Comparative Evaluation of Antimicrobial Efficacy of Chlorhexidine 2% and Triphala Against E. Faecalis When Used as Root Canal Irrigants - An In Vitro Study

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Abstract

Introduction: The purpose of the study was to evaluate and compare the anti-microbial efficacy of 2% Chlorhexidine and Triphala against E. faecalis. **Materials and method**: 20 freshly extracted permanent single rooted teeth were collected and were decoronated to achieve 14 mm of root length. The root canals was prepared using step-back technique, master apical file (MAF) was kept as 50 no. K-file and the enlarged apical foramen was sealed from outside with cynoacrylate. Teeth were mounted vertically in plaster blocks and sterilized. Pure culture of Enterococcus faecalis was grown on Mac conkey's agar plates and suspension of E. faecalis was then prepared in normal saline. The root canals were inoculated using sterile micropipette and were then incubated at 37 ^{0}C for 24 hours. After incubation they were divided into two groups. The samples were irrigated with 2% Chlorhexidine and Triphala respectively. The remaining suspension was aspirated from the root canals and then transferred into Brain Heart Infusion broth and were then incubated at 37 ^{0}C for 4 days. The occurrence of the broth turbidity was indicative of bacteria. Then it was grown on Mac Conkey's agar for 24 hours at 37^{0}C to count the colony forming units of E. faecalis. **Results and conclusion**: Collected data was subjected to statistical analysis which revealed that 2% Chlorhexidine performed significantly better Triphala when used against E. faecalis.

Keywords: Anti-microbial efficacy, Brain heart infusion broth, Chlorhexidine, E. faecalis, Triphala.

INTRODUCTION

Enterococcus faecalis has been found to be one of the most predominant bacterial species associated with failed endodontic cases.[1] The organism can survive in extreme challenges as it resists antimicrobial effect; share extrachromosomal elements encoding virulence traits, which help to colonize, resist host defence mechanisms and produce pathological changes directly through production of toxins or indirectly through induction of inflammation. The ability of this microbe to form biofilms, penetrate into dentinal tubules, survival in low pH, high salinity and high temperature and resistance to many intracanal irrigants makes it one of the most resistant pathogen of all the root canal flora. Its prevalence in such infections ranges from 24% to 77%.[2,3] Thorough debribement and complete elimination of micro-organisms are objectives of an effective endodontic treatment. Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that has been advocated as an effective medication in endodontic treatment.[4] Chlorhexidine is a positively charged lipophilic/hydrophobic molecule that interacts with phospholipids and lipopolysaccharides on the bacterial cell membrane. In endodontics, its mode of antibacterial activity is determined by its concentration (0.2% or 2%).[5] As the global scenario is now changing towards the use of plant and plant products herbs like triphala, neem and green tea etc all being tested as potential root canal irrigants because they possess antibacterial and antifungal properties.[6]

Triphala is an ayurvedic rasayana consisting of Amulaki (emblica officinalis), Bibhitaki

(terminalia bellirica) and Halituki (terminalia chebula) and is rich in citric acid which helps in removing the smear layer.[7] It's chelating property makes it an effective alternative to sodium hypochlorite for root canal irrigation. Triphala and Green tea polyphenols are preferred over the traditional root canals irrigants due to their curative properties such as anti-oxidant, anti-inflammatory and radical scavenging activities.[8,9]

The present study was aimed to evaluate and compare the antimicrobial efficacy of Chlorhexidine 2% and Triphala against Enterococcus faecalis when used as root canal irrigants.

Materials and methodology

This in-vitro study was conducted in the Department of Conservative dentistry and Endodontics, Gian Sagar Dental College and Hospital, Ramnagar in collaboration with Department of Microbiology, Gian Sagar Medical College and Hospital, Ramnagar. The single rooted were included whereas teeth with carious lesion, root resorption, root cracks, endodontically treated teeth, calcified root canals were excluded from this in-vitro study. Twenty freshly extracted permanent single rooted teeth (Figure 1) were collected on the basis of inclusion and exclusion criteria from the Department of Oral and Maxillofacial Surgery of Gian Sagar Dental College and Hospital, Ramnagar, Rajpura and were stored in normal saline.

Figure 1: Twenty selected samples

Teeth were decoronated perpendicular to the long axis of tooth using diamond disc, straight handpiece and micromotor, under continuous water spray to obtain 14 mm of root length. Working length of the tooth was taken using 10 no. K-file (Mani) by pushing the file beyond root apex as shown and then keeping it 1 mm short of anatomic apex. Once the working length was finalized then root canal was prepared using standard protocol and master apical file (MAF) was kept at 50 no. K-file. Irrigation during preparation was performed using distilled water (Figure 2). After root canal preparation, the enlarged apical foramen was sealed from outside with cyanoacrylate to prevent bacterial leakage. To make both handling and identification easier, teeth were mounted vertically in plaster blocks and sterilized in an autoclave for 20 minutes at 121^0C.

Figure 2: Irrigation using distilled water

Pure culture of *Enterococcus faecalis* (ATCC 29212) was procured and was grown on Mac conkey's agar plates for 24 hours at 37^0C. A suspension of *E. faecalis* was then prepared in sterile normal saline and its turbidity was adjusted to 0.5 Mc Farland standard. The root canals were then inoculated with 10-20 uL of prepared *Enterococcus faecalis* suspension using sterile micropipette. The samples were then placed in sterile stainless steel box and placed in incubator for 24 hours at 37^0C. After incubation, they were divided into two experimental groups randomly having 10 samples each according to the irrigation solutions Group I (2% Chlorhexidine) and Group II (Triphala) (Figure 3).

Figure 3: 2% Chlorhexidine and Triphala

The samples were irrigated by respective irrigants using sterile plastic syringe and endodontic irrigating needle (26 gauge side-vent, Amdent). 2 ml of irrigant was delivered which was left in the canal for 5 minutes. Then 1 ml of saline was used as final rinse to wash the canals. The remaining suspension left in the canals was aspirated with the help of syringe and then transferred into the test tubes containing 5 ml of Brain Heart Infusion broth. These tubes were then incubated at 37^0C for 4 days. These tubes were then incubated at 37^0C for 4 days.

All the samples in respective groups which were incubated and were observed following two criteria: Presence of Turbidity and Colony forming units/ml (CFU/ml). The occurrence of the broth turbidity was indicative of bacteria remaining in the root canal. Group I none of samples had shown turbidity (Figure 4). In Group II all samples were turbid (Figure 5). Then it was grown on Mac Conkey's agar for 24 hours at 37^0C (Figure 6) to count the Colony forming units/ml.

Figure 4: Out of 10 samples (Group I) none of the sample had shown turbidity

Figure 5: Out of 10 samples (Group II) all samples had shown turbidity

Figure 6: Turbid samples were grown on Mac Conkey Agar plates

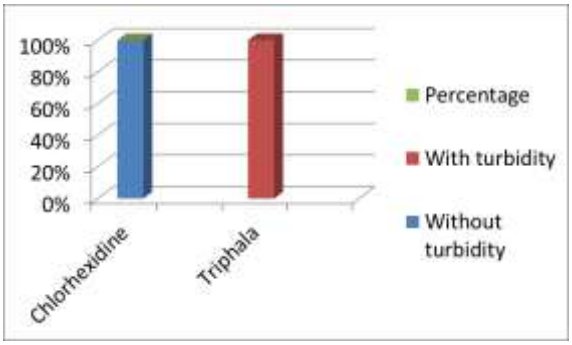
Results

The number of samples in which turbidity was seen in each groups are described in Table 1. The number and percentage of samples which showed turbidity are presented graphically in Graph 1. The colony forming units in Group II was more than 10^5/ml (Graph 2).

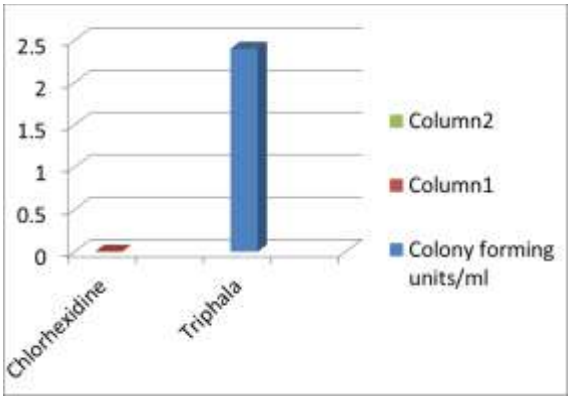
Table 1: Number of turbid samples in each group

Group	Total samples	Turbidity present
Group I (2% Chlorhexidine)	10	0
Group II (Triphala)	10	10

Graph 1: Graphical comparison on the basis of presence of turbidity between two groups.



Graph 2: Graphical comparison on the basis of CFU/ml between two groups



Intergroup comparison was done on the basis of presence of turbidity using Chi-square test (Table 2) and it was concluded that the results were statistically significant.

Table 2- Intergroup comparison on the presence of turbidity among study groups using Chi-square test (SSPS 22.0)

Group compared	Comparison group	Chi-square value	P-value	Significance
Group I (2%Chlorhexidine)	Group II (Triphala)	30.0	<0.001	Significant

Turbid samples were cultured to count the colony forming (CFU) units/ml. intergroup comparison was done on the basis of Colony forming unit/ml (CFU/ml) (Table 3) using Anova test and the results were statistically significant.

Table 3- Intergroup comparison on the Colony forming units/ml among study groups using Turkey test

Group compared	Comparison group	Mean diff	Std. Error	P-value	Significance
Group I (Chlorhexidine)	Group II (Triphala)	-2.40	0.30	<0.001	Significant

Discussion

For the proper eradication of bacteria from the root canals, we require effective chemo-mechanical instrumentation of canal which can be improved by supplementation with suitable irrigants and medicated fluids to wash out a cavity inside the body. The effectiveness of irrigation, defined as removal of debris and elimination of bacteria, depends on the diameter of the root canal, the penetration depth of the irrigating needle, irrigation pressure and the properties of the solution.[5,10] Out of all persistent endodontic microflora, *E. faecalis* has been repeatedly identified as the species most commonly recovered from root canals of teeth with failed root canal treatment and persistent root canal infections.[11] The prevalence of *E. faecalis* in primary endodontic infection is 40% and in persistent endodontic infection is 24-77%.[12] 2% Chlorhexidine is a potent antimicrobial agent which is quite effective in eliminating *E. faecalis* from canal space and dentinal tubules (upto 100-300 um depth).[13] Chlorhexidine is also available in 0.2% concentration, but at this concentration it has bacteriostatic action only.[5] The major limitation of Chlorhexidine is that it is unable to dissolve the organic matter present in the root canals.[4,14] The efficacy of Chlorhexidine against *E. faecalis* has already been well established by Oncag et al (2003) and Bhardwaj et al (2012) and proved to be good antimicrobial agent.[15,16] According to Ma J et al (2015), Chlorhexidine was found to be more effective in improving the antibacterial activities against *E. faecalis*.[17] Another in-vitro study conducted by Oliveria et al (2007) demonstrated that 2% Chlorhexidine gel and 5.25% NaOCl were effective in eliminating *E. faecalis*.[18,19,20]

As the global scenario is now changing towards the use of plant and plant products, herbs like neem, triphala, propolis, tea tree oil, aloe-vera, ginger, garlic, turmeric, morinda citrifolia and green tea all are being tested as potential root canal irrigants because they possess antibacterial and antifungal properties.[7,21,22]

Triphala is a powder that consists of equal parts of *Emblica officinalis, Terminalia chebula,* and *Terminalia belerica*.[23,24,25] In dentistry, it has been used because of their antimicrobial, antiplaque, antigingivitis, anticariogenic and anti-collagenase properties.[26-30] Shakouie *et al* compared the antimicrobial efficacy of triphala with various concentrations of NaOCl against *E. faecalis* and reported that triphala exhibited better antimicrobial activity against *E. faecalis* when compared to 0.5 and 1% NaOCl. Triphala's fruit is rich in citric acid which helps in removing the smear layer. Its chelating property makes it an effective alternative to sodium hypochlorite for root canal irrigation.[31] In an in vitro study conducted by J. Prabhakar et al, Triphala and Green tea polyphenols were found to have significant anti-microbial activity against E. faecalis biofilm formed in tooth substrate.[32] In another study by Madhu Pujar et al, antimicrobial efficiency of triphala, green tea polyphenols and 3% sodium hypochlorite were compared against and E. faecalis and it was observed that triphala and green tea polyphenols showed significantly better antibacterial activity against 2 week biofilm.[33]

According to present study, Group I (2% Chlorhexidine) was significantly better than Group II (Triphala) when used against *E. faecalis* as root canal irrigant.

Conclusion

The present study was conducted to evaluate the anti-microbial efficacy of 2% Chlorhexidine and Triphala against *E. faecalis* when used as root canal irrigants. After the completion of study, results were calculated on basis of turbidity and Colony forming units/ml and it was concluded that 2% Chlorhexidine was better than Triphala when used against *E. faecalis* as a root canal irrigant due to its effective bactericidal property.

However small sample size and in-vitro model of this study form its limitations. Therefore, clinical evaluation using larger sample size is perhaps required to draw more definite conclusions. Since triphala is not commercially available for use in endodontics, future research lies in manufacturing these herbal irrigants for commercial use, so as to attain standards and the ready availability of these herbal alternatives for the speciality of endodontics.

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