

**COMPARATIVE ANTIMICROBIAL ANALYSIS OF HERBAL AND NON-HERBAL
ENDODONTIC IRRIGANTS USING PASSIVE ULTRASONIC IRRIGATION- AN IN
VITRO STUDY*****Dr. Niveditha B., Dr. Veerendra Patil, Dr. Ratnakar P., Dr. Surabhi R. and Dr. Ashwini Marurkar**

India.

***Corresponding Author: Dr. Niveditha B.**

India.

Article Received on 19/04/2018

Article Revised on 09/05/2018

Article Accepted on 30/05/2018

ABSTRACT

Root canal irrigants are used during chemico mechanical procedures as antimicrobial agents, debris removal, lubricate and dissolve organic compounds. Several irrigants, such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), hydrogen peroxide (H₂O₂), normal saline are used. In this study we use 3% NaOCl, 2% CHX, Ozonated oil, Neem extract to check antimicrobial efficacy against *E. faecalis*. **Aim:** The aim was to compare antimicrobial efficacy of herbal and non-herbal endodontic irrigants using ultrasonic irrigation. **Material and method:** 40 extracted human single rooted teeth will be used for this study and grouped as.

Group 1 – 3% NaOCl

Group 2 - 2% CHX

Group 3 - ozonated oil

Group 4 - neem extract.

Teeth will be decoronated to a standardised length of 16mm and they will be chemo-mechanically prepared upto #30 k file and autoclaved before inoculation. Each sample will be inoculated with *E. faecalis*, and incubated at 37°C, 7 days. Following this teeth will be enlarged upto f3 protaper, each group will be irrigated with irrigant as mentioned before using passive ultrasonic irrigation. Microbial samples from each tooth will be collected using sterile paper point, number of viable *E. faecalis* colonies will be counted as Colony Forming Units. **Results:** Although statistically insignificant the study showed that Chlorhexidine 2% has better antimicrobial efficacy, but none of the groups could completely eradicate *E. faecalis*.

INTRODUCTION

Endodontic infections are polymicrobial^[1] and elimination of these infective micro organisms from the root canal primarily determines the success of endodontic treatment. This is achieved by chemomechanical preparation of the canal. Root canal irrigants are used during chemomechanical preparation of the canal not only as an antimicrobial agent but also to flush out loose debris. Traditionally, sodium hypochlorite is used as a root canal irrigant. It is effective mainly on organic components, but not on inorganic material.

Chlorhexidine gluconate is a cationic bisguanide. At low concentrations it shows bacteriostatic effect and higher concentrations bactericidal. When used as an irrigant in the root canal, it is adsorbed on dental hard tissues and is gradually released over prolonged period showing its therapeutic effect (Substantivity). Various forms of OZONE- Gaseous, water and oil based have been used as irrigant & intracanal medicament in root canal disinfection. Ozone therapy has been found to be more

efficient on anaerobic bacteria which are the predominant species in the oral cavity.

Among the microorganisms commonly isolated from root canals *E. faecalis* (gram positive facultative anaerobic bacterium) is frequently isolated from persistent root canal infections.^[2]

The Neem (*Azadirachta indica* A.) tree has been described for its value in traditional Indian medicinal texts.^[3]

The isoprenoid group of constituents of neem have anti-inflammatory^[4,5] anti-bacterial,^[6,7] anti-fungal^[8,9,10] and immunomodulatory properties. The need to evaluate the efficacy of other irrigants raised because conventional irrigants fail to completely eradicate persistent microbes such as *E. faecalis*.

MATERIALS AND METHOD

40 freshly extracted single rooted human teeth were obtained from Dept. Of Oral-Maxillo facial surgery. Teeth were de-coronated to a standardized length of 16mm using diamond disc, they were then enlarged till

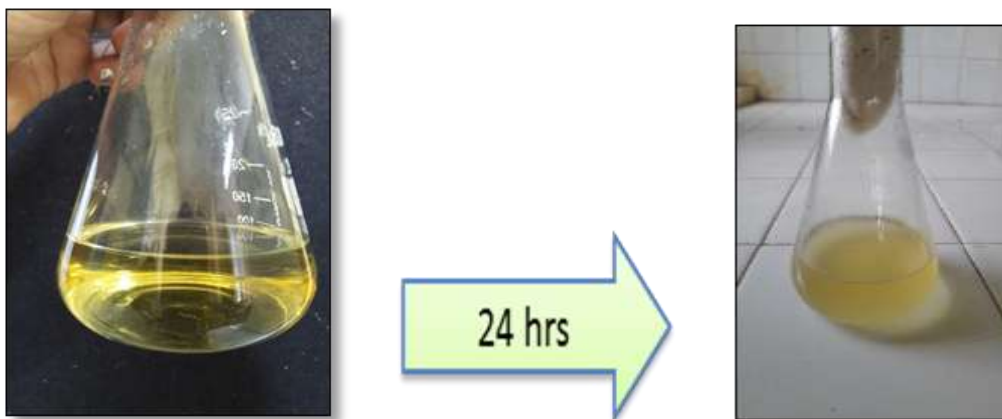
#30 K file, following irrigation with 3% NaOCL. Canals were dried with sterile paper points and apical region was sealed with light-cured resin composites. All the specimens were sterilized by autoclaving (15 min at 121 °C) and divided into 4 groups with 10 teeth each according to the irrigation solutions used. The teeth were stabilized with chemically activated acrylic resin (5 in each block).

- Group I - Sodium hypochlorite (NaOCL)
- Group II- Chlorhexidine 2%
- Group III-Neem

- Group IV - ozonated olive oil

Inoculation of E. faecalis in root canals

All the microbiologic procedures were performed under aseptic conditions. Standardised suspension of E. faecalis (ATCC 29212) was prepared in nutrient broth (100µL in 100ml). Then, 20µl of suspension was inoculated into 40 root samples. The cervical access was sealed using gutta percha and incubated at 37°C for 24 hours.



Preparation of root canals

The root samples were prepared with rotary protaper files upto F3, with intermittent passive ultrasonic irrigation using respective group irrigants. About 5ml of each irrigant was ultrasonically activated for 30sec each.

After the use of last file, #30 sterile paper point was used to blot the root canal and transferred to Eppendorf tubes containing 0.3ml physiologic solution.



After thorough agitation of the tubes, the solution was poured over SDA media for inoculation and this was incubated for 24hrs at 37°C.



RESULTS

24hrs culture of each sample was obtained and the numbers of visible colony forming units were calculated

manually. The number of CFUs in each sample were obtained as following:

E.Faecalis count	Group 1 Sodium hypchlorite	Group 2 Chlorhexidine	Group 3 Neem	Group 4 Ozone
Sample 1	13	100	127	38
Sample 2	128	78	157	113
Sample 3	130	77	65	99
Sample 4	140	91	153	93
Sample 5	70	53	70	101
Sample 6	49	95	109	42
Sample 7	137	81	107	102
Sample 8	121	72	47	111
Sample 9	157	93	83	89
Sample 10	92	49	59	121



NaOCL



CHX



Azadirachta indica (Neem)



Ozonated olive oil

Statistical analysis

The obtained readings were subjected to statistical analysis, and the means of respective irrigants were

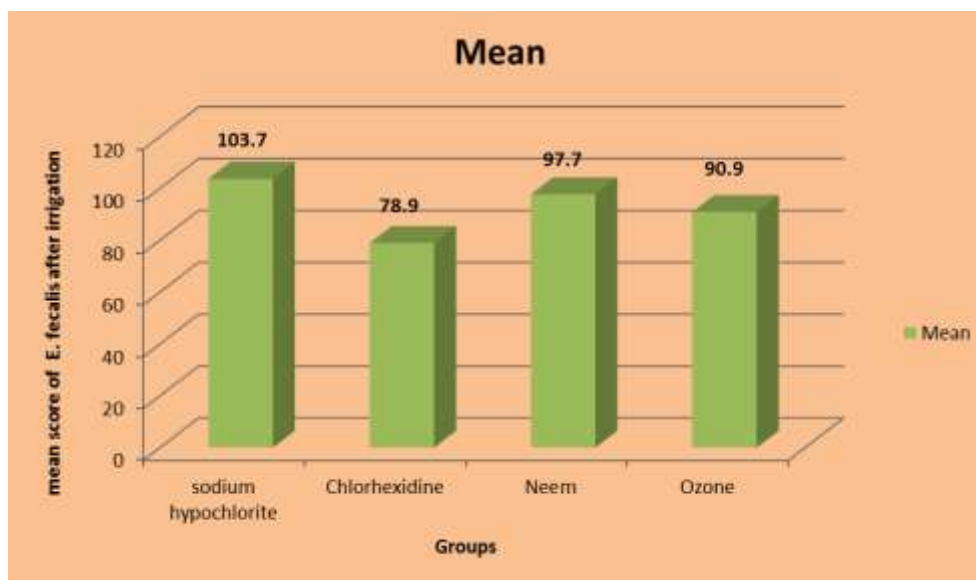
compared using one way ANOVA. The significance level was set as $p < 0.05$.

Groups	No of extracted tooth in each group	Mean	Std. Deviation
1.Sodium hypochlorite	10	103.7	46.4
2.Chlorhexidine	10	78.9	17.2
3.Neem	10	97.7	39.1
4.Ozone	10	90.9	28.5
Total	40	92.8	34.5
P ^a value	0.428 , Non significant		

Above mentioned table shows that though there was a difference in the mean number of CFU of E. Faecalis among different irrigant groups but no statistical

significant difference was found in the number of CFU of E. Faecalis following irrigation with four different

irrigants that is NaOCL, CHX, Azadirachta indica and ozonated olive oil ($p=0.428$).



Mean score/CFU of E. faecalis among different groups after irrigation.

DISCUSSION

The present study showed significant difference with respect to the number of colonies formed. Highest being in group 1 (NaOCL) and least in group 2 (CHX), followed by group 4 (ozonated olive oil) and group 3 (Azadirachta indica). However, the result was statistically insignificant ($p < 0.05$). This can be owed to the size of sample taken and dilution factor (100 μ L in 100ml).

Sodium hypochlorite is the most commonly used irrigating solution in clinical practice because of its tissue dissolution and antimicrobial activity, making it an irrigating solution of choice irrespective of its several undesirable characteristics such as tissue toxicity, risk of emphysema, allergic potential and disagreeable smell and taste.^[11]

Chlorhexidine gluconate is a cationic bisguanide. At low concentrations it shows bacteriostatic effect and higher concentrations bactericidal. It is adsorbed on dental hard tissues and is gradually released over prolonged period showing its therapeutic effect (Substantivity).

Ruqshan Anjum MG^[2] et al, in their in-vitro study concluded with similar result, where in, *chlorhexidine showed least colonies* in comparison with other irrigating solutions.

Kandaswamy D^[12] et al, concluded chlorhexidine to be better efficacious against E. faecalis in their study. Second least count was found in group IV (ozonated olive oil). Ozone therapy has been found to be more efficient on anaerobic bacteria which are the predominant species in the oral cavity. Ozonised oil because of its viscosity remains in the root canal for

prolonged periods,^[13] thus it can also be used as intracanal medicament. Early in vitro studies showed that several polyphenols in olive oil have antibacterial properties against human pathogens, particularly oleuropein, tyrosol, and hydroxytyrosol. The use of ozonated oil is still not widely accepted in dentistry and very few studies have been done.

Group I (NaOCL) and Group III (Azadirachta indica) showed highest colony counts. NaOCL is effective mainly on organic components, but not on inorganic material. A concentrated solution is recommended for its better efficiency. However, it is proven to be highly irritating to periapical tissues.

Azadirachta indica (neem) has been investigated, due to its antimicrobial potential against oral microorganisms. Interest on this substance is based on its properties like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, analgesic and immunostimulant.

Aarti bohora et al^[14] in their study gave a similar conclusion of CHX to be more efficient than neem extract. An study done by **Srinidhi et al^[15]**, concludes that neem has antimicrobial efficacy comparable with 3% NaOCL. However, use of neem as an endodontic irrigant might be advantageous because it is a biocompatible, antioxidant and thus not likely to cause severe injuries to patients that might occur via NaOCL accidents.

CONCLUSION

Although statistically insignificant, with the respective colony count outcome we can conclude that.

- Chlorhexidine 2% has better antimicrobial efficacy.

- Ozonated olive oil has the potential to be used as endodontic irrigant, however, further studies are required to establish the same.

REFERENCES

1. Siqueira JF Jr., Rôças IN. Diversity of endodontic microbiota revisited. *J Dent Res*, 2009; 88: 969-81
2. Ruqshan anjum MG, sujatha I, sharath chandra SM. Antimicrobial efficacy of various irrigating solutions on *E. Faecalis* in root canals: an in-vitro study. *Ijads*, 2015; 1(4): 94-97.
3. Dutta.A, Kundabala.M.Comparative antimicrobial efficacy of Azadirachta indica irrigant with standard endodontic irrigants: A preliminary study . *J Conserv Dent*, 2014 Mar-Apr; 17(2): 133–137.
4. Jain A, Basal E. Inhibition of Propionibacterium acnes-induced mediators of inflammation by Indian herbs. *Phytomedicine*, 2003; 10: 34–8. [PubMed: 12622461].
5. Okpanyi SN, Ezeukwu GC. Anti-inflammatory and antipyretic activities of Azadirachta indica. *Planta Med*, 1981; 41: 34–9. [PubMed: 6972048].
6. Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of Azadirachta indica leaf extract gel A 6-week clinical study. *J Ethnopharmacol*, 2004; 90: 99–103. [PubMed: 14698516].
7. Fabry W, Okemo PO, Ansorg R. Antibacterial activity of East African medicinal plants. *J Ethnopharmacol*, 1998; 60: 79–84. [PubMed: 9533435].
8. Natarajan V, Pushkala S, Karuppiyah VP, Prasad PV. Anti dermatophytic activity of Azadirachta indica (neem) by invitro study. *Indian J Pathol Microbiol*, 2002; 45: 311–3. [PubMed: 12785173].
9. Fabry W, Okemo P, Ansorg R. Fungistatic and fungicidal activity of east African medicinal plants. *Mycoses*, 1996; 39: 67–70. [PubMed: 8786762].
10. Nwosu MO, Okafor JI. Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. *Mycoses*, 1995; 38: 191–5. [PubMed: 8531930].
11. Tyagi SP, Sinha DJ, Garg P, Singh UP, Mishra CC, Nagpal R. Comparison of antimicrobial efficacy of propolis, Morinda citrifolia, Azadirachta indica (Neem) and 5% sodium hypochlorite on Candida albicans biofilm formed on tooth substrate: An in-vitro study. *J Conserv Dent*, 2013; 16: 532-5.
12. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, Morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. *Int Endod J*, 2010; 43: 419-23.
13. Reddy KS, Prasad SD, Sirisha. PD, Prashanth. V. Evaluation of Antimicrobial Efficacy of Ozonated Sesame Oil, Calcium Hydroxide and their Combination as Intracanal Medicament against Candida Albicans: An in-vitro study. *Adv Hum Biol*, 2015; 5(2): 78-87.
14. Aarti Bohora, Vibha Hegde, Sharad Kokate.Comparison of the antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against *E. faecalis*, *C. albicans* and mixed culture - An in vitro study.
15. Srinidhi Surya Raghavendra, Ketaki Dattatray Balsaraf. Antifungal efficacy of Azadirachta indica (neem) - An in vitro study. *Braz J Oral Sci*, 13(3): 242-245.