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An In Vitro Evaluation of Antibacterial Efficiency of Triphala, Green Tea Polyphenols, Morinda Citrifolia, Qmix 2 In 1, 5%Sodium Hypochlorite and 2%Chlorhexidine Gluconate Solution Against Enterococcus Faecalis

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ABSTRACT

Aim: To evaluate the antibacterial efficiency of Triphala, Green tea polyphenols, Morinda citrifolia, QMix 2 in 1, 5%Sodium hypochlorite and 2%Chlorhexidine gluconate solution against Enterococcus faecalis.

Materials and method: 140 extracted human permanent mandibular single rooted premolar teeth were collected. All the teeth were decoronated and biomechanical preparation was completed. Root canal spaces of all teeth were infected with pure culture of E. faecalis bacteria and were randomly divided into seven groups with 20 teeth per each group. Group A:Triphala, Group B:Green Tea Polyphenols, Group C:Morinda citrifolia, Group D:Qmix 2 in 1, Group E:2%Chlorhexidine gluconate solution, Group F: 5%Sodium hypochlorite, Group G: Normal saline were used as root canal irrigants. 2ml of each irrigant with the time of contact of 5 minutes per root canal was used as per their respective groups. Dry sterile paper points were used for culture tests from all the specimens and the number of CFU were counted.

Results: One-way analysis of varience(ANOVA) showed significant difference amoung the seven groups ($P<0.001$). Tukey's post hoc test was done for inter-group comparison and it showed no significant difference between three root canal irrigants ($P>0.05$).

Conclusion: QMix 2in1 and triphala showed superior antibacterial efficiency as root canal irrigants on E. faecalis.

Keywords: Enterococcus faecalis, QMix 2 in 1, Triphala, Antibacterial efficiency, Colony forming units.

INTRODUCTION

Endodontic infections are polymicrobial in nature, Enterococcus faecalis is a facultative anaerobic gram positive bacteria often associated with 22%-27% of failed root canal treatment cases. E. faecalis

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can survive in root canals as a single organism or as a major component of the microbial flora even in harsh conditionss due to its physicochemical properties helping it to modify as per the prevailing nutritional deficiences, high salt concentration and high alkaline environment, thus making them the most resistant species to antimicrobial agents[1] and the success of endodontic treatment vastly depends upon the use of root canal irrigants, but anatomical complexities, microbiological factors often pose serious threat. So, it's a prerequiste to use an ideal root canal irrigant in addition to mechanical preparation of the root canal in order to ensure complete disinfection of the root canal space and to attain success of endodontic therapy.[2]

QMix 2 in 1 [Dentsply Tulsa, Dental Specialities, Johnson City, USA] is a newly introduced root canal irrigant. It contains a mixture of Bisbiguanide antimicrobial agent [2% chlorhexidine], 17%EDTA [EthyeneDiamineTetraAceticacid], Surfactant [N-cetyle-NNN trimethyl ammonium bromide] and distilled water.[2] Sodium hypochlorite(NaOCl) is one of the most widely used root canal irrigant because of its ability to destroy broad spectrum of microorganisms but it has some undesirable characterstics such as high tissue toxicity, allergic potential, disagreeable taste and inability to remove the smear layer.[3] 2% Chlorhexidine gluconate solution(CHX) is another commonly used root canal irrigant, because of its bacteriocidal action, substantivity and low tissue toxicity. However, it is incapable of pulp tissue dissolution and its inability to remove smear layer.[3,4] The constant increase in the antibiotic resistant strains of bacteria and side effects caused by the synthetic chemical root canal irrigants has promted for the use of herbal root canal irrigants in endodontic practice.[5]

Triphala is a plant-derived product and its powder is a combination of three dried plants naming Terminaliabellerica, Terminaliachebula and Emblicaafficinalia with tannic acid being its principal constituent. The advantages of triphala includes; its easy access, low cost, long-term substantivity, less toxicity and absence of microbial resistance.[6] Morinda Citrifolia is commercially known as Noni or Indian mulberry and its juice has broad range of therapeutic effects including antibacterial, anti-inflammatory properties. Green tea polyphenols is a safe herbal product with anti-inflammatory, antioxidant and wide spectrum of antimicrobial properties.[7]

The purpose of this in-vitro study was to evaluate the antibacterial efficiency of Triphala, Green tea polyphenols, Morinda citrifolia, QMix 2 in 1, 5% Sodium hypochlorite and 2% Chlorhexidine gluconate solution against Enterococcus faecalis.

MATERIALS AND METHOD

140 freshly extracted human permanent mandibular single rooted premolar teeth were collected from the Department of Oral and Maxillofacial surgery, Triveni institute of dental sciences, hospital and research center. Bodri, Bilaspur, Chhattisgarh.

Inclusion criteria: Non-carious, non-fractured, matured with closed root apices, non-restored, single rooted teeth with Vertucci class-I root canal configuration.

Exclusion criteria: Carious, fractured, restored, multirooted, open apex, previously endodontically treated teeth.

All the specimens were cleaned off superficial debris, calculus, tissue tags and stored in 0.1% thymol at room temperature until used. All the teeth were decoronated at cementoenamel junction with a diamond disc to obtain a standardized root length of approximately 12 mm for uniform specimens. Pulp tissue was extirpated from all the teeth and working length was determined 1 mm short of the apical foramen. Biomechanical preparation was done in all specimens in crown-down technique using Protaper hand files (Dentsply Maillefer, Switzerland) and the canals were enlarged to an apical size of No.F3 file, 17% EDTA and distilled water were used as root canal irrigants. The specimens were then autoclaved at 121°C to ensure no microbial contamination. Enterococcus faecalis (ATCC-American Type Culture Collection) 29212, Kwik Stik, Microbiologics, France) [Figure 1] was used as the test strain. It was grown in brain heart infusion broth (HiMedia Lab Pvt.Ltd, India) and the bacterial suspension was adjusted to match 0.5 McFarland units 1.5×10^8 CFU/ml and the purity of the culture was confirmed by Gram stain and colony morphology.

Fig 1: Enterococcus faecalis bacteria (ATCC 29212).

An innoculum of 10 µlitre culture suspension of E. faecalis was suspended into the prepared root canal space of all the specimens using a sterile micropipette inside a laminar hood to prevent any air borne contamination and the specimens were incubated anaerobically at 37°C for 48 hours. All the specimens were then randomly divided into seven groups with 20 teeth per each group. Group A: Triphala(Sri Jain Ayurvedic Pharmacy, India)[Figure 2], Group B: Green Tea Polyphenols)(GTP)(Life extension, Quality Supplements extract Inc. USA), Group C: Morinda citrifolia(BioTech Pvt Ltd, India), Group D: Qmix 2 in 1(Dentsply, Tulsa Dental Specialties, USA)[Figure 3], Group E: 2% Chlorhexidine gluconate solution(CHX) (Asep-Rc, Anabond Stedman Pharma Pvt Ltd, India), Group F: 5% Sodium hypochlorite (NaOCl)(LabChem Pvt Ltd, India), Group G: Normal saline (Helios Pharmaceuticals Pvt Ltd, India) were used as root canal irrigants.

Fig 2: Triphala.

Fig 3: QMix 2 in 1.

Preparation of Green tea polyphenols and Triphala for root canal irrigation: 2.4 grams of each powder was taken into two separate glass beakers and was mixed with 40 ml of 10% DiMethylSulfOxide(DMSO)(HiMedia Lab Pvt.Ltd. India) solvent, stirred for 2 minutes and filtered through a Whatman no.1 filter paper and the strained solutions were collected and used as test root canal irrigants. Other root canal irrigants; QMix 2 in 1 solution, Morinda citrifolia, 5% sodium hypochlorite, 2% chlorhexidine gluconate solution and normal saline were readily available in liquid form.

Fig 4: Green Tea Polyphenols.

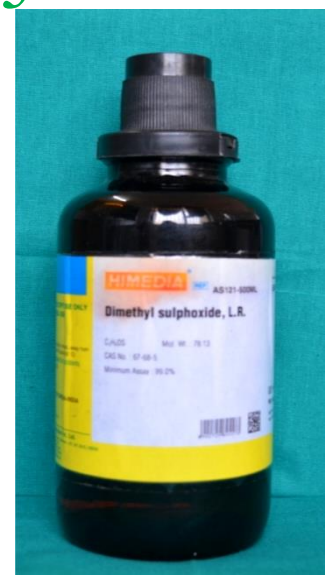


Fig 5: DiMethylSulfOxide(DMSO).

2ml of test root canal irrigant was placed in the root canal space of each specimen as per their respective groups using a 30 gauge ProRinse endoirrigation needles (Dentsply International Inc, Tulsa,OK) placed 1-2mm short of the working length determined. The time of contact of each irrigant in the root canal was 5 minutes and 3ml of distilled water was used to flush each root canal to prevent the potential carry-over effect of the tested root canal irrigants. The excess moisture from the root canals was removed using sterile paper points (DiaDent Group International, Korea). A dry sterile paper point of ISO No.30 was placed into the root canal space of all the specimens to the full working length, left for 10 seconds and the paper points were then transferred into the sterile test tubes containing 2ml of PBS (Phosphate Buffered Saline) and vortexed for 30 seconds. Using a nicrome wire inoculation loop, bacterial suspensions from each test tube samples were placed in 5% sheep blood agar plates (HiMedia Laboratories, Pvt. Ltd, Nashik, India). The agar plates were incubated anaerobically at 37^0C for 48 hours. The entire procedure was carried out in strict aseptic conditions. The cultured plates were then evaluated to confirm the growth of E. faecalis bacteria and the Colony Forming Units (CFU) were counted using Digital colony counter (SECOR, Scientific Engineering Corp, India) and the data was collected and tabulated.

RESULTS

The tabulated data was statistically analysed with computer software; SPSS (Statistical Package for Social Sciences) Version 16 using One-way analysis of varience (ANOVA) and Tukey's post hoc test. One-way ANOVA shows significant difference in the number of E. faecalis Colony Forming Units (CFU) amoung the seven groups, Minimal growth of bacterial CFU (mean 30.00) were seen in Group D and maximum growth of bacterial CFU (mean of 92.05 and 125.00) were seen in Group B and in Group: G (Positive control group) respectively. The P-value was < 0.01 [Table I]. To find exactly which group differs with the other, Tukey's post hoc test was done for inter-group comparison of antibacterial efficiency between the seven groups and no significant difference was observed in E. faecalis CFU in the inter-group comparison of triphala, 2% chlorhexidine gluconate solution and 5% sodium hypochlorite root canal irrigants and the P-value was >0.01 [Table II]. The mean wise comparison of bacterial colony forming units in seven groups was shown in Graph I.

Table I: One-way analysis of variance (ANOVA)

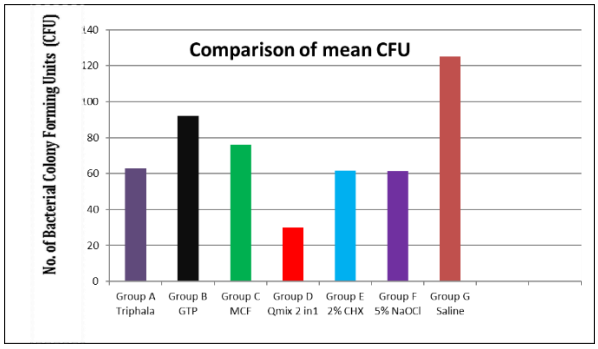
Groups	N	E. faecalis bacterial CFU (Mean ± SD)		Significant Groups at 5% level	Non-significant Groups at 5% level
Group A	20	62.95±1.15	P value < 0.001HS	A *vs* B, C, D, G	A *vs* E, F
Group B	20	92.05±2.31		B *vs* A, C, D, E, F, G	
Group C	20	75.95±1.15		C *vs* A, B, D, E, F, G	
Group D	20	30.00±2.92		D *vs* A, B, C, E, F, G	
Group E	20	61.65±1.57		E *vs* B, C, D, G	E *vs* A, F

Group F	20	61.15±3.08		F *vs* ,B, C, D, G	F *vs* A, E
Group G	20	125.00±2.27		G *vs* A, B, C, D, E, F	

P: Probability HS: Highly Significant SD: Standard Deviation.

Table II: Tukey's post hoc test.

Inter-Group comparison	Group B	Group C	Group D	Group E	Group F	Group G
Group A	P<0.01	P<0.01	P<0.01	**P > 0.05**	**P > 0.05**	P<0.01
Group B		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Group C			P<0.01	P<0.01	P<0.01	P<0.01
Group D				P<0.01	P<0.01	P<0.01
Group E					**P > 0.01**	P<0.01
Group F						P<0.01



Graph I: Vertical Bar Graph showing Mean wise comparison of bacterial CFU.

DISCUSSION

Elimination of microbes from the infected root canals and prevention of re-infection is one of the fundamental aims of endodontic therapy. It is established that pulpal and periapical diseases and failure of endodontic treatment are due to the persistance of microbial flora in the root canal system.[8] In the present invitro study, the prepared root canals were artificially infected with E. faecalis bacteria. E. faecalis was selected because it is believed to be the most common intracanal microorganism which is often associated with failed root canal treatment and was found to be resistant against commonly used root canal irrigants.[8] Thus, a root canal irrigant which is effective against E. faecalis is highly desirable. An ideal root canal irrigant should be able to disinfect the entire root canal space devoid of microbial flora in less time period.

QMix 2 in 1 is a clear solution, ready to use with no chair-side mixing. Mixing of EDTA and Chlorhexidine is known to produce a white precipitate, but in QMix 2 in 1 solution, this is avoided because of its chemical design.[9] In the present study, QMix 2 in 1 solution showed superior antibacterial efficiency on E. faecalis compared to all the other root canal irrigants used and this was in accordance with the studies of Jerin Jose et al,[2] and Veeramachaneni C et al[10] reported superior antibacterial activity of QMix 2 in 1on E. faecalis compared to 2% CHX solution and 5% NaOCl.

Advantages of QMix 2 in 1 solution is attributed to effective functioning of its various individual constituents. Surface-active agent (surfactant) in the solution decreases its surface tension and thereby increasing its wetability which helps for better penetration of solution into the root canal dentin and its good antibacterial activity. The potential benefit of bisbiguanide in QMix is in the prevention of microbial colonization on root canal dentine surface. Calcium chelating agent(17%EDTA) in QMix causes cell wall damage in bacteria by chelating and removing divalent cations (Mg +2 and Ca +2) from bacterial cell membrane increasing the root canal dentin permeability by effectively removing the smear layer.[10,11]

Herbal medicinal products have been used as root canal irrigants and are becoming increasingly popular due to its high antibacterial efficiency,

biocompatibility, antioxidant properties, low-cost, easy availability, lack of microbial resistance, low tissue toxicity and the current worldwide 'back to nature' trend.[12] According to WHO(World Health Organization), Herbal medicine is defined as the plant derived materials or preparations which contains raw or processed ingredients from one or more plants with therapeutic values.[7]

The advantages of triphala includes its easy access, low cost, long-term substantivity, less tissue toxicity and absence of microbial resistance.[13] In the present study, 10% Dimethyl SulfOxide (DMSO) was used as a solvent for Triphala and Green tea polyphenols, although they are readily soluble in normal water. DMSO is a clean, safe, highly polar, aprotic solvent that helps in bringing out the pure properties of all the components of a herbal product being dissolved. Antibacterial inertness of 10% DMSO was confirmed with the disc diffusion test.[5]

In the present study, Triphala[three(tri) fruits(phala)] showed higher antibacterial efficiency with no significant difference in comparison to 5%NaOCl and 2%CHX solution on E. faecalis and this was in accordance with previous studies of Paridhi Garg et al[1] and Prabhakar et al[5] reported the superior antibacterial efficacy of triphala with 80-100% killing of E. faecalis in 5 minutes and it is attributed to its formulation of three medicinal plants in equal proportions resulting in synergistic or additive positive effect.

Green tea polyphenols is available in powder form and it is prepared from the young shoots of tea plant Camellia sinensis. It is made by drying and crushing of fresh leaves of the plant and is produced without passing through the stage of oxidation, fermentation. It contains highest concentration of polyphenols in the form of catechin compounds like: Catechin, Epicatechin, Epigallocatechin and EpiGalloCatechin Gallate(EGCG) and EGCG is found to be the most active component in green tea.[14]In our study, Green tea polyphenols showed least antibacterial efficiency. Abdulkareem J.Al-Azzawi[15] concluded, least antibacterial efficiency of green tea polyphenols against E. faecalis compared to 5% NaOCl, 2% Chlorhexidine solution. Morinda citrifolia is a herbal product available in liquid form and it is indigenous to tropical countries. Antibacterial properties of morinda citrifolia is attributed to the presence of L-asperuloside, alizarin, scopoletin, aucubin.[16] In the present study, Morinda citrifolia showed minimal antibacterial efficiency against E. faecalis compared to triphala and other chemical root canal irrigants used, it can be due to the use of pure extracts of morinda citrifolia which degraded over a period of time and lowered its antibacterial action and this was in accordance with a previous study of Prabhakar AR et al[17] reported the degradation of pure extracts of morinda citrifolia over a period of time leading to decline in its antibacterial efficacy, but in contrary Morinda citrifolia, 5% NaOCl and 2% Chlorhexidine gluconate solution showed similar antibacterial efficiency against E. faecalis and it was identified as a possible alternative to sodium hypochlorite as root canal irrigant.[16,18]

Sodium hypochlorite causes deleterious effect on root canal dentine reducing its elastic modulus and flexural strength.[19] 2% Chlorhexidine gluconate solution causes discolouration of tongue and teeth, burning sensation of oral mucosa and dysgeusia.[4,18] In our study the antibacterial efficiency of 5% sodium hypochlorite on E. faecalis was found to be comparatively similar to 2% chlorhexidine gluconate solution and triphala, but inferior to QMix 2 in 1 solution. Pavlovic et al[20] and Arslan et al[21] concluded no significant difference in the antibacterial efficiency between 5% sodium hypochlorite and 2% chlorhexidine gluconate solution on E. faecalis.

CONCLUSION

Under the limitations of the present study, QMix 2 in 1 solution showed maximum antibacterial efficiency against E. faecalis compared to other root canal irrigants used. However, triphala, 5% sodium hypochlorite and 2% chlorhexidine gluconate solution showed similar antibacterial efficiency on E. faecalis. The use of triphala as a safe alternative root canal irrigant can prove to be highly advantageous considering several deleterious effects of these commonly used chemical root canal irrigants. Further studies are required to prove the potent antibacterial efficiency of QMix 2 in 1 solution and Triphala as root canal irrigants against E. faecalis in clinical situations.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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