Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 100

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Comparison of disinfecting potential of 3%naocl, 2%chx, ozonated olive oil and diode laser in infected root canals by enterococcus faecalis -An in vitro study

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ABSTRACT:
Background: Microorganisms play a key role in the development of pulpal and periapical diseases.[1]E. Faecalis, a facultative anaerobe predominates the infected root canals. Elimination of bacteria from infectedcanals before obturation is a crucial step. Sodium hypochlorite exerts antibacterial effect by disrupting the metabolic function of bacterial cell. Chlorhexidine combines with bacterial cytoplasmic component to form toxic complex which destroys the microorganism. Ozone, a triatomic molecule of oxygen, disrupts the bacterial cell envelope by oxidation of phospholipids and lipoproteins.Diode laserdisinfects the canalwithout altering the shape of the canal. Normal saline and is used for final rinsing and flushing of the canals. **Methodology:** Freshly extracted 150 single rooted teeth were decoronated and root length was standardized to 15 mm from the root apex. Samples were randomly divided into 5 groups with 30 teeth in each and were subjected to following irrigation system. GROUP A: 3% NaOCl, GROUP B: 2% CHX, GROUP C: Ozonated olive oil, GROUP D: Diode Laser andGROUP E: Normal Saline.10 microliters of turbid suspension of *E. faecalis* were inoculated after preparing samples followed by incubating at 37°C for 24 hours. Pre and post irrigation microbial count was obtained using sterile paper points, depositing in Brain Heart Infusion agar and counting the coloniesusing digital counter. **Results:** Significant reduction in colonies of E. Faecalis post irrigation were seen in all the groups except normal saline. **Conclusion:** Diode laser showed highest disinfection followed by 3% sodium Hypochlorite, Ozonated olive oil, 2% Chlorhexidine and normal saline was least effective.
Keywords: Enterococcus faecalis, Disinfection, Diode Laser, Ozonated olive oil.

Received: 05 April, 2023 Accepted: 10 May, 2023

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This article may be cited as: Mundhra R, Nagpal R, Gupta PK, Jaju N, Dhote S, Sneha. Comparison of disinfecting potential of 3%naocl, 2%chx, ozonated olive oil and diode laser in infected root canals by enterococcus faecalis -An in vitro study. J Adv Med Dent Scie Res 2023;11(6):47-52.

INTRODUCTION

Complex morphology of the root canal system involving deltas and lateral canals harbors bacteria, debris and necrotic tissues and its sterilization is one of the biggest challenges to the researchers".[2]

E. Faecalis resists chemo-mechanical effects of root canal treatment and its prevalence ranges from 24%to 77% in teeth with endodontic failure[3]. They cause persistent intra-radicular or extra-radicular infection by surviving inharsh environmentswith poor nutrient supply and high alkaline pH of up to 11.5.They grow as highly antimicrobial resistantbiofilms without synergistic support from any other bacteria.[4]

Sodium Hypochlorite (NaOCl) is universal irrigating solution with low surface tension, available in varying concentration between 0.5 to 5.25 % which exerts its antimicrobial effect due to its high pH that interferes with bacterial cytoplasmic membrane, active chlorine causes oxidation of bacterial enzyme, forms toxic complex which destroys the microorganism. Although its foul smell, taste and its cytotoxicity when injected into the peri radicular tissues are major drawbacks.[5]

Chlorhexidine gluconate has been recommended as a root canal irrigant. because of its broad-spectrum antimicrobial action, substantivity and low toxicity.[6] but is incapable of tissue dissolution.

Ozone (O3)at low concentrations of 0.1 ppm, it inactivates the bacterial cells including their spores. On combination with a proton, hydrogen trioxide (HO3) is formed which gives the hydroxyl radical (OH). Theseradicals changeosmotic permeability of the cell membranes resulting in cell damage.

Laser lightpenetrates deep into the dentinal tubules, eliminatesmicroorganisms and aids in smear layer removal.[4] The laser fiber should be rotationally moved into the canals from apical to coronal third to prevent overheating and charring of the dentinal walls.[7] The threshold temperature level of the root surface is 7°C.

Normal saline causes gross debridement and lubrication of root canals.

AIM

The aim of the study was to evaluate the effectiveness of 3% NaOCl, 2%CHX,Ozonated olive oil and Diode Laser in disinfecting theroot canals infected with Enterococcus faecalis.

MATERIALS AND METHODS

150 freshly extracted single rooted teeth were cleaned and stored in normal saline.Length of 15 mm was measured from the root apex and De-coronation was done using diamond disc. Access opening followed by patency filing was done using 10 K file till it was seen beyond the apex. The working length was established 1 mm short of the apex and the canals were prepared uptoprotaper gold F2 file using RC Help and 2.5% of NaOCl and finally irrigated using Normal Saline. Apical foramens were sealed using two coats of nail varnish to prevent any bacterial leakage. (Fig:1)

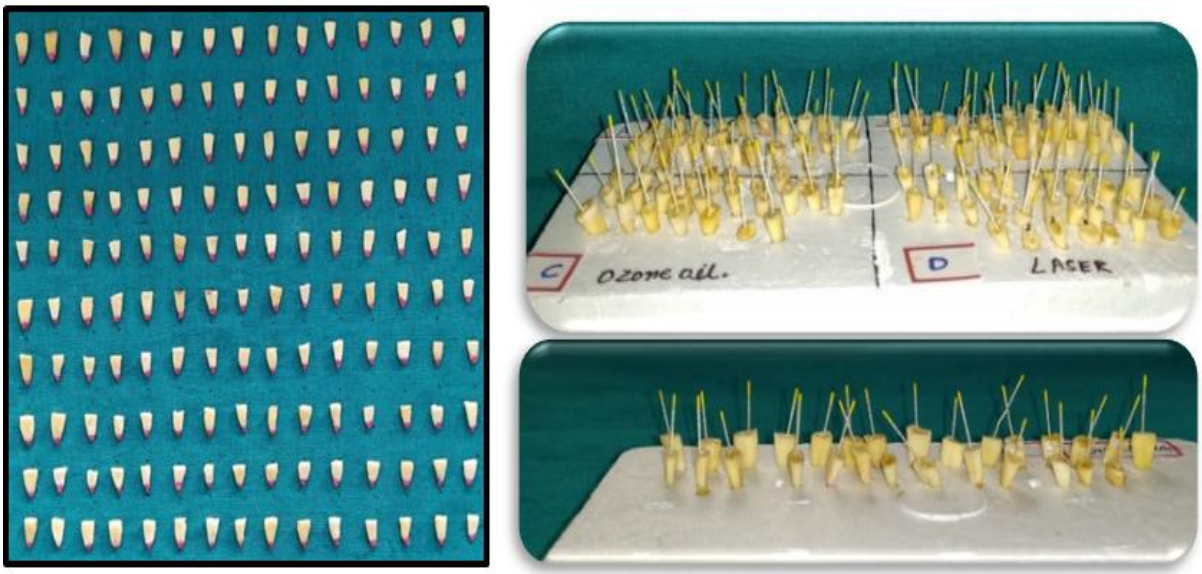


FIG 1: DECORONATED SAMPLES WITH SEALED APICAL FORAMEN
FIG 2: PAPER POINTS INTRODUCED INTO SAMPLES FOR PRE- MICROBIAL COUNT

Sterilized samples were then randomly divided into 5 groups with 30 teeth in each group. Samples were infected by inoculating 10 microliters of turbid suspension of *E. faecalis*andincubated at 37°C for 24 hours. Pre-irrigation microbial count was obtained from the samples by inserting sterile paper points (Fig 2.) and deposited in BHI agar plates which were divided into 4 quadrants with each half taken for pre and post microbial count from one sample. Specimens in each group were then subjected to the following irrigation system for 2 minutes.

GROUP A: 3ml of 3% NaOCl
GROUP B: 3ml of 2% CHX
GROUP C: 3ml of Ozonated olive oil for.
GROUP D: Gallium-aluminum-arsenic Diode laser (Fig:3) with following parameters
Wavelength: 980 nm
Fiber Diameter: 200 micro meters
Power: 2.5 Watt
Time: 5 seconds in 4 cycles with interval of 10 seconds
Technique: Tip was moved in continuous motion from root tip to
Upwards by keeping it 1mm away from apex.
GROUP E: 3ml of Normal Saline.

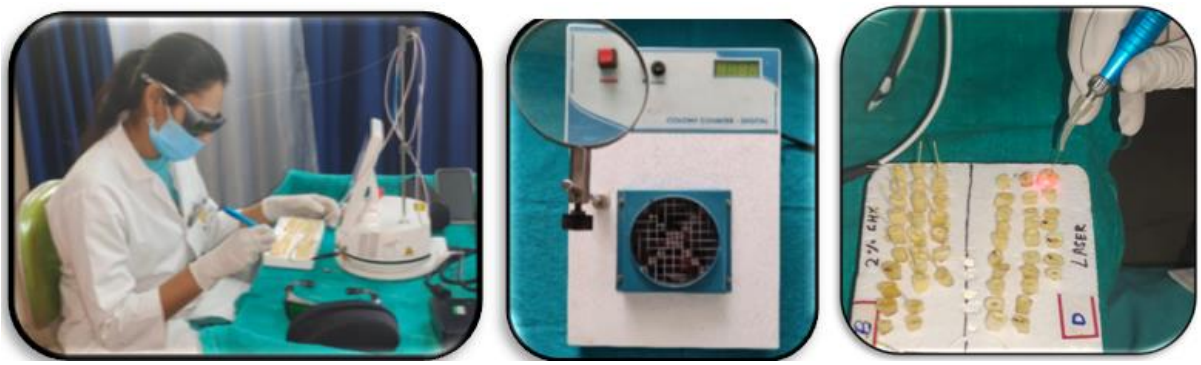


FIG 3: DISINFECTION USING GALLIUM-ALUMINIUM- ARSENIC- DIODE LASER
FIG 4: DIGITAL COLONY COUNTER

Final rinsing was done using Normal saline**.** Post irrigation samples of paper pointswere deposited in the BHI agar and incubated at 37°C for 48 hours. Following formula was used:
No. of colonies x 4 (considering 1 plate for a sample)
Volume inoculated
Mean reduction in total bacterial count, pre and post irrigation was calculated in each group using digital colony counter unit.(Fig.4)

MICROBIAL CULTURE

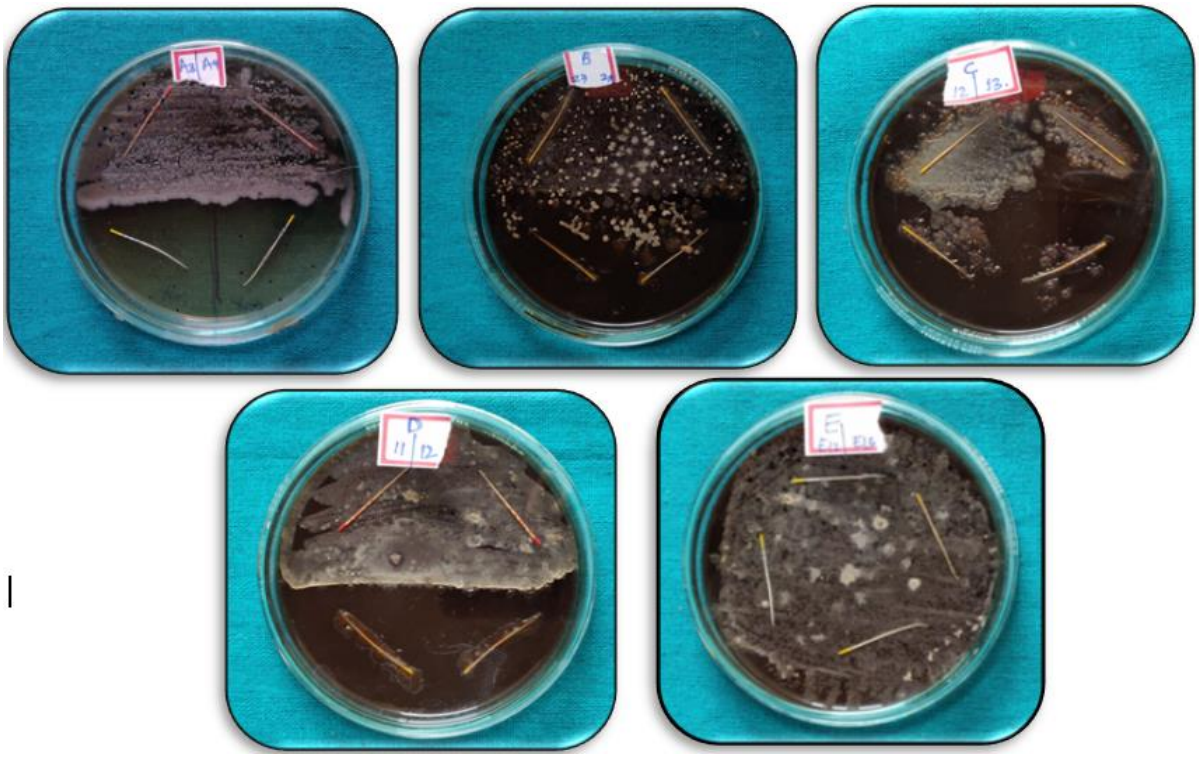


FIG 5: PRE AND POST MICROBIAL CULTURE OF SAMPLES (GROUP A TO GROUP E)

STATISTICAL ANALYSIS

To compare the difference between Pre and Post Microbial Count Paired T Test was applied. To compare between the Microbial Count between Groups, ANOVA with PostHoc Tukeys was applied. Confidence interval at 95% and P<0.05.

RESULT

TABLE 1: MEAN PRE AND POST MICROBIAL COUNT

GROUPS (N=30)	MEAN (Pre-microbialcount)	MEAN (Post-microbial count)	STD. ERROR. MEAN (Pre-count)	STD. ERROR. MEAN (Post-count)	MEAN DIFFERENCE
GROUP A 3%NaoCl	181.33	34.26	28.58	5.62	157.72

GROUP B 2%CHX	184.93	53.20	27.14	6.81	142.68
GROUP C Ozone oil	194.66	58.26	22.46	9.89	145.26
GROUP D Diode Laser	196.26	38.66	23.36	8.15	166.62
GROUP E Saline	191.86	175.8	3.17	3.18	20.01

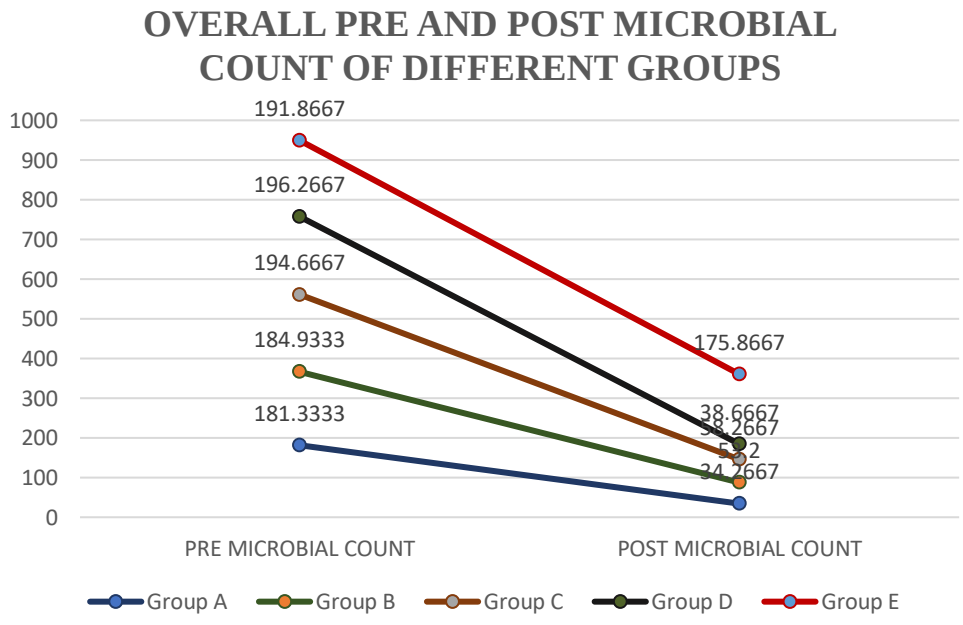
Table 1.shows the mean of differences in the pre and post microbial colonies count along with the standard deviation of each group. The mean of differences between the pre and post microbial count from highest to lowest are Group D i.e.; 166.6 followed by Group A i.e.; 157.7, Group C i.e., 145.2, Group B i.e.; 142.6 and Group E i.e.; 20 CFU/ microlitre. It was found that there was a statistically significant difference between Pre and Post Microbial count ($p<0.05$)

TABLE 2: PAIRWISE COMPARISON OF MICROBIAL COUNT BETWEEN GROUPS

Multiple Comparisons						
Dependent Variable: MICROBIAL COUNT DIFFERENCE						
Tukey HSD						
(I) GROUPS	**(J) GROUPS**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Group A	Group B	-15.33333	6.26004	.108	-32.6261	1.9594
	Group C	-10.66667	6.26004	.435	-27.9594	6.6261
	Group D	10.53333	6.26004	.448	-6.7594	27.8261
	Group E	-131.06667*	6.26004	.000	-148.3594	-113.7739
Group B	Group C	4.66667	6.26004	.945	-12.6261	21.9594
	Group D	25.86667*	6.26004	.001	8.5739	43.1594
	Group E	-115.73333*	6.26004	.000	-133.0261	-98.4406
Group C	Group D	21.20000*	6.26004	.008	3.9072	38.4928
	Group E	-120.40000*	6.26004	.000	-137.6928	-103.1072
Group D	Group E	-141.60000*	6.26004	.000	-158.8928	-124.3072

Table 2. shows the Pairwise comparison between the Pre and Post microbial count between groups. In this comparison, it was found that the difference in mean between Group A-Group B, Group A – Group C, Group A – Group D, Group A- Group E was -15.33,-10.66,-10.53,-131.06 respectively and this difference in was only statistically significant between Group A- Group E ($p<0.05$) however it was not statistically significant between rest groups ($p>0.05$)

The results of above study are further illustrated in the form of graphical representation below:



DISCUSSION

Endodontic triad given by AAE consists of biomechanical preparation, microbial control & complete obturationof the root canal system.E. Faecalis has serine protease, gelatinase and collagen binding protein (Ace) due to which they proficiently invade, bind and live within dentinal tubules. Their biofilms are 1000 times more resistant to phagocytosis, antibodies and antimicrobials.[8]**Shehab et al[4]** concluded that these organisms are highly resistant to a wide range of antimicrobial agents and easy to maintain and culture.

In the present study, the antimicrobial activity was evaluated by anaerobically incubating the pre irrigation and post irrigation samples for 48 hours and reduction in the count was calculated. It is time consuming, difficult and requires microbial facilities.[8]Samples used effectively prepared and enlarged up to F2 protaper file in order to inoculate the microbial suspension easily and allow the introduction of fiber optic tip of diode laser into the canal.

Sodium hypochlorite is well known for its unique property of tissue dissolution. It forms undissociated hypochlorous acid (HClO) in solution containing active chlorine.[9] Its antimicrobial action is time dependent i.e.; the time duration for which it is retained inside the root canal.**Radcliffe et al[10]**concluded that E. Faecalis is resistant to 0.5% NaOCl. While 2.5% had moderate effect against these bacteria, its effect depends on the time. 5.25% sodium hypochlorite aided in complete eradication of E. Faecalis at 2 minutes.Despite its advantages, NaOCl has extensive drawbacks; it is cytotoxic if accidently injected into periapical tissues, foul taste and smell, bleaches clothes, and corrosion of metals.[11]**Sjogren U et al[12]**and **Siqueria JF et al[13]**concluded that sodium hypochlorite does not eradicate all bacteria nor does it completely remove the smear layer.Very less studies have determined the efficacy of 3% NaOCl and compared it with other irrigating solutions at 2 minutes.

Chlorhexidine (CHX)has broad spectrum antimicrobial action. It gets adsorbed to negative charged surfaces in the oral cavity like tooth, mucosa, pellicle and restorations and is released slowly to inhibit microbial activity from 48 hours up to 12 weeks.[14]**Vianna MEet al** concluded that its antimicrobial action is due to the cationic molecule negatively charged microbial cell wall, thereby altering the cell's osmotic equilibrium.[15] It does not dissolve organic tissues and might cause transient taste disturbances, reversible discoloration and burning sensation on initial use.

Ozone is a highly unstable oxygen compound, acts on glycolipids, glycoproteins and other amino acids. It inhibits enzymatic control system of the cell, enhances membrane permeability causing immediate function loss.**Ozonized oil** on hydrolysis gives hydrogen peroxide, aldehydes and ketones. Hydrogen peroxide, an oxidant attacks cellular components and disrupts bacterial cytoplasmic membrane, causes oxidation of enzymes and DNA damage. Aldehydes have alkalization of carboxyl, sulfhydryl and hydroxyl groups in nucleic acids. Their oily consistency increases their contact time with and their duration of action**.**

Nagayoshi, Kitamura et al.[16] concluded that even if it penetrates into periapical tissues it eliminates the toxic waste products of bacteria, encourages regeneration and complete healing of the osseous structures. **In following conditions it is contraindicated:**

- Pregnancy
- Glucose 6 Phosphate dehydrogenase deficiency
- Hyperthyroidism
- Severe anemia
- Severe Myasthenia
- Ozone allergy
- Recent myocardial infarction
- Acute alcohol intoxication

Diode Laser used in this study was **Gallium aluminum and arsenicdiode laser**, which emitted 980 nm wavelengths. On the basis of in vitro study by **Gutknecht et al. [17]** tip diameters of 200 micrometer, power setting of 2.5 W, with 5s irradiation pulse and 10s of rest in total 4 cycles with continuous motion of fiber tip from apex to upwards were selected.

Lee and his colleagues[18]found that continuous movement of fiber tip reduced the thermal effect. **Cohen et al[19]**concluded its tip should not be left in root at fixed position.Repetition of irradiation cycles in turn cause repetition of stress due to which non-lethal reversible damage caused by heat gets converted into irreversible lethal cell damage.Lasers eliminate the bacteria due to its thermal and photo disruptive effects.[20] Irradiation causes denaturation of proteins, destruction of cell wall integrity, cessation of cell growth and successive cell lysis.**Moritz A et al [21]** concluded that its effectiveness depends on its power, amount of energy and time of irradiation. Too high power and energy for longer duration can cause charring of the radicular dentin.

Normal saline was used as control in this study. **Park et al[22]**concluded that neutral irrigant like saline do not render the canals free of pulp tissue debris or bacteria.

The results of this study are in agreement with the studies of **Castelo-Baz et al** where 980 nm diode laser alone was superior in eradicating E. Faecalis and **Niranjan Ashok Vatkar et al [23]** where both Nd: YAG and Diode lasers were effective in eliminating the vitality of E. Faecalis.

By far no studies have compared the efficacy of these two emerging disinfection systemsi.e., Ozonated olive oil and Diode Laser with the commonly used traditional irrigating solutions.

Clinical significance of present study is: Ozonated olive oil has potential to overcome the drawbacks of NaOCl and CHX like transient discoloration and burning sensation.Diode laser has the highest efficacy

in reducing the count of E. Faecalis which might have clinical application for successful endodontic therapy.

Limitations of this study are as follows:

- ✓ It is an in vitro study which had no simulating periradicular tissues.
- ✓ Time duration for disinfection along with volume of solution used was standardized to 2 minutes and 3 ml respectively for all the systems but total laser irradiation was for 1 minute in order to maintain laser parameters and prevent its lethal effect.
- ✓ Only single test microorganism i. e. E. Faecalis was used in the study. However, infected root canal systems have wide array of microorganisms.

CONCLUSION

This study concluded that Gallium aluminum arsenic diode laser with irradiation power of 2.5 W, irradiation time of 5s in 4 cycles showed highest disinfection in infected root canals by E. Faecalis when compared with 3% NaOCl, 2% CHX, Ozonated olive oil and Diode laser.3% NaOCl was second most effective followed by Ozonated olive and 2% CHX was least effective. However, they were significantly effective than Normal Saline.

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