ORIGINAL RESEARCH

Comparative Evaluation of Antimicrobial Efficacy of 2% Sodium Hypochlorite, 2% Chlorhexidine, Ozonated Water, and Turmeric Extract against *Enterococcus faecalis*: An *In Vitro* Study

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ABSTRACT

Aims and Objectives: The main motive of endodontic treatment is elimination of microorganisms and their by-products from the root canal system which is obtained to some extent. The microorganism which persistently harbor in root canal is a Gram-negative facultative anaerobe; *Enterococcus faecalis* (*E. faecalis*). The aim of this study was to determine whether irrigation with sodium hypochlorite (NaOCl), chlorhexidine, ozone gas, and turmeric extract was effective against *E. faecalis.*

Materials and Methods: Cultures of *E. faecalis* were grown in BHI broth at 37°C. Four round wells, 4 mm deep and 8 mm diameter, named as groups A, B, C, and D. 50 µl of the irrigants was added to the wells with the help of micropipette as follows: Group A - 2% NaOCl, Group B - 2% chlorhexidine, Group C - ozonated water, and Group D - turmeric extract.

Results: Maximum antimicrobial activity was shown by 2% chlorhexidine, followed by 2% NaOCl. Turmeric extract and ozonated water showed comparable zone of inhibitions to NaOCl and chlorhexidine.

Conclusion: About 2% of chlorhexidine has a significant antimicrobial effect against *E. faecalis*. Microbial inhibition potential of turmeric extract and ozonated water observed in this study opens perspectives for its use as a root canal irrigant.

Keywords: *Enterococcus faecalis*, Irrigants, Sodium hypochlorite, Chlorhexidine, Ozonated water turmeric extract.

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INTRODUCTION

E. faecalis has been frequently found in reinfected root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases.[1]

Sodium hypochlorite (NaOCl) has been the most widely used root canal irrigating solution. The main advantages of NaOCl are its ability to dissolve necrotic tissue and its antibacterial properties against microorganisms.[2]

Chlorhexidine is a cationic molecule, which can be used during treatment. It has a wide range of antimicrobial activity. Furthermore, because of its cationic structure, chlorhexidine has a unique property of substantivity.[3]

Ozone is being presented as a possible alternative antiseptic agent due to its antimicrobial power and low cytotoxicity; however, there is little information regarding the time and concentration to use.[4]

High tissue toxicity, unpleasant smell and taste, allergic potential, and inability to remove the smear layer are the disadvantages of NaOCl.[5] As there is a need of irrigating agents which have both antimicrobial property and exert minimal tissue irritating effects. Hence, the use of herbal irrigants is gaining interest.

The constant increase in antibiotic-resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives. Noni fruit juice (*Morinda citrifolia)*, neem leaf extract (*Azadirachta indica*), ginger, triphala, and green tea polyphenols had been evaluated previously as endodontic irrigants.[6] Although NaOCl is the most commonly used irrigant, chlorhexidine has recently been introduced as an alternative irrigating solution. However, it does not describe any possible effect of the association between them.

The advantages of using herbal extracts are that they have few side effects, economical, and better tolerated by patients and are renewable in nature. Herbal extracts like turmeric have active components such as alkaloids, volatile essential oils, glycosides, resins, and tannins, which exert an antimicrobial, anti-inflammatory, and antioxidant properties.

Objectives

The objective of this study was to compare the antimicrobial efficacy of NaOCl (2%), chlorhexidine (2%), ozonated water, and turmeric extract against *Enterococcus faecalis in vitro*.

METHODOLOGY

This *in vitro* study was carried out in Microbiology Lab of Aditya Dental Institute. The study was conducted using disc diffusion method.

Preparation of Microbial Inocula

Agar plate was prepared using brain heart agar (BHI). Cultures of *E. faecalis* were grown in BHI broth at 37°C. Culture was grown overnight in BHI broth, adjusted to 0.5 turbidity reading on McFarland scale (1.5 × 108 bacteria/ml). Brain heart agar plates were taken and "Lawn cultures" were prepared on the agar plates by swabbing the sterile swab across the media.

Wells Preparations in Agar Plates

The antibacterial sensitivity test was performed by agar well diffusion method. Four round wells, 4 mm deep and 8 mm diameter, were punched using sterile cork borer, named as groups A, B, C, and D. 50 µl of the irrigants was added to the wells with the help of micropipette as follows:

Group A: 2% NaOCl
Group B: 2% Chlorhexidine
Group C: Ozonated water
Group D: Turmeric extract (50% Aqueous turmeric extract).

A total of three such inoculation agar plates with medicament were prepared. Agar plates were incubated at 37°C for 24 h in an incubator.

The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm with the help of Vernier caliper.

RESULTS

The results were tabulated and statistically analyzed using analysis of variance (ANOVA). Mean zone of inhibitions was recorded.

All medicaments used in this study showed well-defined zones of inhibition around their respective well as shown in Figure 1.

Maximum antimicrobial activity was shown by 2% chlorhexidine (22.67 mm), followed by 3% NaOCl (20.3 mm) [Graph 1].

Turmeric extract and ozonated water showed comparable zone of inhibitions to NaOCl and chlorhexidine.

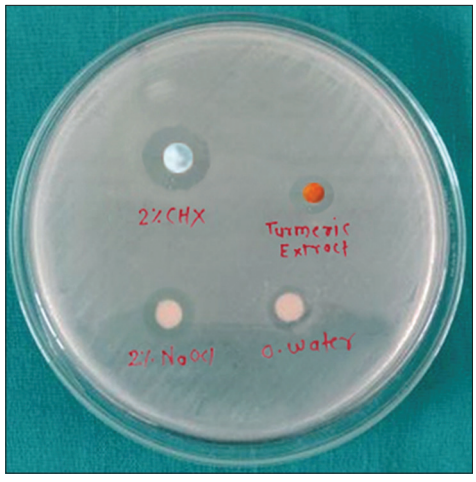
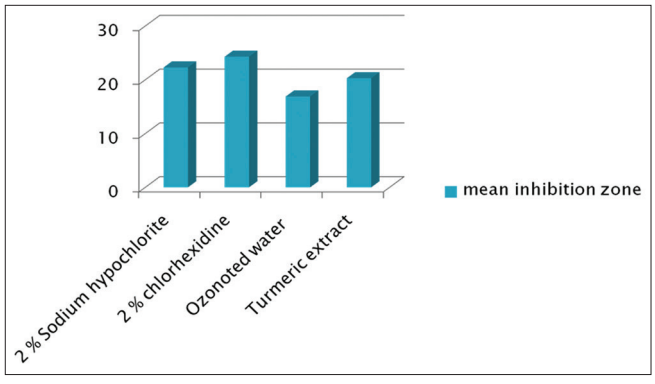


Figure 1: Bacterial inhibition zones around each well



Graph 1: Mean inhibition zone in all groups

The statistical analysis with ANOVA showed that there was no significant ($P > 0.05$) difference between the zone of inhibition diameters among 2% NaOCl, 2% chlorhexidine, ozonated water, and turmeric extract against *E. faecalis* Table 1.

DISCUSSION

The final goal of root canal treatment is to clean the root canal system free of debris and microbiota, thereby leading to well-sealed microbial tight filling. This is achieved by "chemomechanical preparation."

Stuart *et al*. and Portenier *et al*. have identified *E. faecalis* frequently in cases with post-treatment disease.[7,8] *E. faecalis* is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment.[7] It is commonly found in a high percentage of root canal failures, and it is able to survive in the root canal as a single organism or as a major component of the flora. *Enterococci* survive very harsh environments including extreme alkaline pH (9.6) and salt concentrations.[9] They resist bile salts, detergents, heavy metals, ethanol, azide, and desiccation.[10] They can grow in the range of 10–45°C and survive a temperature of 60°C

Table 1: Mean inhibition zone in all groups

Group	Agent	Number of plates	Mean (mm)	Range (Min-max)	Mean±SD
a	2% sodium hypochlorite	3	22.3	19–22	20.3±3.6
b	2% chlorhexidine	3	24.3	21–28	24.3±2.08
c	Ozonoted water	3	16.9	20–25	16.9±3.01
d	Turmeric extract	3	19.8	20–26	19.8±2.94

ANOVA test (Analysis of variance) (*P* value>0.05)

for 30 min. *E. faecalis* is associated with different forms of periradicular disease including primary endodontic infections and persistent infections. The prevalence of *E. faecalis* is increased in oral rinse samples from patients receiving initial endodontic treatment, those midway through treatment, and patients receiving endodontic retreatment when compared to those with no endodontic history.[7]

Several chemical irrigants have been suggested for use in the treatment of infected root canal. The most commonly used chemical is NaOCl in varied concentrations. It is preferred over others for its unique ability to dissolve the pulp tissue and its antimicrobial efficacy.[2]

In our study, the antimicrobial efficacy is more with chlorhexidine followed by NaOCl. Previous studies showed similar results. Gomes *et al.* testing various concentrations of NaOCl and CHx during different periods, in cell suspensions of *E. faecalis*, found that even though all tested irrigants were effective in killing *E. faecalis*, the time required depended on the concentration and type of irrigant used.[4]

The comparison between NaOCl and ozonated water in Graph 1 showed that NaOCl has more antimicrobial effect on E. *faecalis* which is, in contrast, to study conducted by Nagayoshi *et al*. studied that ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation, especially when combined with sonication, and showed a low level of toxicity against cultured cells.[11]

NaOCl has limited antimicrobial efficacy, and this has led to problems in penetration to the most peripheral parts of the root canal system. Long-term exposure of dentin to high concentrations of NaOCl can have a detrimental effect on dentin elasticity and flexural strength, thereby predisposing the tooth to vertical fracture, which has a worse prognosis.[12]

Due to the potential adverse effects and the safety concerns, herbal preparations have gained more popularity in the last few years. Although our study results show that turmeric has less antimicrobial effect as compared to NaOCl, it can be good option to overcome the adverse effects of NaOCl. Results of our study were similar to the study conducted by Chaitanya *et al*. comparing NaOCl with turmeric extract.[13]

Ozone is being presented as a possible alternative antiseptic agent due to its antimicrobial power and low cytotoxicity. However, there is little information regarding the time and concentration to use. The study showed that ozone water has less antibacterial efficacy as compared to NaOCl and CHx. However, Hems *et al*. evaluating the ability of ozone to kill an *E. faecalis* strain verified that its antibacterial efficacy was not comparable to that of NaOCl.[4]

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