. citrifolia* for 60 s using a 5-mL syringe and a 30G double-side vented needle, positioned 2 mm short of the working length
- Group C2: The root canals were rinsed with 5 mL of *M. citrifolia* for 60 s, followed by activation with the diode laser as mentioned earlier.

Quantification of surviving bacteria

After treatment, remaining cells were detached using a combination of sonication and vortex mixing, with three cycles of 30 s each. The teeth were then rinsed with 1 mL of normal saline. Subsequently, the rinsed saline was transferred onto mitis salivarius agar and spread-plated with an inoculation loop, then incubated at 37°C for 48 h. After incubation, the colonies were counted, and the mean number of CFUs in each group was calculated and compared [Figures 1 and 2].

The CFUs were determined using the following formula:

CFU/mL = (Number of bacterial colonies counted on plate × dilution factor)/volume of culture plate.

RESULTS

Statistical analysis was performed using SPSS Statistics 22 software (IBM Corp., Turkey). The descriptive statistics (mean, standard deviation, and percentages) was obtained, and Intergroup comparison was done by using analysis of variance followed by paired *t*-test. The level of significance was set at 5% ($P < 0.05$ = statistically significant).

Statistical analysis

The effectiveness of each irrigant before and after laser activation against *E. feacalis and S. mutans* was evaluated.

The mean and standard deviation of CFUs in all the groups before and after laser activation which denotes no significant difference between the groups [Table 1].

Table 2 depicts intergroup comparison with same irrigants before and after laser activation which denotes no significant difference.

DISCUSSION

Root canal infections are mixed and semispecific infections characterized by a predominance of obligate anaerobic bacteria. The semispecific nature of these infections is attributed to the relationship between certain bacterial groups and specific forms of periradicular disease. Both species investigated in this study have been shown to be associated with primary root canal infections as well as persistent infections, which can lead to treatment failure. They may present clinical challenges either as resistant microorganisms that survive intracanal procedures or as secondary invaders that can enter the root canal during or after treatment.[1] It has been noted that Gram-positive bacteria, particularly facultative ones, may exhibit greater

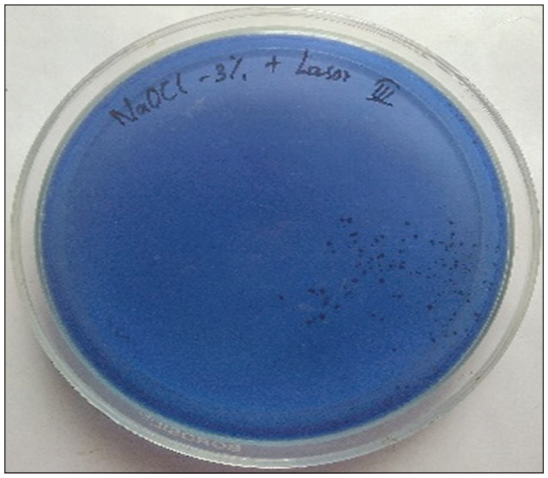

Figure 1: Image of spread plate containing colony forming units after sodium hypochlorite activation with diode laser

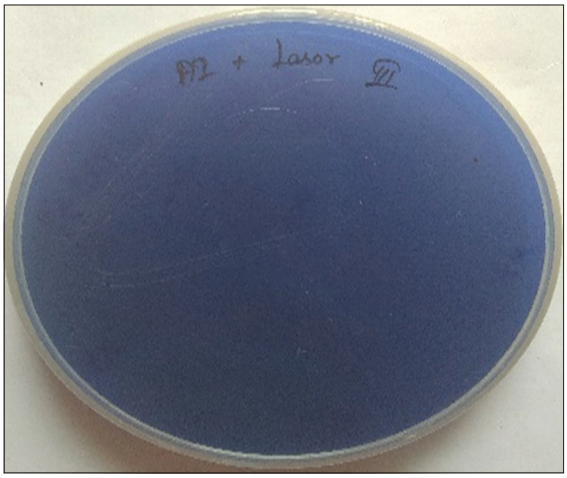

Figure 2: Image of spread plate containing colony forming units after Azaradichta indica activation with diode laser

Table 1: The mean and standard deviation of colony forming units

Groups	*n*	Mean	SD	*P*
Group A1	10	51.50	60.43	0.048*
Group A2	10	59.00	91.38	
Group B1	10	7.20	15.53	
Group B2	10	0.00	0.00	
Group C1	10	23.40	31.04	
Group C2	10	20.60	27.04	

*Statistically significant ($P<0.05$). *P* value based on ANOVA. ANOVA: Analysis of variance, SD: Standard deviation

Table 2: Paired-*t*-Test

Groups	*n*	Mean	SD	*P*
Group A1	10	51.50	60.43	0.843
Group A2	10	59.00	91.38	
Group B1	10	7.20	15.53	0.177
Group B2	10	0.00	0.00	
Group C1	10	23.40	31.04	0.845
Group C2	10	20.60	27.04	

*Statistically significant ($P<0.05$). *P* value based on paired *t*-test. SD: Standard deviation

resistance to the effects of chemomechanical procedures.[15] In these instances, isolates from the *Streptococcus* group were the most prevalent. Moreover, *Enterococcus* species have been found to proliferate in certain canals following standard chemomechanical procedures.[15] Indeed, Leach *et al*.[7] has noted that *E. faecalis* and members of the *Streptococcus anginosus* group are the most commonly recovered microorganisms from teeth with failed endodontic treatment. While *E. faecalis* is occasionally found in primary root canal infections, literature suggests that it plays a significant role in endodontic failure. Its occurrence in root-filled teeth with periradicular lesions can range from 30% to 40% of cases.[7] In our study, the root canals were inoculated with *E. faecalis* and *S. mutans*.

The results showed no differences between conventional NaOCl irrigation, *A. indica* irrigation, *M. citrifolia* irrigation, and activation with a diode laser. This can be partly attributed to the exposure time of NaOCl. There is currently no consensus on the required disinfection duration with NaOCl to eliminate *E. faecalis* from the root canals. The 1-min disinfection time used in this study was selected based on recommendations for the final disinfection protocol.[20-22] However, the results indicate that the 1-min exposure may not have been adequate for the antimicrobial action of 3% NaOCl. In fact, the comparable effectiveness of herbal irrigants and NaOCl irrigation could be attributed to the mechanical effects of fluid movement and replacement from continuous irrigation at a flow rate of 5 mL/min, combined with the inherent antibacterial properties of the irrigants themselves.[23]

Regarding the high-power diode laser, the current results align with previous studies that also showed challenges in effectively eliminating Gram-positive *E. faecalis* using diode and Nd:YAG lasers.[24,25] Laser-induced bacterial eradication results from the thermal heating of the surrounding environment to temperatures exceeding lethal levels of microorganisms, along with localized heating within the bacteria.[24] The survival of *E. faecalis* and *S. mutans* species, and their lower reduction rates, can be attributed to the high resistance of these bacteria to heat, which is due to their cell wall structure.[25] Disinfecting agents like NaOCl need direct contact with bacteria, which is often challenging in peripheral areas of the root canal, such as anastomoses, fins, and the most apical regions of the main root canal.[26]

CONCLUSION

There is no significant difference in the antibacterial activity of *A. indica* and *M. citrifolia* when compared to NaOCl, indicating that they can be used as alternatives to chemical root canal irrigants. Furthermore, diode laser activation and conventional syringe irrigation showed comparable antibacterial effects.

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Conflicts of interest
There are no conflicts of interest.

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