Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/3cj5ad33

A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECTS OF HERBAL ROOT IRRIGANT CARVACROL VERSUS SODIUM HYPOCHLORITE USING PASSIVE AND ULTRASONIC IRRIGATION TECHNIQUE – AN IN VITRO STUDY

Dr. Kanika Jain[1], Dr.Aakriti[2], Dr. Anukriti Pathak[3]

[1*]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Kalka Dental College, Meerut, Uttar Pradesh
[2]Assistant Professor, Department of Dentistry, Laxmi Chandravansi Medical College and Hospital, Palamu, Jharkhand
[3]Senior lecturer, Department of Conservative Dentistry and Endodontics, Inderprastha Dental College, Ghaziabad, Uttar Pradesh

***Corresponding Author:** Dr. Kanika Jain
* Senior Lecturer, Department of Conservative Dentistry and Endodontics, Kalka Dental College, Meerut, Uttar Pradesh

ABSTRACT:
AIM & OBJECTIVE- Microorganisms are the actual cause of all the pathologies of the pulpal and periapical tissues. Enterococcus faecalis is the most commonly found bacteria in failed root canal treatments. Chemical irrigation of canals along with biomechanical preparation helps in the elimination of microorganisms. The present study was aimed to evaluate the antimicrobial effect of herbal root canal irrigant Azadirachta indica with sodium hypochlorite (NaOCl) using passive and ultrasonic irrigation technique.
MATERIAL & METHODS - The antimicrobial efficacy of irrigant was assessed by counting the colony forming units of E. faecalis which was taken from root surface by the sterile paper point and inoculated on blood agar and incubated for 24 hours at 370C. It was done by incubating irrigated teeth samples with paper point for 30 minutes. Then, those paper points were used for inoculating the E. faecalis colonies on the blood agar. Blood agar plates were incubated for 24 hour at 370C. Then, Colony forming unit and percent reduction can be calculated.
RESULTS – Azadirachta indica has antimicrobial effect against E faecalis but it can not be ultrasonically activated.
CONCLUSION – Due to various toxic effects of sodium hypochlorite, Azadirachta indica can be used as an intracanal irrigant as it has antimicrobial effects against E faecalis.

KEY WORDS - Enterococcus faecalis, Biomechanical Preparation, Antimicrobial Efficacy, Azadirachta indica, Sodium hypochlorite.

INTRODUCTION:

Microorganisms are the actual cause of all the pathologies of the pulpal and periapical tissues. Dental caries is the most common pathway for microbes to enter the root canal system. There are persistent infections where microbes are resistant to antimicrobial procedures, generally comprised of gram-positive bacteria, especially *E. faecalis*.[1] *E. faecalis* is a normal inhabitant of the oral cavity, mostly

associated with acute periradicular periodontitis or acute periradicular abscesses. It is present in 4–40% of primary endodontic infections and 24–77% of failed root canal treatment cases.[2] It can grow at 10–45°C at pH 9.6 and overcomes the challenges of survival within the root canal system in several ways.[3]

Mechanical preparation of the root canal along with chemical irrigation is essential for the success of root canal treatment. The irrigant must possess antibacterial properties, tissue-dissolving ability, low surface tension, substantivity, lubrication, and should be biocompatible.[4] The gold standard among various irrigants is sodium hypochlorite (NaOCl) because of its antimicrobial efficacy with tissue-dissolving properties. Effective irrigant delivery and agitation are important. Therefore, recently, new techniques have been advocated in dentistry in which irrigant solutions are activated ultrasonically. Active irrigation initiates dynamics and flow within the fluid, thus improving root canal disinfection.

However, chemical irrigants have certain shortcomings, the major disadvantages being high toxicity, reduction in elastic and flexural strength of dentin, corrosiveness to instruments, and unpleasant taste. This has led to the search for herbal alternatives such as *Azadirachta indica*, carvacrol, *Morinda citrifolia*, etc.[5] The advantages of using herbal irrigants include low toxicity, lack of microbial resistance, and easy availability. Some products have gained attention due to these advantages, supported by research and the current worldwide "back to nature" trend.[6]

The herbal irrigant used in this study is Carvacrol, one of the active components of oregano oil. It has broad-spectrum antibacterial activity, which works by ATP inhibition and increased non-selective permeability of the bacterial cell membrane. This, in turn, helps inhibit bacterial colonization and makes bacteria more sensitive to antibacterial agents.[7] Its anti-inflammatory activity can be explained by its significant inhibition of human neutrophil elastase activity with a very low IC_{50} value.[8] Therefore, this new material seems to show promising results as an irrigant.
In this study, we are comparing Carvacrol as a herbal irrigant against *E. faecalis* with sodium hypochlorite, which is considered the ideal root canal irrigant, using both passive irrigation and ultrasonic active irrigation techniques.

MATERIAL & METHODS:

Sixty freshly extracted teeth were randomly divided into three groups (n = 18) and two subgroups (n = 9) and stored in normal saline to prevent dehydration. All samples were decoronated at the cementoenamel junction (CEJ) level using a high-speed diamond fissure bur, and access cavities were prepared.
The working length was determined, and apical preparation was performed using the crown-down technique with an apical size of #25. Neo-Endo rotary files of sizes 20-0.06 and 25-0.06 were used to reach the working length. Irrigation was carried out with normal saline between each instrument.
The roots were then autoclaved for sterilization, and three roots were randomly selected as the negative control group and transferred to an incubator. *E. faecalis* (ATCC 29212) was cultured under aerobic conditions on 5% defibrinated sheep blood agar medium for 24 hours. After this, three roots were selected as the positive control group, and the remaining roots were divided into three experimental groups.

GROUP A – 100 µl of 5.25% NaOCl was used for irrigation.
SUBGROUP-1– Sodium hypochlorite was maintained within canal for 5 min. Subsequently, canals were irrigated and washed by 1 ml normal saline for 3 min to eliminate NaOCl and the sample was collected with paper point no. 20. Canals were not dried before sampling.
SUBGROUP-2 – Sodium hypochlorite was maintained in canal and ultrasonically irrigated for 5 min.

Subsequently, canals were irrigated and washed by 1 ml normal saline for 3 min to eliminate NaOCl and the sample was collected with paper point no. 20. Canals were not dried before sampling. The same was repeated with Group B (100 µl of 0.6% Carvacrol emulsion) and Group C (100 µl of *Azadirachta indica* solution).

The antimicrobial efficacy of irrigant was assessed by counting the colony forming units of *E. faecalis* which was taken from root surface by the sterile paper point and inoculated on blood agar and incubated for 24 hours at 37°C. It was done by incubating irrigated teeth samples with paper point for 30 minutes. Then, those paper points were used for inoculating the *E. faecalis* colonies on the blood agar. Blood agar plates were incubated for 24 hours at 37°C.

- Percentage reduction in colony = (Initial colony – final colony count × 100) / Initial colony count
- Inoculation factor = 1 / Inoculum
- Dilution factor = 1 / Dilution
- Colony forming units/ml = No. of colonies × 1/dilution × 1/inoculum

RESULTS:

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviation. Also, One Way Analysis of Variance (ANOVA) followed by post hoc Tukey's Test was performed with the help of Critical Difference (CD) at 5% and 1% level of significance to compare the mean values of more than two groups. $p < 0.05$ was taken to be statistically significant. (Table 1)

Table 1: Comparison of reduction in microbial count using sodium hypochlorite using active versus passive irrigation system

	Reduction in microbial count						
Sodium Hypochlorite	Mean	Std. Deviation	Mean Difference	95% Confidence Interval		t-test value	p-value
Passive Irrigation	57.56%	3.61%	-26.67%	-31.92%	-21.42%	-10.765	0.001*
Active Ultrasonic Irrigation	84.22%	6.50%					

Unpaired t-test ***Significant difference**

As shown in Table 2, the mean Reduction in microbial count was compared between Passive Irrigation and Active Ultrasonic Irrigation groups using the unpaired t-test. The mean Reduction in microbial count was significantly more among Active Ultrasonic Irrigation group compared to Passive Irrigation.

Table 2: Comparison of reduction in microbial count using Carvacrol using active versus passive irrigation system

	Reduction in microbial count						
	Mean	Std. Deviation	Mean Difference	95% Confidence Interval		t-test value	p-value
Carvacrol	Mean	Std. Deviation		Lower	Upper		
Passive Irrigation	84.00%	4.00%	0.00%	-4.00%	4.00%	0.001*	1.000
Active Ultrasonic Irrigation	84.00%	4.00%					

Unpaired t-test **#Non-significant difference**

Table 3 depicts comparison of the mean reduction in microbial count between Passive Irrigation and Active Ultrasonic Irrigation groups using the unpaired t-test. There was no significant difference in mean Reduction in microbial count between Passive Irrigation and Active Ultrasonic Irrigation groups.

Table 3: Inter-group comparison of mean reduction in microbial count was done using the post-hoc Bonferroni test

		Mean Difference	Std. Error	p-value
Sodium Hypochlorite	Carvacrol	-26.44%	1.64%	0.001*

Post-hoc bonferroni test ***Significant difference**

The inter-group comparison of mean reduction in microbial count was done using the post-hoc Bonferroni test. (Table 4) The mean reduction in microbial count was significantly more among Carvacrol group compared to Sodium Hypochlorite which was significantly more than Azadirachta Indica.

Table 4: Mean reduction in microbial count was compared between Sodium Hypochlorite and Carvacrol groups.

	Reduction in microbial count					
Active Ultrasonic Irrigation	Mean	Std. Deviation	95% CI		F-value	p-value
Sodium Hypochlorite	84.22%	6.50%	79.23%	89.22%	203.679	0.001*
Carvacrol	84.00%	4.00%	80.93%	87.07%		

One-way ANOVA test ***Significant difference**

The mean Reduction in microbial count was compared between Sodium Hypochlorite and Carvacrol groups using the one-way ANOVA test. There was a significant difference in mean Reduction in microbial count between Sodium Hypochlorite and Carvacrol groups.

DISCUSSION:

The eradication of bacteria by endodontic treatment from the root canal is difficult because of biofilm formation on the dentin walls of the main root canal. [7] So, one of the major goals in root canal treatment is removal of biofilms and to create a microbe-free environment inside the root canal. Mostly, Gram-positive bacteria are present in root canal that resist the root canal disinfection and are present in failed root canal cases that are mostly *Enterococcus faecalis.* Its prevalence in such infections ranges from 24%–77%. [9] It is a facultative anaerobic gram-positive cocci and is a normal inhabitant of the oral cavity. It is associated with asymptomatic chronic periradicular lesions significantly more often with acute periradicular periodontitis or acute periradicular abscesses. It can grow at 10–45°C at pH 9.6 and overcomes the challenges of survival within root canal system in several ways such as gene-coded resistance, biofilm penetration into dentinal tubules, survival in low pH, high salinity, and high temperature. [10] It can alter host response by maintaining pH homeostasis. [11]

Cleaning and shaping of root canal comprises the most important step of endodontic treatment. The objective of this step is to debride and disinfect/sterilize the root canal system and to shape/contour the root canal walls for the purpose of sealing the root canal completely with a condensed, inert filling material. [12] Mechanical preparation of root canal along with chemical irrigation is essential for the

success of root canal treatment. The irrigant must possess antibacterial property, tissue dissolving properties, low surface tension, substantivity, lubrication and should be biocompatible. [13]

The gold standard among various irrigants is sodium hypochlorite (NaOCl) because of its antimicrobial efficacy with tissue-dissolving properties. On ionization, it produces hypochlorous acid which releases chlorine, which forms chloramines on reaction with amino groups of proteins. It acts as a lubricant, solvent for pulp tissues, and potent antimicrobial. [14] Various other therapeutic and chemical solutions are used in disinfection of root canal like 2% Chlorhexidine, EDTA, Hydrogen peroxide, Sodium hypochlorite. 2% Chlorhexidine, which is a cationic bisbiguanide, has shown to have greater substantivity as it binds to hydroxyapatite and has sustained antibacterial effects up to 12 weeks in root canal. [15] But, on the other hand, it doesn't show smear layer removal and it doesn't dissolve pulpal tissue. Another irrigant is EDTA that has shown smear layer removal as it causes chelation of calcium ions but it can interfere with tissue-dissolving properties of sodium hypochlorite. [16] Even Hydrogen peroxide has good tissue-dissolving properties but is not biocompatible. [7]

Effective irrigant delivery and agitation are important. Therefore, recently new technique has been advocated in dentistry in which irrigant solutions are activated ultrasonically. This would help to improve effectiveness of irrigant as well as enable the irrigant to reach into the complex isthmuses of the root canal. [17] Active irrigation initiates dynamics and flow within the fluid, thus improving root canal disinfection. Ultrasonic devices can be used in irrigation by two ways: concurrent combination of ultrasonic irrigation/instrumentation and passive ultrasonic irrigation devices. [18] This method reduces the rate of potential mishaps in the root canal system and also improves the exchange of substances in the canal, permits heating of irrigant substance, and eliminates dentinal debris, thereby achieving greater cleaning effect. [19]

However, chemical irrigants have some shortcomings, of which the major disadvantages are high toxicity, reduction in elastic and flexural strength of dentin, corrosive to instruments, unpleasant taste, decreases the mechanical properties and cutting efficiency of nickel–titanium instruments, allergic reactions, and inability to remove the smear layer. [20] Even with constant increase in antibiotic-resistant strains of bacteria as well as the side effects experienced due to synthetic drugs led to the search of herbal alternatives like *Azadirachta indica*, Carvacrol, *Morinda citrofolia*, etc. [21] The advantageous reasons for using herbal irrigants are low toxicity, lack of microbial resistance, and easy availability.

Azadirachta indica (Neem) is the most common and traditional plant of India of great importance. It consists of medicinal properties and that is why it is highly commercially available. Neem leaf is believed to have immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic properties. [19] It has the ability to prevent the production of prostaglandins. Its immunomodulatory function enables antigen presentation to immunocompetent cells. [22]

This present study is done to evaluate the antimicrobial ability of herbal root irrigant *Azadirachta indica* vs sodium hypochlorite using passive and ultrasonic irrigation technique in an in vitro method against *E. faecalis* to explain their antimicrobial efficacy. In the present study, experimental teeth were sterilized in an autoclave for 15 minutes at 121˚C and 15 lb pressure. Culture technique was used to detect *E. faecalis* inside the root canal because it is one of the most reliable methods to detect viable bacteria, particularly when samples are taken immediately after antibacterial treatment in which viability might not be ascertained by most culture-independent methods. [23]

The results, while using different irrigating solutions inside the tooth model, revealed significant bacterial reduction with a particular irrigation time period using colony-forming unit counting. As

can be seen in our study, when we compare the antimicrobial efficacy of sodium hypochlorite with that of *Azadirachta indica*, *Azadirachta indica* has shown much less reduction when compared to sodium hypochlorite. Another aspect of this study was to understand the effect of ultrasonic activation of these various irrigating solutions on the reduction of *E. faecalis* colonies. As we know from literature, sodium hypochlorite has shown a tremendous increase in its bactericidal properties when activated ultrasonically. Not much difference in the bactericidal action of *Azadirachta indica* using active ultrasonic technique can be seen against *E. faecalis.*

Within the limitations of our study, the results presented can be useful for deciding which irrigant will be better to disinfect canal while using herbal irrigants. It has been suggested that, knowing the adverse effects of chemical irrigants, one should now go for herbal irrigants as they do not have such adverse effects. In herbal irrigants, we would suggest *Azadirachta indica* as the better irrigant as it works by inhibiting ATPase activity and increasing the non-selective permeability of bacterial cell membranes.

This in vitro study had several advantages and limitations. The strength points being canal instrumentation by single operator and utilization of colony-forming counting system. In most of the published literature, antimicrobial efficacy against *E. faecalis* has been assessed only in the zone of inhibition system. However, the main goal of our study was to evaluate the antimicrobial efficacy using colony-forming unit counting.
Despite all attempts to standardize the groups using the inclusion and exclusion criteria, it was seen that complete standardization of extracted teeth is not possible in terms of canal shape, canal size, and hardness. [24] We focused mainly on herbal irrigants only as it is already established that toxic effects of herbal irrigants are less as compared to chemical irrigants. [25] Further studies are necessary for three-dimensional evaluation of adequate debridement, properties of the irrigants, and techniques utilized to assess the antimicrobial effect of herbal root irrigants inside the root canals and to substantiate the results of the present study.

CONCLUSION:

Within the limitations of this study, it was concluded that herbal irrigants are not only known for their flavor and fragrances but also can be used for their antimicrobial efficacy in root canal treatment. Neem has shown to have antimicrobial efficacy but it is less potent than sodium hypochlorite. Therefore, Neem can be used as an irrigating solution in root canal treatment as it has no cytotoxic effects as compared to that of sodium hypochlorite. On ultrasonic activation, sodium hypochlorite proved to be more potent as an irrigating solution as compared to the herbal irrigant since they could not be activated ultrasonically. Proper understanding of the action of different irrigating solutions is necessary for appropriate irrigant selection in different case scenarios in order to prevent undesirable toxic effects in root canals. Research should continue to further understand the role of different herbal irrigants and to assess their working inside the root canal, so as to overcome the toxic or undesirable effects of the pre-existing chemical irrigants and at the same time achieve at least equal, if not superior, disinfection in the root canal system.

FUNDING: Nil.
CONFLICTS OF INTEREST: None

REFERENCES:

1. Baumgartener JC, Falkwar WA. Detection of immunoglobulins from explant cultures of periapical lesions. J Endod. 1991;17(3):105.
2. Rocas IN, Siquera JF, Santos KRN. Association of E. faecalis with different forms of periradicular diseases. J Endod. 2004;30:315-20.

3. Sedgey CM, Lennan SL, Clewell DB. Prevalence, phenotype, and genotype of oral enterococci. Oral Microbiol Immunol. 2004;19:95-101.
4. Hubble TS, Hatton JF, Nallapreddy SR, Murray BE. Influence of Enterococcus faecalis proteases and collagen binding protein (Ace) on adhesion to dentin. Oral Microbiol Immunol. 2003;18:121-6.
5. Ingle JL, Zeldow BJ. An evolution of mechanical instrumentation and negative culture in endodontic therapy. J Am Dent Assoc. 1958;57:471.
6. Dakin D II. On the use of certain antiseptic substances in treatment of infected wounds. Br Med J. 1915;2:318.
7. Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentin and tooth surface strain. Int Endod J. 2001;34:120-32.
8. Polaquini SR, Svidzinski TI, Kemmelmeier C, Gasparetto A. Effects of aqueous extracts from neem on hydrophobicity, biofilm formation, and adhesion in composite resins by Candida albicans. Arch Oral Biol. 2006;51:482-90.
9. Gill AO, Holley RA. Disruption of Escherichia coli, Listeria monocytogenes, and Lactobacillus sakei cellular membranes by plant oil aromatics. Int J Food Microbiol. 2006;46:3590-5.
10. Kacem R, Meraihi Z. Effects of essential oil extracted from Nigella sativa seeds and its main components on human neutrophil elastase activity. Yakugaku Zasshi. 2006;126(4):301-5.
11. Kawashima N, Wadachi R, Suda H, Yeng T, Parashos P. Root canal medicaments. Int Dent J. 2009;59(1):5-11.
12. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill Enterococcus faecalis in vitro. J Endod. 2004;30:218-9.
13. Lee W, Lim S, Son H, Bae K. Sonicated extract of Enterococcus faecalis induces irreversible cell cycle arrest in phytohemagglutinin-activated human lymphocytes. J Endod. 2004;30:209-12.
14. McHugh CP, Zhang P, Michalek S, et al. pH required to kill Enterococcus faecalis in vitro. J Endod. 2004;30:218-9.
15. Silva FB, Almeida JM, Sousa SM. Natural medicaments in endodontics – a comparative study on anti-inflammatory action. Braz Oral Res. 2004;18:174-9.
16. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod. 2004;30:785-7.
17. Gulabivala K, Ng YL, Gilbertson M, Eames I. The fluid mechanics of root canal irrigation. Physiol Meas. 2010;31(12):49-84.
18. Van der Sluis L, Versluis M, Wu M, Wesselink P. Passive ultrasonic irrigation of root canals: a review of the literature. Int Endod J. 2007;40(6):415-26.
19. Briseno M, Wirth R, Hamm G, Standhartfinger W. Efficacy of different irrigation methods and concentrations of root canal irrigants on bacteria in the root canal. Dent Traumatol. 1992;8(10):6-11.
20. Polaquini SR, Svidzinski TI, Kemmelmeier C, Gasparetto A. Effects of aqueous extracts from neem on hydrophobicity, biofilm formation, and adhesion in composite resins by Candida albicans. Arch Oral Biol. 2006;51:482-90.
21. Gill AO, Holley RA. Disruption of Escherichia coli, Listeria monocytogenes, and Lactobacillus sakei cellular membranes by plant oil aromatics. Int J Food Microbiol. 2006;46:3590-5.
22. Nayak A, Nayak RN, Saumya G, Bhatt K, Kudalkar K. Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extract of neem (Azadirachta indica): an in vitro study. Int J Res Ayurveda Pharm. 2011;2:230-5.
23. Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. Int Endod J. 2003;36:75-85.
24. Siqueira JF Jr, Guimarães-Pinto T, Rôças IN. Effects of chemomechanical preparation with 2.5% sodium hypochlorite and intracanal medication with calcium hydroxide on cultivable bacteria in infected root canals. J Endod. 2007;33(7):800-5. doi: 10.1016/j.joen.2006.11.023. PMID: 17804315.

25. Khalilak Z, Fallahdoost A, Dadresanfar B, Rezvani G. Comparison of extracted teeth and simulated resin blocks on apical canal transportation. Iran Endod J. 2008;3(4):109.