EVALUATION OF ANTIBACTERIAL EFFECTIVENESS OF VARIOUS CHEMICAL AND HERBAL ROOT CANAL IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS: AN IN-VITRO STUDY

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ABSTRACT
A study was conducted to evaluate the antibacterial efficiency of various chemical and herbal root canal irrigants against Enterococcus faecalis. One hundred forty extracted human mandibular premolar teeth were decoronated and biomechanical preparation was done in crown-down technique. 10µl culture suspension of E. faecalis (ATCC 29212) was placed into the prepared root canal space of all teeth. After 48 hours of incubation, all teeth were randomly divided into seven groups with 20 samples per each group. Group A (n=20): Triphala, Group B (n=20):Camellia sinensis (Green tea polyphenols), Group C (n=20): Biopure MTAD solution, Group D (n=20): QMix 2in1 solution, Group E (n=20): 2% Chlorhexidine gluconate solution, Group F (n=20): 5% Sodium hypochlorite, Group G (n=20): Normal saline (Positive control) were used as root canal irrigants. The time of contact of each irrigant in root canal space was 5 minutes and canals were then flushed with distilled water, excess moisture was removed using paper points. Dentinal shavings were harvested from the walls of root canal space in all samples using Gates-Glidden drills and were transferred into test tubes containing saline, were serially diluted and placed in 140 tryptic soy agar plates, incubated at 37°C for 48 hours. Colony forming units of E. faecalis were then counted using digital colony counter. Results of the present study showed statistically significant difference among the seven groups in One-way ANOVA test, as P-value was < 0.001. Tukey’s post hoc test showed inter-group comparison between Group A,
Group E and Group F were statistically non-significant, as P-value was > 0.05. Based on our study results, we would like to conclude that QMix 2 in1 solution showed maximum antibacterial efficiency on E. faecalis compared to other root canal irrigants. Triphala, 5% sodium hypochlorite and 2% chlorhexidine gluconate solutions exhibited higher and similar antibacterial efficiency against E. faecalis. Key words: Antibacterial activity, Colony forming units, E. faecalis.

INTRODUCTION

Enterococcus faecalis is a facultative anaerobic gram-positive cocci bacteria seen in 22-27% of failed root canal treatments due to persistent or secondary endodontic infections with recalcitrant periradicular lesions.1 E. faecalis can survive as a single organism or as a major component of the microbial flora adapting to the ecologically harsh conditions of the root canal space due to its physicochemical properties helping it to modify according to nutritional deficiencies, high salt concentration, extracellular superoxide production and extreme alkaline environment.2 Elimination of microbes from the infected root canal system and prevention of re-infection is one of the fundamental aims of endodontic therapy and failure of endodontic treatment is due to the persistence of microbial flora in the root canal system. Disinfection of root canal space is an important step for the success of root canal treatment.1 So, it’s a prerequisite to use an ideal root canal irrigant with the ability to dissolve vital and non-vital pulp tissues, potent antibacterial efficiency, removes smear layer, in addition to precise mechanical instrumentation of root canals to ensure complete disinfection of the root canal system.3,4

5%Sodium hypochlorite solution is the most commonly used root canal irrigant in endodontic treatment, because of its ability to eliminate broad spectrum of microbes, but has some undesirable characteristics such as unpleasant taste, foul odour and its inability to remove smear layer.5 2% Chlorhexidine gluconate solution shows bacteriocidal action, substantivity and low tissue toxicity, but it is incapable of pulp tissue dissolution, inability to remove smear layer, causes burning sensation of oral mucosa and dysgeusia.5

BioPureMTAD (Mixture tetracycline citric acid detergent) is a root canal irrigant introduced by Torabinejad M in 2003. It is available in powder-liquid system (single doses/multiple doses). Part A is liquid, supplied in syringes and it contains 4.25% Citric acid and 0.5% Polysorbate 80 detergent (Tween 80). Citric acid, a demineralising agent helps in the removal of smear layer. Tween 80, a nonionic surfactant is known to decrease the surface tension, thereby enhancing the flow and penetration of root canal irrigant deeper into the dentinal tubules. Part B is powder, supplied in bottles and it contains 3% Doxycyclinehyclate a broad-spectrum bacteriostatic antibiotic. Once the powder-liquid are mixed according to its manufacturer’s instructions, the final solution obtained was used as irrigant.6 QMix 2 in1 solution is a newer root canal irrigant introduced by Haapasalo M in 2011, contains a mixture of Bisbiguanide (2% Chlorhexidine), 17% EDTA (EthyleneDiamineTetraAceticacid), Surfactant (N-cetylen-NNN-trimethylammonium bromide) and distilled water. QMix 2 in1 is a clear solution, ready to use with no chair-side mixing, pH of the solution is slightly above neutral and is biologically safe.3,7
Due to constant rise in the resistant strains of microorganisms and adverse effects caused by chemical root canal irrigants, promoted for the use of herbal root canal irrigants in endodontic therapy. According to World Health Organization (WHO), Herbal medicine is defined as the plant derived materials or preparations which contain raw or processed ingredients from one or more plants with therapeutic values. Green Tea Polyphenols (GTP) is a herbal medicinal powder prepared from the young shoots of tea plant Camellia sinensis, Polyphenols are present in the form of Catechin, Epicatechin, Epigallocatechin and EpigalCatechinGallate (EGCG). EGCG was found to be the most active component in green tea. Triphala [Tri(three) phala(fruits)] is a herbal medicine. 1gm of triphala powder contains 0.333gms of Terminalia belerica (Bibhitaka), 0.333gms of Terminalia chebula (Haritaki) and 0.333gms of Emblica officinalis (Amalaki) in equal proportions and the major constituents of the formula include tannins, gallic acid, ellagic acid and chebulinic acid. Advantages of triphala include its low cost, easy availability, antibacterial, antioxidant, non-toxic, shows long-term substantivity and absence of microbial resistance. The aim of this in-vitro study was to evaluate antibacterial efficiency of QMix 2 in1 solution, Biopure MTAD, Triphala, Camellia sinensis (Green tea polyphenols), 5%Sodium hypochlorite and 2% Chlorhexidine gluconate solution as root canal irrigants against E. faecalis.

MATERIALS & METHOD

One hundred forty human mandibular premolars extracted for orthodontic purposes or for being periodontally compromised were collected after obtaining written informed consent from each patient. Mandibular premolars typically present with single root and a single root canal. Inclusion criteria: Non-carious, non-fractured, matured with closed root apices, non-restored, single rooted/single root canal teeth. Exclusion criteria: Carious, fractured, restored, multirooted, multicanaled, open root apex, defects within root portions, previously endodontically treated teeth. The collected teeth were cleaned off superficial debris, calculus, tissue tags and stored in 10% formalin at 37°C and were used within four weeks of extraction. All teeth were decoronated at cemento-enamel junction with a diamond disc (DFS, Germany) attached to slow speed micromotor handpiece (NSK, Nakanishi Inc, Japan) to obtain uniform samples of approximately 12mm in length. Necrotic pulp tissue was removed with barbed broach no.15 (DentsplyMalleifer, TulsaDental, Tulsa, USA). A no. 10 K(Kerr)-file (DentsplyMalleifer, Ballaigues, Switzerland) was passively placed into each root canal until its tip was just visible at the apex and the working length was established by subtracting 1mm from this length. Biomechanical preparation was done using Nickel-Titanium rotary ProTaper Universal files (DentsplyMalleifer, Ballaigues, Switzerland) in crown-down technique upto no. F3 file, 17% EDTA (Rc Help Prime Dental Products, Pvt Ltd. India) and distilled water were used for root canal irrigation. All teeth were then autoclaved at 121°C to ensure no microbial contamination. Enterococcus faecalis (ATCC[American Type Culture Collection] 29212, Kwik Stik, Microbiologics, France) was used as the test strain. Under strict aseptic conditions, E. faecalis was suspended in 5ml of tryptic soy broth (HiMedia Laboratories Pvt Ltd, India) and incubated...
anaerobically at 37°C for 48 hours. The growth of E. faecalis changes the turbidity of broth and the optical density was adjusted to match the turbidity equivalent to 0.5 McFarland units $1.5 \times 10^8$ CFU/ml and purity of the culture was further confirmed by sub-culturing 50µicrolitre broth in tryptic soy agar plates (HiMedia Laboratories Pvt. Ltd, India). 10µicrolitre culture suspension of E. faecalis was placed into the prepared root canal space of all samples using a sterile micropipette (Labtop, India) inside a laminar air flow cabinet (Bionics scientific technologies Pvt.Ltd, India) to prevent any airborne contamination and the samples were then placed in sterile test tubes, anaerobically incubated (Thermo Fisher scientific Pvt Ltd, India) at 37°C for 48 hours.

All samples were then irrigated with 3ml of distilled water to remove incubation broth and root canals were dried with sterile paper points (DiaDent Group International, Korea).

Samples classification:
All teeth were randomly divided into seven groups, with 20 teeth per each group depending on root canal irrigants used. Group A: Triphala (Zandu, Emami limited, Silvassa, Dadra and Nagar Haveli, India) [Figure 1], Group B: Camellia sinensis [Green Tea Polyphenols (GTP)] (Life extension, Quality Supplements extract Inc. USA), Group C: Biopure MTAD (Dentsply Tulsa Dental, International Inc, Tulsa, OK, USA) [Figure 2], Group D: Qmix 2in1 solution (Dentsply Tulsa Dental Specialties, International Inc. Johnson city, USA) [Figure 3], Group E: 2% Chlorhexidine gluconate solution (CHX) (Vishal Dentocare Pvt.Ltd, India), Group F: 5% Sodium hypochlorite solution (NaOCl) (Neelkanth Health Care Pvt Ltd, India), Group G: Normal saline (Amanta Healthcare Ltd, India) (Positive control).

Figure 1: Triphala (Zandu, Emami limited, Silvassa, Dadra and Nagar Haveli, India)

Figure 2: Biopure MTAD (Dentsply Tulsa Dental, International Inc, Tulsa, OK, USA)
Figure 3: QMix 2in1 solution (Dentsply Tulsa Dental Specialties, International Inc. Johnson City, USA)

Manipulation of Triphala and Camellia sinensis (Green tea polyphenol) with DMSO solvent:\textsuperscript{10,11} 0.1ml of 100\% DMSO (DiMethylSulfoXide) (HiMedia Laboratories Pvt. Ltd, India) solvent was diluted with 99.9ml of distilled water to obtain 100ml of 0.1\% DMSO. 2.4 grams of triphala and 2.4 grams of green tea polyphenol powders were taken into two separate glass beakers (Borosil, India) and each was mixed with 40ml of 0.1\% DMSO, stirred for 2 minutes, filtered through Whatman filter paper no.41 to obtain 40ml of each strained solutions and both the herbal irrigants were used in similar concentrations as root canal irrigants in their respective groups to avoid any favouritism. Biopure MTAD (Powder-Liquid) was freshly mixed according to its manufacturer’s instructions and the final solution obtained was used as root canal irrigant.\textsuperscript{6,12} QMix 2in1 solution, 5\% sodium hypochlorite solution, 2\% chlorhexidine gluconate solution and normal saline were all available in ready to use liquid form as test root canal irrigants in their respective groups.

2ml of test root canal irrigant was placed in the root canal space of teeth as per their respective groups using a 30 gauge ProRinse irrigation needles (Dentsply International Inc, Tulsa, OK) placed 1-2mm short of the working length determined. The time of contact of each test irrigant in the root canal space was 5 minutes. 3ml of distilled water was then used to flush out each root canal to terminate the effect of tested root canal irrigants and excess moisture was removed using sterile paper points (DiaDent Group International, Korea). Dentinal shavings were collected from all specimens at a depth of 400\µm using Gates Glidden drills No.4 and 5 (Mani Inc, Japan) respectively along the walls of the root canal space.\textsuperscript{13} The collected dentinal shavings from each specimen were weighed using a digital weighing balance and only 2mgms was transferred into each sterile test tube containing 1ml of tryptic soy broth and incubated anaerobically at 37\°C for 24 hours, the contents of each test tube were then serially diluted, 100\µl of broth in 100\µl of sterile saline for 5 times up to 10^{-5} dilution. Under strict aseptic conditions, 500\µl of each dilution was then placed in one hundred forty tryptic soy agar plates (HiMedia Laboratories Pvt. Ltd, India) and incubated anaerobically at 37\°C for 48 hours. The agar plates were then checked for the growth of E. faecalis and number of colony Forming Unit’s (CFU’s) were counted by digital colony counter (Secor, Scientific Engineering Corp, India) [Figure 4] using classical microbial counting technique\textsuperscript{14} and data was recorded.
RESULTS

The obtained data was tabulated for statistical analysis using SPSS (Statistical Package for Social Sciences) computer software, version 19. The frequency of every score for each tested group was counted to give descriptive analysis. The mean CFU values of Group A was 62.15, Group B:92.25, Group C:75.65, Group D:30.10, Group E:61.75, Group F:61.15, Group G:128.30 and inferential statistical analysis was done using One-way analysis of variance (ANOVA) test to determine if there was any statistically significant difference in the mean values and standard deviation of colony forming units among seven groups and it revealed statistically significant difference, as P-value (Probability) was <0.001 [Table 1].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sample no.</th>
<th>E. faecalis CFU (Mean ± SD)</th>
<th>F ratio</th>
<th>P-value</th>
<th>Significant Groups at 5% level</th>
<th>Non-significant Groups at 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>n = 20</td>
<td>62.15 ± 1.15</td>
<td>3681.06</td>
<td>&lt;0.001 (HS)</td>
<td>A vs B, C, D, G</td>
<td>A vs E, F</td>
</tr>
<tr>
<td>Group B</td>
<td>n = 20</td>
<td>92.25 ± 2.31</td>
<td></td>
<td></td>
<td>B vs A, C, D, E, F, G</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>n = 20</td>
<td>75.65 ± 1.15</td>
<td></td>
<td></td>
<td>C vs A, B, D, E, F, G</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>n = 20</td>
<td>30.10 ± 2.92</td>
<td></td>
<td></td>
<td>D vs A, B, C, E, F, G</td>
<td></td>
</tr>
<tr>
<td>Group E</td>
<td>n = 20</td>
<td>61.75 ± 1.57</td>
<td></td>
<td></td>
<td>E vs B, C, D, G</td>
<td>E vs A , F</td>
</tr>
<tr>
<td>Group F</td>
<td>n = 20</td>
<td>61.15 ± 3.08</td>
<td></td>
<td></td>
<td>F vs B, C, D, G</td>
<td>F vs A, E</td>
</tr>
<tr>
<td>Group G</td>
<td>n = 20</td>
<td>128.30 ± 2.27</td>
<td></td>
<td></td>
<td>G vs A, B, C, D, E, F</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: One-way Analysis of Variance (ANOVA)

*P : Probability, †SD : Standard Deviation, ‡F : Ratio of the between group variance to the within group variance, §HS : Highly Significant, ¦CFU : Colony Forming Unit.
Group D showed least number of colony forming units of E. faecalis. Group Band Group G showed maximum number of CFU of E. faecalis. To find any inter-group differences between the seven groups, Tukey’s post hoc test was done and it showed inter-group comparison between Group A, Group E and Group F were statistically non-significant as P-value was > 0.05 [Table 2]. Comparison of the mean CFU’s of E. faecalis among the seven groups was shown in Graph 1.

<table>
<thead>
<tr>
<th>Inter-Group comparison</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
<th>Group G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Group C</td>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Group D</td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Group E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Group F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Table 2: Tukey’s post hoc test. P: Probability

Graph 1: Vertical bar graph comparing the mean Colony Forming Units (CFU’s) of E. faecalis among the seven groups

QMix 2in1 solution exhibited maximum antibacterial efficiency and Biopure MTAD, Camellia sinensis (Green tea polyphenols) showed least antibacterial efficiency as root canal irrigants against E. faecalis.
DISCUSSION
The model proposed by Haapasalo and Orstavik\textsuperscript{15} was modified in the present study by adapting to the use of freshly extracted human teeth as samples rather than the previously used bovine teeth and this modification was considered appropriate because of the marked difference in the diameter between the root canals of bovine and human teeth. Research studies on human extracted teeth would definitely be more suitable to simulate with clinical situations.\textsuperscript{16} In the present study, biomechanical preparation was done in all sample using rotary ProTaper Universal files and the root canals were enlarged up to no. F3 file (0.30mm apical size), Khademiet al\textsuperscript{17} indicated minimal apical size needed for the better penetration of root canal irrigants in the apical third of the root canal is 0.30mm.

In the present study, QMix 2in1 solution exhibited maximum antibacterial efficiency against E. faecalis compared to other root canal irrigants used. Jerin Jose et al,\textsuperscript{3} Veeramachaneni C et al,\textsuperscript{7} Stojicic S et al\textsuperscript{18} reported superior antibacterial activity of QMix 2in1 solution on E. faecalis compared to 2%CHX and 5%NaOCl solutions. Advantages of QMix 2in1 solution as root canal irrigant is attributed to effective functioning of its various individual constituents; Surfactant helps in deeper penetration of solution into root canal dentin, thereby increasing its wetability. Bisbiguanide acts as antimicrobial agent, thus preventing microbial colonization in root canal dentin. 17%EDTA helps in the removal of smear layer and causes damage to the cell wall of bacteria by chelating and removing divalent cations (Mg+2 and Ca+2) from bacterial cell membrane.\textsuperscript{7,18} Mixing EDTA and Chlorhexidine (Bisbiguanide) in a single solution is known to produce a white colour precipitate, but in QMix 2in1 this was avoided because of its inherent chemical design.\textsuperscript{19} Ma J et al\textsuperscript{20} reported higher antibacterial efficiency of QMix 2in1 against E. faecalis residing deep in root canal dentin compared to 1% and 5%NaOCl, 2%CHX solutions and recommended the use of QMix 2in1 solution as both primary and final root canal irrigant in endodontic therapy.

In the present study, Triphalashowed higher antibacterial efficiency against E. faecalis and our findings coincided with the studies of Paridhi Garg et al\textsuperscript{2} and Prabhakar et al\textsuperscript{8} reported superior antibacterial efficiency of triphala as root canal irrigant with 80-100% killing of E. faecalis in 5 minutes and this is attributed to its formulation of three medicinal plants in equal proportions resulting in synergistic positive effect. Shirur KS et al\textsuperscript{21} concluded in their study that triphala and 5%NaOCl were equally effective against E. faecalis and tannins constituent of triphala is known to be bacteriocidal to both gram-positive and gram-negative pathogens.

In the present study, 0.1%DMSO was used as solvent for triphala and green tea polyphenols, although they were readily soluble in water. DMSOs a clean, colourless liquid and lesser its concentration, lower its toxicity. It is an organic polar aprotic molecule with amphipathic nature that is ideal for dissolving poorly soluble polar and non-polar molecules and it is widely used to solubilize drugs of therapeutic applications and for cryopreservation of cells.\textsuperscript{22} <1% DMSO is accepted as non-toxic to any living tissues and is completely inert.\textsuperscript{11} 0.1% DMSO is considered to be biologically safe, antibacterially inert and it helps in bringing out the pure properties of all the components of a herbal product being dissolved.\textsuperscript{10}
The antioxidant potential of Camellia sinensis (Green tea polyphenols) is directly related to the combination of aromatic rings and hydroxyl groups that make their structure. The antibacterial activity is a result of binding and neutralization of free radicals by the hydroxyl groups, leading to destruction and dissolution of the bacterial cell wall.\textsuperscript{23} EGCG, which is the most abundant polyphenol in green tea, is an effective antimicrobial agent inhibiting bacterial growth and suppressing their virulence against both the planktonic and biofilm forms of E. faecalis.\textsuperscript{24} But in the present study, green tea polyphenols as root canal irrigant showed least antibacterial efficiency on E. faecalis compared to other test irrigants used except the control group. Abdulkareem J. Al-Azzawi\textsuperscript{25} evaluated the antibacterial effectiveness of green tea polyphenols, 5.25% NaOCl, 2% CHX solution and siwak extracts as root canal irrigants against E. faecalis and concluded green tea polyphenols was least effective compared to other tested irrigants.

In our study, Biopure MTAD showed poor antibacterial efficiency against E. faecalis compared to triphala and other chemical root canal irrigants used. 1%, 5% and 6% NaOCl solutions were more effective as root canal irrigants on E. faecalis compared to MTAD.\textsuperscript{26, 27} Biopure MTAD was found to be more effective as a final root canal irrigant with 1.3% NaOCl solution used as initial irrigant and cross-resistance of E. faecalis to tetracycline is common.\textsuperscript{6, 8} MTAD, a tetracycline derivative has the ability to intrinsically stain teeth during odontogenesis, can chelate calcium ions and get incorporated into teeth, resulting in discolouration of both primary and permanent dentitions.\textsuperscript{28} MTAD is highly expensive and is contraindicated in pregnancy.\textsuperscript{6, 12}

In the present study, antibacterial efficiency of 5% NaOCl and 2% CHX solution against E. faecalis was higher and it was comparatively similar to triphala as root canal irrigant. Pavlovic et al\textsuperscript{29} and Arslan et al\textsuperscript{30} concluded no significant difference in the antibacterial efficiency between 5% NaOCl and 2% CHX solutions on E. faecalis. E.L. Pashley et al\textsuperscript{31} reported higher the concentration of sodium hypochlorite, higher its antibacterial efficiency against E. faecalis, but it also increases the risk of cytotoxicity and accidental extrusion of NaOCl beyond the root apex into periapical tissues causes severe immediate pain, swelling and bleeding. Sodium hypochlorite also causes deleterious effect on root dentin reducing its elastic modulus and flexural strength.\textsuperscript{32} Improper use of 2% CHX solution as root canal irrigant causes contact dermatitis, desquamative gingivitis, discoloration of teeth and tongue.\textsuperscript{33} Invitro studies definitely cannot duplicate the environment that exists invivo. However, these invitro evaluations provide information that aids clinicians in selecting ideal root canal irrigant for specific clinical situations.

**CONCLUSION**

Within the limitations of this study, QMix 2in1 solution showed maximum and superior antibacterial efficiency against E. faecalis compared to all other root canal irrigants used. Triphala, 5% sodium hypochlorite and 2% chlorhexidine gluconate solutions exhibited higher and similar antibacterial efficiency against E. faecalis. Using triphala as a safe herbal alternative root canal irrigant proves to be advantageous considering several undesirable characteristics of commonly used chemical root canal irrigants (NaOCl, CHX) in endodontic treatment. Further
research is needed to conclusively recommend QMix 2in1 solution and Triphala as root canal irrigants in routine endodontic therapy.

REFERENCES