Original Article

Antimicrobial efficacy of two commercially available herbal products with and without ultrasonic activation in primary endodontic infections: A randomized clinical trial

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Abstract

Background: Effective irrigation is crucial for successful endodontic treatment. Traditional irrigants like sodium hypochlorite (NaOCl) have been widely used, but there is a growing interest in exploring natural alternatives for their potential antimicrobial properties.

Objective: The study aims to compare the antimicrobial efficacy of Neem, Bitter Gourd, and NaOCl, with and without ultrasonic activation in managing primary endodontic infections.

Materials and Methods: Ninety patients were randomly assigned six groups (n = 15) Group 1: NaOCl, Group 2: NaOCl with passive ultrasonic irrigation (PUI), Group 3: Neem juice, Group 4: Neem juice with PUI, Group 5: Bitter gourd juice, and Group 6: Bitter gourd juice with PUI. Bacteriological samples were collected before (S1) and after (S2) shaping, plated on brain heart infusion agar, and colony counting was done after 24 h.

Statistical Analysis Used: Shapiro–Wilk test, one-way ANOVA, *post hoc* Tukey analysis, and paired *t*-test.

Results: All the groups demonstrated a significant reduction in bacterial count. Groups with PUI (2, 4, 6) demonstrated higher mean bacterial reduction than their counterparts without PUI (1, 3, 5).

Conclusion: Neem and Bitter gourd juices, particularly when used with PUI, demonstrated antimicrobial efficacy comparable to NaOCl with PUI.

Keywords: Antibacterial activity; bitter gourd juice; Neem juice; passive ultrasonic irrigation; sodium hypochlorite

INTRODUCTION

Endodontic treatment refers to the removal of organic tissue, microorganisms, and their microbial by-products from the root canals.[1] However, mechanical instrumentation itself cannot debride the canals. Irrigation plays an important role during canal disinfection especially in hard-to-reach

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areas such as isthmuses, fins, anastomoses, and other irregularities. In addition, it should be ensured that the irrigant is in direct contact with the canal walls for an effective antimicrobial action. Activated irrigation brings about agitation further enhancing irrigant flow leading to improved penetration and cleansing effectiveness.[2] Currently, passive ultrasonic irrigation (PUI) is one of the most established methods for irrigant activation. Bharadwaj *et al*. observed that ultrasonic irrigation with 1% sodium hypochlorite (NaOCl) eliminated *Enterococcus faecalis* more efficiently.[3]

NaOCl is a widely used endodontic irrigant possessing good antimicrobial efficacy. It is a strong base (pH > 11) acting as an organic solvent thereby dissolving pulpal tissue.[4-6] Due to the detrimental effects of NaOCl on dentin elasticity and flexural strength, newer herbal irrigants are being evaluated as having reduced adverse effects.

Herbal irrigants are continuously researched for use as an endodontic irrigant.[7-9] Neem (*Azadirachta indica*) and Bitter gourd (*Momordica charantia*) are two solutions that have potent anti-microbial properties and can be used for irrigation. Furthermore, activation further increases their anti-microbial efficacy.[10,11]

A. indica or Neem represents the mahogany family, Meliaceae which possesses antiviral, anti-fungal, anti-bacterial, and anti-carcinogenic properties. *M. charantia* (Bitter gourd) belongs to the family *Cucurbitaceae* and has broad-spectrum anti-bacterial and anti-fungal properties.[12]

The antimicrobial efficacy of neem and bitter gourd has been proven *in vitro*. However, *in vivo* studies conducted along with ultrasonic activation (UA) are lacking. Therefore, this study was conducted to assess the antimicrobial viability of Neem, Bitter gourd, and NaOCl with and without UA in primary endodontic infections.

MATERIALS AND METHODS

The study adhered to the Consolidated Standards of Reporting Trials guidelines and was registered with the Clinical Trials Registry of India (Reg. No: CTRI/2021/03/032049). The sample size was calculated by the G Power Software (version 3.0.10). The effect size was measured to be 0.59, with a 5% precision level, 95% confidence interval, and 80% power. A total of 90 patients, i.e. 15 per group were found to be appropriate. Every patient was provided with a detailed explanation of the study and associated risks. This was followed by obtaining informed consent from all participants. The workplace of this study was the postgraduate clinic of the Department of Endodontics, spanning from August 2021 to January 2022. The entire procedure was done by a single operator to avoid bias. The study was approved by the Institutional Ethical Committee (Ref. No: 9861/2019).

Adult participants without any contributory medical history and having intact permanent teeth not subjected to any previous restoration were included. Patients with clinically and radiographically diagnosed necrotic/infected pulp, as well as teeth with sufficient coronal structure for achieving adequate isolation and restoration, were deemed to be eligible for participation. The study excluded individuals with known systemic diseases, pregnant individuals, teeth diagnosed with acute apical abscess, retreatment cases, individuals on antibiotic regimen for 3 months, calcified teeth, presence of intraoral/extraoral sinus, open apex, resorption, and with periodontal pockets >5 mm.

5% Betadine solution (Povidone Iodine 5% V/V, Win medicare Products Ltd, India) was used to disinfect the oral cavity and experimental tooth. Local anesthesia (Biocaine-ADR, Biochem Pharmaceutical Industries, India) was administered and rubber dam (HygenicR Dental Dam Kit, Coltene Whaledent, Switzerland) isolation of the experimental tooth was performed. Before access cavity preparation, the selected tooth along with the clamp and dam were disinfected with 2.5% NaOCl (Bharat Chemical, India) for 30 s. This was followed by neutralization of the effect of NaOCl with 5% sodium thiosulfate. Control samples from coronal surface of the tooth as well as from the rubber dam clamp and sheet were obtained using sterile paper points (Metabiomed, India) to avoid any prior bacterial contamination. Caries was removed and access cavity was prepared using endo access bur #2 (Dentsply Maillefer, Switzerland) mounted to a high-speed handpiece (NSK Pana Air, Japan) under continuous water coolant. Working length determination was done using an electronic apex locator (Morita Root ZX Mini) and confirmed radiographically. Normal saline (5 ml) (0.9% v/w, Lifusion, India) was injected into the canal to obtain a Pre-treatment Sample (S1). Circumferential pumping of a #10 K-file (Mani, Inc., Japan) 1 mm short of the determined working length was done subsequently. Post immersion into transport media (Peptone water, HiMedia Laboratories, India), a sterile paper point (Metabiomed, India) was introduced in the canal for 60 s. It was promptly transferred to test tube containing the transport media. Three samples were collected for each tooth in a similar manner. This procedure was repeated for all 90 samples and they were randomly divided into six experimental groups as follows:

- Group I: NaOCl (2.5% NaOCl) ($n = 15$)
- Group II: NaOCl with UA (2.5% NaOCl + UA) ($n = 15$)
- Group III: Neem juice (*A. indica*) ($n = 15$)
- Group IV: Neem juice (*A. indica*) with UA (Neem juice + UA) ($n = 15$)
- Group V: Bitter gourd juice (*M. charantia*) ($n = 15$)
- Group VI: Bitter gourd juice (*M. charantia*) with UA (Bitter gourd + UA) ($n = 15$).

During the study, both participants and researchers were kept unaware of the irrigant being used. The irrigant was provided in preloaded syringes and patients were randomly assigned to different groups. This double-blinding approach ensured that both administration of the irrigant and evaluation of outcomes remained unbiased and objective. Step back technique was employed for performing the biomechanical preparation. In Group I, III, and V, canals were irrigated with respective irrigants using conventional syringes and needle. In Groups II, IV, and VI, the canals were irrigated with respective irrigants and gently activated with U-files (Mani, Inc., Japan) in two planes concurrently, i.e., for 20 s each in both the buccolingual and mesiodistal directions keeping 2 mm short of the working length.

Post-instrumentation sample (S2) was procured as was done for S1. Canals were then dried using paper points. Calcium hydroxide (Prevest DenPro, India) was placed as an intracanal medicament and the tooth was temporized. Preincubation of both samples (S1, S2) was performed for 30 min. Samples were subjected to vigorous shaking in a vortex mixture for a minute. Decimal dilutions up to 10^{-6} were prepared in 4.5 ml of peptone water. Using an aseptic technique, fifty microliter aliquots of these dilutions were then inoculated onto B HI agar (HiMedia Laboratories, India) using a micropipette. Colony enumeration was done post 24 h using the conventional bacterial counting technique. The obtained data were statistically analyzed.

Patients were recalled 7 days post the first appointment. As previously, rubber dam isolation and disinfection were done. Sterile round diamond bur (Mani, Inc., Japan) was used to remove the temporary restoration. The canal was dried with paper points and obturated by cold lateral condensation method using 2% gutta-percha with resin-based sealer (AH Plus, Dentsply, Germany). Permanent composite restoration (Spectrum, Dentsply, India) was completed on the same visit.

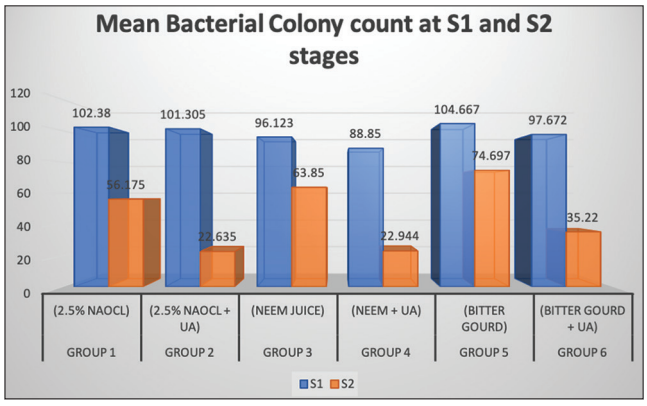

Figure 1: Bar diagram of mean bacterial count at stages S1 and S2 for all the six experimental groups

Statistical analysis

The obtained data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS, IBM, Chicago, IL, USA) software, version 21. Shapiro–Wilk test was used to check for data normality. Inferential statistics was done using one-way ANOVA. *Post hoc* Tukey analysis and paired *t*-test. The level of statistical significance was set at $P < 0.05$.

RESULTS

No tooth demonstrated a positive result for bacterial presence on examining the sterility control sample.

On intragroup comparison, a statistically significant reduction in bacterial count was found from S1 to S2 in all six groups [Table 1].

In intergroup comparison, the mean percentage reduction of bacterial counts was maximum in Group 2 (2.5% NaOCl with UA), followed by Group 4 (Neem juice with UA), Group 6 (Bitter gourd juice with UA), Group 1 (2.5% NaOCl), Group 3 (Neem juice), and least reduction was seen in Group 5 (Bitter gourd juice) [Table 2].

Ultrasonically activated groups presented superior antibacterial activity than nonactivated groups. Amongst the activated groups, 2.5% NaOCl and Neem juice had comparable antimicrobial activity [Figure 1].

DISCUSSION

The key to successful endodontic treatment is microbial elimination from infected canals.[13] However, this can be an intricate task owing to complexities in canal anatomy. Lately, innumerable mechanical filing systems and disinfection regimes have undergone research with the aim of diminishing microbial load.[14] Rotary instruments act only on the canal's central body, barring canal fins, isthmi, and cul-de-sacs which remain intact. The untouched regions harbor tissue debris along with microbes and their by-products, ultimately resulting in persistent apical periodontitis.[3]

Table 1: Comparison of mean and standard deviation of bacterial colony count (CFU/mL×10⁶) at S1 and S2 stages (intragroup) for various groups

	S1		S2		*P*
	Mean	SD	Mean	SD	
Group 1 (2.5% NaOCl)	102.380	6.646	56.175	16.573	0.0001*
Group 2 (2.5% NaOCl + UA)	101.305	12.842	22.635	6.123	0.0001*
Group 3 (neem juice)	96.123	23.102	63.85	18.24	0.0001*
Group 4 (neem + UA)	88.850	15.756	22.944	13.287	0.0001*
Group 5 (bitter gourd)	104.667	15.238	74.697	10.397	0.0001*
Group 6 (bitter gourd + UA)	97.672	18.422	35.22	13.111	0.0001*

*Level of significance set at $P<0.05$. SD: Standard deviation, UA: Ultrasonic activation, NaOCl: Sodium hypochlorite

Table 2: *Post hoc* pair wise comparison for mean absolute reduction of bacterial count (S1–S2) for various groups

	n	Mean	SD	95% CI for mean	*F, P*
Group 1 (2.5% NaOCl)	15	45.0767	15.79223	4.07754–36.3313	34.367, 0.001*
Group 2 (2.5% NaOCl + UA)	15	77.7754	4.58555	1.18398–75.2360	
Group 3 (neem juice)	15	33.0869	12.02414	3.10462–26.4282	
Group 4 (neem+UA)	15	73.7378	15.01034	3.87565–65.4253	
Group 5 (bitter gourd)	15	28.2973	7.54317	1.94764–24.1200	
Group 6 (bitter gourd + UA)	15	64.1562	11.18465	2.88787–57.9623	

*Level of significance set at $P<0.05$. SD: Standard deviation, CI: Confidence interval, UA: Ultrasonic activation, NaOCl: Sodium hypochlorite

Neem is widely utilized in Ayurveda, Unani, and Homeopathy, serving as a valuable resource in contemporary medicine. Fresh leaves are collected and their extract is commonly used as a juice. Neem exhibits antimicrobial properties by impeding microbial growth and demonstrating the capability to degrade cell walls. It also acts as a free radical scavenger because of being a rich antioxidant source along with anti-inflammatory through regulation of proinflammatory enzyme activities comprising of cyclooxygenase and lipoxygenase.[15] The antibacterial action of 2% NaOCl, propolis, neem leaf extract, turmeric, and liquorice against *Enterococcus faecalis* and *Candida albicans* was compared in a study and neem extract exhibited the most substantial zone of inhibition against the tested microorganisms.[16]

Bitter gourd is widely used as food and medicine. It contains 5-a-stigmasta-7, 25-dien-3-b-ol, elasterol, and lanosterol as its active ingredients which may also be accountable for antibacterial activity.[17] Jagessar *et al*. investigated the antibacterial and anti-fungal activities of *M. charantia* against *Staphylococcus aureus*, *C. albicans, Escherichia coli* and found antimicrobial inhibitory activity against all microorganisms.[12]

Molecular-based clinical research indicates that although chemomechanical preparation reduces bacterial load in root canals, infections may often endure. To enhance antimicrobial effects, alternative methods like final irrigation with UA have been recommended.[18]

PUI is efficacious in the eradication of pulp tissue and dentin debris than conventional methods. Ultrasound generates increased speed and volume of irrigant flow within the canal during irrigation. As a result, it removes more debris, minimizes apical packing, and enhances the flow of irrigants into accessory canals.[6] Susila and Minu compared Activated and Conventional nonactivated irrigation protocols and reported that mechanical active irrigation devices perform better at reducing bacterial load. It also enhanced the cleaning of the canal and isthmus.[2]

In the present study, NaOCl with UA demonstrated the highest bacterial reduction, followed by Neem and bitter gourd juice with UA. The least reduction was seen with Bitter Gourd juice. These outcomes are similar to few *in vitro* examinations.[1,11,19,20]

PUI resulted in superior antibacterial activity of all three irrigants. The possible reasons could be the activation of the irrigant because of the implosion of microcavitation bubbles and hydrodynamic effects.[6,14]

A significant increase in the antibacterial efficacy of NaOCl with UA could be due to the inherent advantage of UA and increased temperature of NaOCl which increases chemical reactivity. Neem juice with UA was comparable to 2.5% NaOCl with UA as ultrasonication allowed for deeper penetration of irrigant.[21] Cavitation could have led to the temporary weakening of the bacterial cell wall.[10] Thus, a synergistic action between the chemical effects of Neem with mechanical effects of UA resulted in enhanced antimicrobial activity.

Bitter gourd with UA also resulted in antimicrobial activity comparable to that of Neem but had a significant difference when compared to 2.5% NaOCl with UA. Antimicrobial efficacy is predominantly attributed to triterpenes, and it relies on the interaction between lipid components of triterpenes and the overall surface charge of microbial membranes. Moreover, it has the potential to traverse the cell membrane, entering the cell and engaging with intracellular sites essential for antibacterial effectiveness.[22] UA leads to bacterial biofilm deagglomeration by means of acoustic streaming rendering the subsequent planktonic microorganism vulnerable to bactericidal movement of the herbal irrigant.[23] Therefore, results obtained with UA are better than bitter gourd alone.

Bacterial reduction obtained by 2.5% NaOCl with manual syringe irrigation was comparable to Neem juice. This is in accordance with few *in-vitro* experiments.[24,25]

Bitter gourd juice exhibited the least bacterial count reduction. Its bactericidal effect comes from flavonoids which possess antimicrobial properties. The result could be attributed to the deactivation of active antimicrobial agents due to the presence of active enzymes in the microorganisms and low affinity of target molecules.[22]

CONCLUSION

Neem juice with UA can be recommended as a potential irrigant as its antimicrobial activity was comparable with that of 2.5% NaOCl with UA. Moreover, PUI significantly enhanced the antimicrobial activity of the tested herbal solutions.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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