

COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY OF CURCUMIN, LINEZOLID AND CHLOROHEXIDINE WHEN USED AS INTRACANAL IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS. – AN IN VITRO STUDY

Dentistry

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ABSTRACT

Aim: To comparatively evaluate antibacterial efficacy of curcumin, linezolid and chlorohexidine when used as intracanal irrigants against *Enterococcus Faecalis*. **Materials and Methods:** 50 single rooted freshly extracted human teeth with type I canal anatomy were randomly divided into 5 groups, each consisting of 10 specimens based on the irrigants used. GROUP I (Negative control) Saline, GROUP II (Positive control) 3% Sodium Hypochlorite, GROUP III – 95 % Curcumin extract, GROUP IV – Linezolid, GROUP V - 2% Chlorhexidine. Root canals were prepared with crown down technique using protaper hand instruments and K files up to 25 size apically. Canals were treated with 17% EDTA for 1 min to facilitate removal of smear layer. Root apices were sealed with nail varnish and teeth were sterilized by autoclaving. A turbid suspension of *Enterococcus faecalis* was placed into the canal using a sterile 27 gauge needle syringe and incubated at 36.5 degree celsius for 24 hours. All the samples were irrigated with the test solution for 30 sec. 1 mm hole was drilled 3mm short of apex to collect dentinal shaving. Shavings were allowed to fall on the entire agar plate with brain heart infusion agar culture medium. Agar plates with dentinal shaving were incubated for 48 hours at 36.5 degree celsius. After 48 hours, the colonies were counted using colony counter. **Results:** Linezolid group (0.4216) had least mean colony count against *E Faecalis* when compared to saline, sodium hypochlorite and curcumin solution with statistical significance. **Conclusion:** Within the limitation, the present study concludes linezolid showed maximum antibacterial efficacy against *E Faecalis* followed by chlorohexidine, sodium hypochlorite and curcumin

KEYWORDS

Antibacterial Efficacy, Chlorohexidine, Curcumin, *E. Faecalis*, Intracanal Irrigants, Linezolid, Mean Colony Count, Sodium Hypochlorite.

INTRODUCTION

Complete elimination of microorganisms from the complex root canal system is the main objective of root canal therapy. Persistence of micro-organism and reinfection are the main cause of failed root canal therapy. Currently, more than 300 species are recognized as normal inhabitants of the oral cavity. But only limited numbers of species have been isolated from infected root canals.⁽¹⁾

Enterococcus Faecalis is one of the most predominant microorganisms found in the failed cases of root canal treatment. *Enterococcus Faecalis* is a non spore forming, gram positive cocci that occur singly, in pairs or as short chains. *Enterococcus Faecalis* cells are ovoid and 0.5 to 1 μm in diameter. It is a facultative anaerobic, normal commensal, adapted to the ecologically complex environment of the oral cavity and gastrointestinal and vaginal tracts.^(2,3,4)

The successful elimination of this microorganism and its substrate can be achieved only through meticulous removal of contaminants from the canal system as well as from anatomic irregularities such as root canal fins, webs, cul de sacs etc. Biomechanical preparation and chemical preparation of the canal system are used concomitantly in order to debride the canal system.⁽⁵⁾

Various chemicals have been investigated to disinfect the root canal system. Sodium hypochlorite is capable of dissolving necrotic tissue and vital pulp tissue. Sodium hypochlorite in 5.25% concentration is an extremely effective antimicrobial agent. It is inexpensive, has a long shelf life, provides lubricating effect for instrumentation of the canal walls, exerts bleaching action on discolored teeth and increases the permeability of dentinal tubules for easier penetration by an intracanal medicament.⁽⁶⁾ In spite of these properties, sodium hypochlorite produces allergic reaction and can cause problems if accidentally extruded periapically.⁽⁷⁾

Chlorhexidine gluconate is a wide spectrum antimicrobial agent. It is unique in its ability to bind oral tissues for extended period from which it is released slowly (substantively) and it is relatively non toxic.⁽⁸⁾ However it lacks tissue dissolving property and cannot remove the smear layer.

Linezolid are a new class of synthetic antibiotics which inhibit protein synthesis by binding to the 50s ribosome subunit and preventing formation of the initiation complex. They have significant bacterio-

static activity against multiresistant gram-positive pathogens such as *E faecalis* through inhibition of protein synthesis.^(9,10,11,12) This strongly suggests that linezolid has promise for the clinical treatment of resistant gram-positive infections.

Turmeric consists of 3-5% curcuminoids. Curcumin is the most important fraction which is responsible for the biological activities of turmeric. It has wide spectrum of biological actions such as anti-inflammatory, antioxidant, anticarcinogenic, antiviral, antimicrobial activity, antineoplastic, antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory and antithrombotic, wound healing, antidiabetogenic, antistressor and antilithogenic actions.^(13,14) It's Antibacterial activity against *E faecalis* as an intracanal medicament has already been speculated.⁽¹⁵⁾ Antimicrobial efficacy of nanacurcumin coated gutta percha against *E Faecalis* has already been reported.⁽¹⁶⁾ It's wide spectrum of biological actions provides a basis for exploring its antibacterial activity against *E faecalis* as an intracanal irrigant.

Hence this study was conducted to comparatively evaluate the antibacterial efficacy of curcumin, linezolid and 2% chlorohexidine when used as intracanal irrigants against *Enterococcus faecalis*.

MATERIALS AND METHODS

The present study was conducted in the department of Conservative Dentistry and Endodontics, PMNM Dental College & Hospital, Bagalkot and department of Microbiology, HSK Medical College, Bagalkot. 50 single rooted extracted human teeth with type I canal anatomy were collected and stored in tap water till the period of study. Access cavity was prepared on the lingual surfaces of anterior teeth and occlusal surface of the premolar teeth using diamond points with high speed airtor hand piece. Root canals were prepared with crown down technique using protaper hand instruments (Dentsply/ Tulsa Dental) and K files up to 25 size apically. Canals were treated with 17% EDTA for 1 min to facilitate removal of smear layer. Root apices were sealed with nail varnish and teeth were sterilized by autoclaving. A turbid suspension of *Enterococcus faecalis* (0.5, 10⁸ organisms/ml) was obtained by growing the cells in brain heart infusion broth for 6 hrs at 36.5 degree celsius. The bacteria laden suspension was placed into the canal using a sterile 27 gauge needle with syringe and incubated at 36.5 degree celsius for 24 hours. The teeth were then divided into 5 groups of 10 teeth each.

GROUP I - Negative control group, Saline

GROUP II - Positive control group, 3% Sodium Hypochlorite((Vishal Dent Care Pvt Ltd.India)

GROUP III – Ethanol solution of 95 % Curcumin extract The minimum inhibitory concentration of Curcumin against *E. faecalis* was evaluated using tube dilution method. i.e. 1024µg/ml. of curcumin was used in the study

GROUP IV – Linezolid (Glenmark)

GROUP V - 2% Chlorhexidine (Vishal Dent Care Pvt Ltd.India)

In all the groups the canals were passively irrigated with respective test solution using sterile 27 gauge needle for 30 secs. With the 1mm diameter No.2 sterile round bur, 1 mm hole was drilled 3mm short of apex to collect dentinal shaving. Shavings were allowed to fall on the entire agar plate with brain heart infusion agar culture medium. Agar plates with dentinal shaving were incubated for 48 hours at 36.5 degree celsius. After 48 hours, the viable organisms were expressed as colony forming units (CFU/ml) from fallen dentinal shaving. The colonies were counted using colony counter.

RESULTS AND STATISTICAL ANALYSIS

Data were computerized and analyzed using SPSS 13.0 software. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by pair-wise comparison of groups by Tukey multiple post hoc procedure. Significance of difference was set at $p \leq 0.05$.

The mean colony count of saline group was maximum (38.7000) followed by curcumin group (22.6000), NaOCL group (11.0000), chlorhexidine group (0.6749) and Linezolid group (0.4216). (Chart 1)

The results were statistically very highly significant when saline group was compared with other groups. The results were statistically highly significant when NaOCL group was compared with curcumin, linezolid and chlorhexidine groups. The results were statistically very highly significant when curcumin group was compared with linezolid and chlorhexidine groups. No significant difference was found between linezolid and chlorhexidine groups. (Table 1)

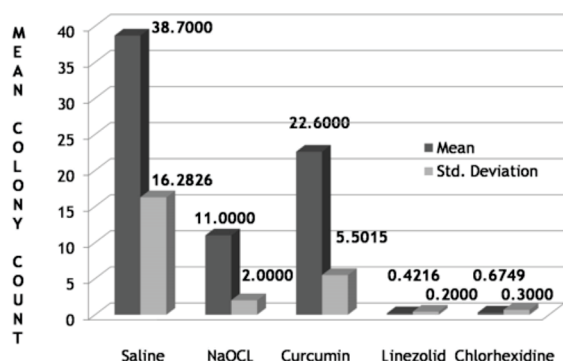


Chart 1

Table 1

Inter-group comparison

group	group	Mean difference	Statistical significance
Saline(-ve cntrl)	NaOCL(+ve cntrl)	27.7000	VHS
	curcumin	16.1000	VHS
	linezolid	38.5000	VHS
	chlorhexidine	38.4000	VHS
NaOCL(+ve cntrl)	curcumin	-11.6000	HS
	linezolid	10.8000	HS
	chlorhexidine	10.7000	HS
curcumin	linezolid	22.4000	VHS
	chlorhexidine	22.3000	VHS
linezolid	chlorhexidine	-1.0000	NS

DISCUSSION

The prime objective of endodontic treatment is sterilization of root canal system. It depends on the complete elimination of micro-

organisms from the complex root canal system. Failed root canal therapy occurs when microorganism persists in the root canal and cause reinfection.¹

The effectiveness of irrigant largely depends upon the type of bacteria present within the tubules. Although obligate anaerobic bacteria dominate the root canal microbiota, some facultative strains such as *E. faecalis* have been involved in persistent infections, influencing the prognosis of pulp space therapy.⁴ This organism is capable of surviving even under unusual environmental stresses. It displays innate resistance to a variety of antibiotics such as betalactams, aminoglycosides, vancomycin and trimethoprim-sulfa methoxazole combinations. Hence this study was conducted to comparatively evaluate the antibacterial efficacy of curcumin, linezolid and chlorhexidine when used as intracanal irrigants against *Enterococcus faecalis*.

The present study compares bactericidal effect of endodontic irrigants at apical third of the root canal. Apical third is the most critical zone where micro-organisms can persist & not get eliminated. The dentinal chips from the apical third were infected by *Enterococcus faecalis* and was spread over the agar plates. This method of culturing the bacteria on the agar plates is simple and is routinely used. It has been used in the past by researchers (RA Buck, PD Eleazer) to test the effectiveness of endodontic irrigants at various tubular depths in human dentin.³

According to the present study linezolid showed best antibacterial activity against *E. faecalis* among all groups. Linezolid group resulted in least mean colony count when compared to other groups. The results were statistically significant when compared to saline, sodium hypochlorite, and curcumin groups. The result may be attributed to its unique mechanism of inhibiting initiation of bacterial protein synthesis by binding to the 23S subunit of the 50S subunit. There are no literature evaluating antibacterial efficacy of linezolid in comparison to curcumin and chlorhexidine as intracanal irrigant against *E. faecalis*. But linezolid was having more antibacterial efficacy than Chlorhexidine against *E. faecalis* when used as intracanal medicament according to the study done by Pavaskar et al.¹⁷ Studies should be carried out to determine antibacterial efficacy of linezolid against *E. faecalis* when used as intracanal irrigant.

Chlorhexidine showed antibacterial efficacy against *E. faecalis* almost similar to linezolid. No statistical significance was seen between chlorhexidine and linezolid groups. It showed superior antibacterial efficacy when compared to saline, sodium hypochlorite and curcumin groups with statistical significance. This result may be attributed to its broad spectrum activity against both gram +ve and gram -ve micro-organisms and also to property of substantivity or residual effect. This result is in accordance with the study done by Dakshita Joy Sinha et al. in which maximum antibacterial efficacy was exhibited by 2 % chlorhexidine, followed by 5 % NaOCL and curcumin.¹⁸

The antibacterial efficacy of curcumin against *E. faecalis* was lesser than linezolid, chlorhexidine & NaOCL groups with statistical significance. This result is in accordance with the study done by Dakshita Joy Sinha et al.¹⁸ curcumin can be considered as a viable herbal alternative to sodium hypochlorite for clinical use because of low toxicity. Further studies should be carried out to evaluate its antibacterial efficacy against *E. faecalis* when used as an intracanal irrigant.

CONCLUSION

Within the limitation, the present study concludes linezolid showed highest antibacterial efficacy against *E. faecalis* followed by chlorhexidine, sodium hypochlorite and curcumin. The antibacterial efficacy of Chlorhexidine was almost similar to linezolid against *E. faecalis*. Further studies should be carried out to evaluate antibacterial efficacy of linezolid and curcumin against *E. faecalis* when used as intracanal irrigants.

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