

Comparative Evaluation of Antibacterial Efficacy of Newer Intracanal Medicament Nitrofurantoin and Ozonated Oil Plus Calcium Hydroxide on *E.faecalis* – An in Vitro Study

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Abstract:- Background: Multiple antibacterial agents have been mixed and used as an intracanal medicament to eliminate *E.faecalis*, which has been most frequently identified in the cases of failed root canal treatment and periapical lesions. This study is aimed at using newer intracanal medicament Nitrofurantoin and Ozonated oil plus calcium hydroxide against *E.faecalis* bacteria. **Aim:** To compare the antimicrobial efficacy of newer intracanal medicament Nitrofurantoin and Ozonated oil plus calcium hydroxide on *E.faecalis* - an in vitro study. **Materials and methodology:** Thirty extracted premolars were collected and instrumented with rotary files (size 25-4% taper). Then, root canals were contaminated with a bacterial solution of *E.faecalis* for 21 days, and divided into two groups. In the root canals of group A nitrofurantoin intracanal medicament was placed and in group B ozonated oil plus calcium hydroxide intracanal medicament was placed and sealed for 7 days. Then on the 8th day, the contaminated root canals were flushed with normal saline to remove the medicament. To evaluate the degree of contamination, the specimens of the dentin chips from the full length of the root canal were harvested using a sterile rotary instrument (size 25-6% taper) and placed in BHI broth and same were subjected for dilution and colony count after 24 hours. Data was recorded and subjected to statistical analysis. **Results and conclusion:** within the limits of the study, Nitrofurantoin showed greater antibacterial efficacy on *E.faecalis* when compared to the mixture of Ozonated oil plus calcium hydroxide as an intracanal medicament.

Keywords:- Nitrofurantoin, Ozonated oil, Calcium hydroxide, *E.faecalis*, antibacterial efficacy

I. INTRODUCTION

There are several factors that may cause a persistent periradicular infection as a consequence of root canal treatment like intraradicular infection, extraradicular infection, or foreign body reaction and cysts [1]. These infections are the result of bacterial infection of the root

canal, which will end in reinfection and failure of root canal treatment [2]. Enterococcus faecalis (EF), which is a facultative bacteria, is the most predominant and most resistant microorganism leading to persistent periradicular lesions and eventually endodontic failure [3–5]. It is found in root canal failures in nearly 24–70% by culturing methods [6–8] and in 67–77% by molecular methods [9– 12]. In other studies, it was retrieved as a major component, about 90% [13, 14].

This microorganism has many special properties that enables it to survive in root canal and cause reinfection such as the ability to tolerate periods of starvation, deeply invade dentinal tubules [15], the ability to adapt to changing environment and antimicrobial resistance. For these reasons, a wide variety of antibacterial intracanal medicaments have been used.

Calcium hydroxide is considered the first choice intracanal medicament owing to its bactericidal property, stability and capacity to stop inflammatory exudates compared to other medicaments especially in multiple-visit endodontics.[16] These favourable actions of calcium hydroxide are attributed to its alkalinity (12.5– 12.8) and presence of calcium ions. The former denatures and detoxify the bacterial enzymes, whereas the later is important for tissue mineralization by stimulating the fibronectin gene expression.[17,18] Further, the dissociation of Calcium hydroxide in to calcium and hydroxyl ions is another rate-limiting step in making Calcium hydroxide as a dentist's choice of intracanal medicament.[19] In order to achieve effective dissociation of ions, appropriate choice of a vehicle is warranted. Studies have shown that the velocity of dissociation of these ions at the periapical tissues and root canal is greatly influenced by the vehicle used to prepare the paste.[20,21]

Oily vehicle-containing paste is employed in clinical situations like chronic long standing lesions and inflammatory root resorption that necessitates gradual, uniform and slow ionic liberation. In order to achieve

optimum results through an endodontic treatment, the antimicrobial capacity of the pastes used is also taken into consideration. Utilization of medical ozone in various forms like ozonated water, ozonated oil or gas is gaining popularity among the dentists. This is mainly due to its efficacy in achieving faster healing and antimicrobial properties.[22] Use of ozonated oil has shown its antimicrobial efficacy on the bacterial species associated with periradicular diseases.[23]

Nitrofurantoin (Nit) is a synthetic nitrofurantoin compound [24]. It is effective against most gram-positive and gram-negative organisms [25] and is a well-known antibacterial agent widely used as an oral antibiotic treatment for urinary tract infections (UTIs) [26]. It is also the drug of choice for the treatment of infections caused by multidrug resistant pathogens [27–29].

Thus the objective of this study is to compare the antimicrobial efficacy of a newer intracanal medicament Nitrofurantoin and a mixture of Ozonated oil plus calcium hydroxide against *E.faecalis* bacteria.

II. MATERIALS AND METHOD

A. Selection of Tooth specimens:

Single rooted , Type- I root canal configuration, non-carious permanent mandibular premolar with no resorption or anomaly were included in this study.

B. Preparation of teeth:

Thirty extracted lower premolar single-rooted teeth were collected and stored in saline until required. The teeth were decoronated using a rotary diamond saw with water irrigation. The roots were divided into two groups of 15 roots each. The root lengths were measured and the working length was determined as 1 mm less than the file length when the tip was visible at the apical foramen. Coronal root structure was removed perpendicular to the long axis of the root to produce roots with a working length of 15 mm. The canals were instrumented with engine-driven nickel titanium rotary files (NEOENDO FLEX) used in a sequential crown down technique to a standardized apical size of 25.4% taper instrument. 2.5% sodium hypochlorite was used as an irrigant during instrumentation with a sterile needle and 2.5 ml sterile syringe. The roots were then rinsed in water for 30 minutes, then rinsed in 17% ethylenediaminetetraacetic acid

(EDTA) for 5 minutes in an ultrasonic bath to remove the smear layer and rinsed in water for a further 30 minutes.[30] Then, the teeth were stored in saline until used. Each root was dried and three layers of clear nail varnish were placed over all external root surfaces with care not to occlude the root canal entrance and teeth were allowed to dry. Then, the roots were autoclaved at 121°C for 15 minutes.

C. Root canal infection:

Each root canal was inoculated with a bacterial solution up to the canal entrance using a sterile endodontic needle and 2.5 ml sterile syringe. Each canal was sealed with a dental wax and all samples were incubated in a closed container at 37°C for a period of 21 days. The canals were reinoculated with fresh bacterial samples every 3 days.

D. Root canal medication:

After 21 days, the canal contents were aspirated and each canal was rinsed with saline, then dried with sterile paper points and divided in two groups.

In Group A- Nitrofurantoin; Group B- mixture of 1 mg of Calcium Hydroxide with 2 mL of Ozonated oil were placed using sterile syringe.

The roots were left for 7 days at 37°C after sealing with paraffin wax. On the 8th day, to investigate the degree of infection of the radicular dentin, specimens of the dentin chips from the full length of the root canal were harvested using a sterile rotary Nickel titanium instrument (size 25 6% taper) and placed in BHI broth. They were then subjected for **colony count** after 24 hours. Data were recorded and subjected to statistical analysis.

III. RESULT

The results of the present study showed that Nitrofurantoin showed almost none of Colony Forming Units (CFU) in their respective group. Whereas mixture of Calcium hydroxide and Ozonated oil group showed mean CFU- 48. Statistical analysis was carried out using Students Unpaired ‘t’ test using SPSS windows 12.0 version. The P value 0.000 show that the difference in the colony count between Group A and B is highly significant.

Table 1: Colony forming units recorded in Group A and B

Group A	0	0	1	2	0	0	1	1	0	0	0	0	0	0	1
Group B	58	18	36	51	46	52	59	17	50	56	58	57	58	56	52

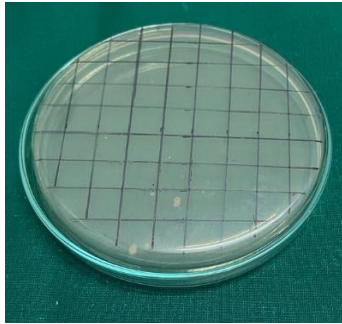


Fig 1 : Colonies formed in Group A

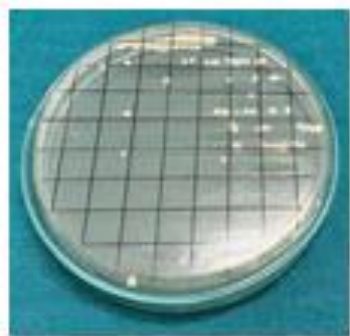


Fig 2: Colonies formed in Group B

IV. DISCUSSION

- Many antibacterial agents have been mixed and used as an intracanal medicament to eliminate *E.faecalis*, which has been most frequently identified in the cases of failed root canal treatment and periapical lesions. This study aimed at comparatively evaluating the use of newer intracanal medicament Nitrofurantoin (Nit) and Ozonated oil plus calcium hydroxide against *E.faecalis* bacteria.
- Nitrofurantoin was selected in this study because of its broad spectrum of antibacterial activity and is both bactericidal and bacteriostatic against microorganisms [31]. It is the drug of choice against *E.faecalis*, and has been used for an extended period in UTI's and chronic and recurrent infections caused by *E.faecalis* [32].
- Nitrofurantoin has a unique mechanism of action. It denatures bacterial ribosomal proteins after being reduced by bacterial flavoproteins; this phenomenon is repeated with other bacterial macromolecules. This results in suppression of many essential processes inside the bacteria like aerobic energy metabolism, cell wall synthesis, DNA synthesis, protein synthesis, and synthesis of RNA. [33]
- Because of this suppression mechanisms, there is a very poor possibility of developing bacterial resistance to Nitrofurantoin. Thus, bacterial resistance to Nitrofurantoin is very rarely seen since its introduction and FDA approval in 1953 until now. It is very rare to encounter cross-resistance with antibiotics or transferable resistance in bacteria [34].
- Calcium hydroxide has wide utility in endodontics owing to its anti-microbial and biological activity. Diffusion of calcium ions and higher pH levels are said to be the rate

limiting factors determining the anti-microbial effect of calcium hydroxide.[35]

- The study conducted by Kesha Vasavada and Sonali Kapoor, concluded that the release of calcium ions was higher in the ozonated olive oil group which is suggestive of higher mineralization activity post ozonation. [36]
- The study conducted by Mewan Salahalddin A. Alrahman , 1 Bestoon Muhammed Faraj,1 and Kawa F. Dizaye concluded that at a concentration of 25 mg/mL, Nitrofurantoin paste is effective in eradicating *E.faecalis* completely when it is used as an intracanal medicament.[33]
- Zhanel et al. [37] showed that nitrofurantoin is active against all isolates of *E.faecalis* found in UTI, showing their susceptibility to Nitrofurantoin. Butt et al. [38] found that, for a period of three years, nitrofurantoin was an effective antibacterial in vitro agent and can be used for the treatment of enterococcus urinary tract infections, as they showed that one hundred and twenty-seven (88%) isolates of enterococci were susceptible to Nitrofurantoin.
- Abdulla and Abdulla [39] demonstrated that nitrofurantoin was effective against EF (cultured from UTI) in 97.3%, while ciprofloxacin was effective in only 35.7%. Rahbar et al. [40] found that nitrofurantoin had the lowest resistance rate compared to other antibiotics like ciprofloxacin against EF (cultured from UTI) (97% vs. 33.38%, respectively). Toner et al. [41] concluded that EF had a sensitivity test 100% to Nitrofurantoin.
- Sorlozano-Puerto et al. [42] showed that for four years, EF had a sensitivity to nitrofurantoin ranging from 95% to 100%.

➤ Significance of Study:

The uniqueness of this study lies in the fact that it is the first of its kind in vitro study performed to evaluate the antimicrobial efficacy between these two newer intracanal medicaments with type- I root canal configuration.

➤ Limitations of the Study:

The limitation in the present study is that we studied the antibacterial effects of Nit only against EF, which is the principal constituent of the microorganisms involved in persistent endodontic infections. This study includes inadequate sample size and limited duration of evaluation to prove the long-term success of these medicaments. Further in vivo and in vitro studies with these medicaments in all types of root canal configurations with a larger sample size and long-term evaluation deems necessary to use them as materials of choice.

V. CONCLUSION

Thus to conclude, within the limits of the study, Nitrofurantoin showed greater antibacterial efficacy on *E.faecalis* when compared to the mixture of Ozonated oil plus calcium hydroxide as an intracanal medicament.

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