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ORIGINAL ARTICLE

MICROBIOLOGICAL EVALUATION OF HERBAL AND NON HERBAL IRRIGATING SOLUTIONS ON ENTEROCOCCUS FAECALIS: AN IN-VITRO STUDY

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ABSTRACT:
Aim and Objectives: This study was designed to evaluate the antimicrobial efficacy of Triphala; 5.25 % sodium hypochlorite, 2 %chlorhexidine (CHX) gluconate intracanal irrigating solutions against Enterococcus faecalis. **Materials and Methods:** Agar plates were prepared using brain heart infusion (BHI) agar. Cultures of E. faecalis were grown in BHI broth at 37°C.Results were derived from agar well diffusion method. Plates were inoculated for 24h at 37°C and microbial zones of inhibition were recorded. **Results**: one way ANOVA test was done ,sodium hypochlorite showed highest inhibition zone , triphala and CHX gluconate showed statistically significant result(P<0.05). **Conclusion:** Triphala can be used as an irrigating solutions in endodontic failure cases.
Keywords: Triphala, 5.25 % Sodium hypochlorite ,2 % Chlorhexidine gluconate gel, Enterococcus faecalis.

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INTRODUCTION

Bacteria and their products play the foremost role in the instigation and exacerbation of pulp and periapical diseases. Miller introduced the role of bacteria in the pathogenesis of pulp diseases for the first time in 1894. [1] Hence, the main principle of root canal therapy is to eliminate microorganisms and their by-products from the root canal system as well as to prevent failure through re-infection. It has been stated that the most apparent cause of the failure in root canal treatment is the persistent of microorganisms in the apical portion of the canal even after complete biomechanical preparation . Facultative bacteria like Enterococcus faecalis is the most commonly isolated species from the root canals of the teeth with failed endodontic treatment[2] and its prevalence in such infections ranges from 24% to 77%.[3] It can survive harsh environments like extreme alkaline pH (9.6) and a temperature of 60°C for 30 min.[4] It possesses certain virulence factors such as lytic enzymes, cytolysin, pheromones, and lipotechoic acid[5] suppresses the action of lymphocytes, potentially contributing to endodontic failure.[6] It is very small to invade and live within dentinal tubules and can endure prolonged periods of starvation.[3] When nutritional supply becomes available it can utilize serum as a nutritional source that originates from alveolar bone and periodontal ligament.[7] E. faecalis passively maintains pH homeostasis by a proton pump. It has been shown to synthesize a variety of stress proteins when exposed to adverse environmental conditions.[8]

Chemomechanical preparation helps to remove the bacteria from the canal walls, remove debris and can lubricate the canals. In particular, the irrigation procedure is also a crucial step in endodontic treatment. Most commonly used irrigating solutions are Sodium hypochlorite in various concentration, Chlorhexidine gluconate, EDTA ,Citric acid and Saline . However, Sodium hypochlorite is frequently used as irrigating for non-surgical endodontic procedures but it has high toxicity, unpleasant taste, causes corrosion of instruments, and is unable to remove the inorganic portion of smear layer and reduction in elastic modulus and flexural strength of dentin.[9]

Chlorhexidine is another most commonly used irrigant but it does not dissolve organic substrate and necrotic tissue from the root canal system [10] . It also causes cytotoxicity owing to all this harmful effects of most commonly used irrigating solutions, Herbal plant extracts can be used. In endodontics, recent trend attends to use

biologic medication extracted from natural plants which are least toxic as compared to chemical irrigating solutions .Many studies have stated that Herbal drugs such as triphala ,curcuma longa , azadirachta indica etc can be used as root canal irrigants . Triphala which is basically a mixture of three herbs such as Terminalia beleria, Terminalia chebula and Embilica officinalis with tannic acid as its principal constituent .Tannic acid has both bacteriostatic and bacteriocidal effect on microorganism,[11] as well as it has anti-inflammatory and antioxidative property .Hence the purpose of the study was to evaluate the antimicrobial efficacy of chemical and herbal can also be used as irrigating solutions.

Methodology

This study was conducted at Department of Conservative and Endodontics,Sharad Pawar Dental College and Department of Microbiology ,Jawaharlal Nehru Medical College, Sawangi.

Materials used al used MMMMM	Manufacturing company
E. faecalis stains (ATCC 29212)	(Himedia Laboratories, Mumbai, India)
BHI Broth	(Himedia Laboratories, Mumbai, India)
2 % Chlorexidine	Gluco –CheX,P.P.H. Cerkamed
5.25% Sodium hypochlorite	Prime dental products pvt ltd
Dimethyl sulfoxide	DMSO (SD Fine chemicals, Chennai, India
Triphala	Naturally available

METHOD

Twenty agar plates were prepared by using Brain Heart Infusion (BHI) in 90mm diameter Petri dishes. These petri dishes were incubated at 37 ˚C for overnight before use to verify that they had remain sterile. A suspension of pure culture of E. faecalis –American type culture collection(ATCC) 29212 was prepared by adding colonies from pure culture of E.faecalis to freshly prepared BHI broth and incubated for overnight.To adjust the turbidity of broth 0.5 McFarland standard, was used as a reference . The antimicrobial sensitivity was performed by agar well diffusion method. Twenty BHI agar plates with single well having the diameter of 6 mm and dept 4mm were prepared .These plates were inoculated with BHI Broth culture of E. faecalis with sterile cotton swabs to provide an even lawn culture. 10 ml of each test solution 5.25 % sodium hypochlorite ,2.5%chlorhexidine ,triphala, curcuma longa solutions respectively and normal saline was used as control in one of the well. BHI agar plates were marked on the bottom of the plate to identify the irrigating solution. Twenty BHI agar plates were incubated at 37˚C for 24hrs. After 24hrs, microbial zones of inhibition were measured across the diameter in millimeters (mm) with a pair of Vernier Calipers.

RESULTS

Enterococcus faecalis was inoculated on agar and the antibiotic disc were placed. After incubation, inhibition zones were measured. Table 1 depicts the readings of inhibition zones for different irrigating solutions tested in the present study.

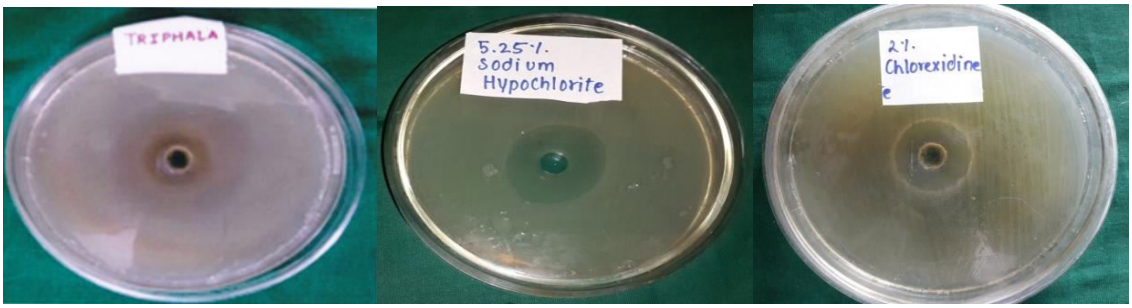

Figure 1: Inhibition zones for different irrigating solutions

Table 1: Inhibition zones (in mm) for different irrigating solutions

	Triphala	Sodium hypochlorite	Chlorhexidine
Inhibition zones in mm	16.0	24.0	21.0
	18.0	24.0	22.0
	21.0	25.0	22.0
	18.0	25.0	23.0
	17.0	25.0	21.0

Table 2: Mean and median inhibition zones for all the irrigating solutions along with their 95% CI

Irrigating solutions	Mean	Standard deviation	95% Confidence interval	Median	95% Confidence interval
Triphala	18.0	1.8	16.6-19.6	18.0	16.0-21.0
Sodium hypochlorite	24.6	0.5	24.2-25.0	25.0	24.0-25.0
Chlorhexidine	21.8	0.8	21.2-22.4	22.0	21.0-23.0

Table 3: One-way ANOVA for table 2

Source	Sum of squares	Degrees of freedom	Mean square	F statistic	p-value
Irrigating solutions	750.0000	3	250.0000	36.6300	<0.001
Error	109.2000	16	6.8250		
Total	859.2000	19			

Table 2 depicts the descriptive statistics of the above readings. Among the four antibiotics, Sodium hypochlorite and Chlorhexidine had larger mean inhibition zones (24.2-25.0 mm and 21.2-22.4 mm) respectively followed by in Triphala (16.6-19.6 mm). Turmeric had least inhibition zone among all the antibiotics. The median values do not differ significantly from mean values and hence parametric tests can be used. Inhibition zone of Sodium hypochlorite was significantly larger than that of Triphala ($p<0.01$). But there was no significant difference between inhibition zones of Sodium hypochlorite and Chlorhexidine ($p>0.05$).Inhibition zone of Triphala was not significantly different than that of Chlorhexiidine ($p>0.05$).

STATISTICAL ANALYSIS

Data was entered in Microsoft Excel and analysed using SPSS 18.0. Data was described using mean (standard deviation) and median and their 95% Confidence Intervals. 95% Confidence Intervals were obtained by using bootstrapping technique as sample size was very small. As mean and median values were almost similar, parametric test was used. One-way ANOVA was used to test null hypothesis that whether mean inhibition zones of antibiotics differed significantly. Tukey's post-hoc test was used to find out which pairs differed significantly from one another contributing to significance of ANOVA. P-value <0.05 was used as level of significance.

DISCUSSION

Long-term conventional success of root canal treatment depends on complete removal of causative microorganisms from complex root canal anatomical areas. An attempt was made to select representative bacteria that have been classically isolated from necrotic canals. E. faecalis was preferred as a test organism because it is a facultative Gram-positive organism that rapidly colonizes dentinal tubules. It has been extensively used in endodontic research because it has been found to be evident in 63% of teeth with endodontic failures.[12] Virulence factor of E. faecalis in failed endodontically treated teeth can be related to the ability of E. faecalis to invade dentinal tubules and adhere to collagen in presence of human serum.[13] E. faecalis remains to be the most recurrently identified species in canals of root filled teeth with periapical lesions as established by different molecular methods from time to time.[14-18] This may be due to its ability to endure the effects of a wide range of antimicrobial solutions and intracanal medicaments used during endodontic treatment procedures. It also endures drawn out periods of nutritional deprivation. The root canal is hardly a nutrient rich medium, but E. faecalis may stay alive on serum components from the dentinal fluid. Therefore, even in a well debrided and coronally well-sealed root canal, remaining or surviving cells of E. faecalis may still grow and utilize local sources of energy and nutrients.[19]

In this study, anti-microbial activity of various root canal irrigating solutions were compared to eliminate endodontic pathogen responsible for root canal failure. This study was a reserved attempt to evaluate and compare the antimicrobial efficacy of 5.25%sodium hypochlorite , 2% chlorhexidine and triphala extract solution on E. faecalis.

Sodium hypochlorite has long been used as a root canal irrigator because of its enormous antimicrobial activity[20] Sodium hypochlorite (NaOCl) has been universally used as a root canal irrigant. However, this irrigant has various side effects such as unpleasant taste and odor, tissue toxicity, inability to remove smear layer, inability to fully eradicate microbes from the infected canals, allergic potential, risk of emphysema on overfilling, and discoloration of clothes.[21]In this study, sodium hypochlorite showed the largest zone of inhibition, which may be because of its volatile nature and high diffusibility on agar plate.

In current study 2% CHX also showed the consistent antimicrobial activity against E .fecalis ,CHX is active against a wide range of micro-organisms, such as Gram-positive and Gram-negative bacteria, bacterial spores, lipophilic virus, yeast being bacteriostatic at lower concentrations and bactericidal at high concentration[22]. It shows substantial antimicrobial effect against common endodontic pathogens like S. aureus, Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, E. faecalis, C. albicans and S. mutans Lessa FC, Nogueira et al , Vianna ME et al, Dammaschke T et al. [23-25]The antimicrobial effect is because of reaction of CHX molecule with negatively charged groups on the cell surface, causing an irreversible loss of cytoplasmic constituents, membrane damage and enzyme inhibition. At higher concentrations CHX results in extensive cell damage, coagulation of cytoplasm and precipitation of proteins and nucleic acids.[26]

Due to potential side effects, safety concerns, and ineffectiveness of conventional endodontic irrigants, we are now in quest of herbal alternatives.

Triphala [(tri) 'three' (phala) 'fruits'] is a traditional Ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants, Terminalia chebula, Terminalia bellirica, and Phyllanthus emblica, also known as the 'three myrobalans'. "Triphala" has good antioxidant property & could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and

phenolic compounds[27]. It exhibits anti-viral, anti-bacterial, antifungal and anti-allergic properties. [28]

CONCLUSION

Although the present study has shown 5.25 % Sodium hypochlorite to be the most effective antibacterial agent, concern over the possible cytotoxic effects has shifted the focus onto triphala extract. This study confirms and supports the use of triphala for antimicrobial treatment of root canal infection.apart from this study ,triphala has shown more antimicrobial efficacy than 2.5 %sodium hypochlorite.

LIMITATIONS

This is a vitro study therefore it is possible that the interferences from the study might not co-relate completely with similar situation clinically. Even though critical care to be taken at every step, human error cannot be ruled out from the final result.

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