European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 11, 2020

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

[1]Dr. Srikumar G.P.V, [2]Dr. R. Shirish Kumar, [3]Dr. Archana Jalheria, [2]Dr. Gangaraju Shakapuram, [5]Dr. Baji B, [6]Dr. Suryasowjanya Doranala

[1]Professor & Head, Department of Conservative Dentistry and Endodontics, Triveni Institute of Dental Sciences, Hospital & Research Centre, Bilaspur, Chhattisgarh, India
[2]Senior Lecturer, Department of Conservative Dentistry & Endodontics, Meghna Institute of Dental Sciences, Nizamabad, Telangana, India
[3]MDS, Consultant Prosthodontist, Smile Dental Care, Ahmedabad, Gujarat, India
[4]Senior Lecturer, Department of Conservative Dentistry & Endodontics, Meghna Institute of Dental Sciences, Nizamabad, Telangana, India
[5]Senior Lecturer, Department of Conservative Dentistry & Endodontics, Sri Sankara Dental College, Varkala, Kerala, India
[6]Senior Resident, Department of Conservative Dentistry & Endodontics, Government Dental College & Hospital, Hyderabad, Telangana, India

Corresponding Author:
Dr. Srikumar G.P.V
M.D.S, Professor & Head,Department of Conservative Dentistry & Endodontics,Triveni Institute of Dental Sciences, Hospital & Research Centre,Bilaspur, Chhattisgarh, India
E-mail Id: drsrikumar2611@gmail.com

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 o
ther 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 ofBisbiguanide (2%Chlorhexidine),17%EDTA(EthyleneDiamineTetraAceticacid),Surfactant(N-cetyle-NNN-trimethylammonium bromide) and distilled water. QMix 2in1 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, promted for the use of herbal root canal irrigants in endodontic therapy.[8]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.[9]Green Tea Polyphenols (GTP) is a herbal medicinal powderprepared from the young shoots of tea plant Camellia sinensis, Polyphenols are present in the form of Catechin, Epicatechin, Epigallocatechin and EpiGalloCatechinGallate(EGCG). EGCG was found to be the most active component in green tea.[9]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, showslong-term substantivity and absence of microbial resistance.[9,10]

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 beingperiodontally 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 micromotorhandpiece (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 (DentsplyMaillefer, 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 (DentsplyMaillefer, 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 LaboratoriesPvt.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 1.5×10^8 CFU/ml and purity of the culture was further confirmed by sub-culturing 50µicrolitrebroth 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 samplesusing a sterile micropipette(Labtop, India) inside a laminar air flow cabinet(Bionics scientific technologies Pvt.Ltd, India) to prevent any airborne contaminationand 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 wererandomly 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 NagarHaveli, 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 DentocarePvt.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 NagarHaveli, 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:[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 weretaken into two separate glass beakers (Borosil, India) andeach 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 concentrationas 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.[6,12]QMix 2in1 solution, 5%sodium hypochlorite solution, 2%chlorhexidine gluconate solution and normal saline wereall 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 teethas 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 spacewas 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 400µm using GatesGlidden drills No.4 and 5(Mani Inc, Japan) respectively along the walls of the root canal space.[13] The collected dentinal shavingsfrom each specimen were weighed using a digital weighing balance and only 2mgms was transferred into each steriletest 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, 100µl of broth in 100µl of sterile saline for 5 times upto 10^{-5} dilution. Under strict aseptic conditions, 500µlof each dilution was then placed in one hundred forty tryptic soy agar plates(HiMedia LaboratoriesPvt. Ltd, India) and incubated anaerobicallyat 37ºC 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 microbialcounting technique[14]and data was recorded.

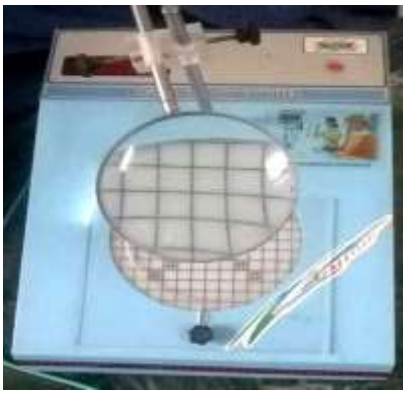


Figure 4: Digital colony counter (SECOR, Scientific Engineering Corp, India)

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].

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

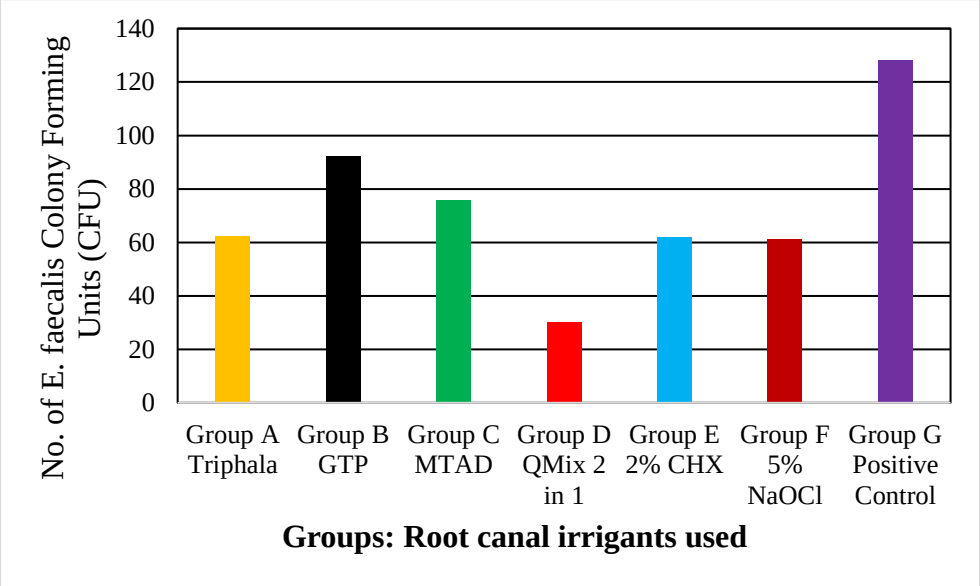
Table 1: One-way Analysis of Variance (ANOVA)

*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.

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 > 0.05	P < 0.01
Group F						P < 0.01

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 maximumantibacterial 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[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.[16]In the present study, biomechanical preparationwas done in all samplesusing rotary ProTaper Universal files and the root canals were enlarged upto no. F3 file(0.30mm apical size), Khademiet al[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 solutionexhibited maximum antibacterial efficiency aganist E. faecalis compared to other root canal irrigants used. Jerin Jose et al,[3]Veeramachaneni C et al,[7]Stojicic S et al[18]reported superior antibacterial activity of QMix 2in1 solution on E. faecalis compared to 2%CHX and 5%NaOCl solutions. Advantages of QMix 2in1 solutionas 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%EDTAhelps in the removal of smear layer and causesdamage to the cell wall of bacteria by chelating and removing divalent cations (Mg+2 and Ca+2) from bacterial cell membrane.[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.[19]Ma J et al[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 bothprimary and final root canal irrigant in endodontic therapy.

In the present study, Triphalashowed higher antibacterial efficiency aganistE.faecalis and our findings coincided with the studies of Paridhi Garg et al[2] and Prabhakar et al[8]reportedsuperior antibacterial efficiency of triphala as root canal irrigant with 80-100% killing of E.faecalis in 5 minutes and thisis attributed to its formulation of three medicinal plants in equal proportionsresulting in synergistic positive effect.Shirur KS et al[21]concluded in their study thattriphala and 5%NaOCl were equally effective against E. faecalis and tanninsa constituent of triphala is known to be bacteriocidal to both gram-positive and gram-negative pathogens.

In the present study, 0.1%DMSOwas used as solvent for triphala and green tea polyphenols, although they were readily soluble in water. DMSOis 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 applicationsand for cryopreservation of cells.[22]<1% DMSO is accepted as non-toxic to any living tissues and is completely inert.[11]0.1% DMSO is considered to bebiologically safe, antibacterially inert and it helps in bringing out the pure properties of all the components of a herbal product being dissolved.[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.[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.[24]But in the present study,green tea polyphenols as root canal irrigant showed least antibacterial efficiency on E. faecalis compared toother test irrigants used except the control group. Abdulkareem J.Al-Azzawi[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.[26,27]Biopure MTAD was found to be more effective as a final root canal irrigant with 1.3% NaOCl solution used as initial irrigantand cross-resistanceof E. faecalis to tetracycline is common.[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.[28]MTAD ishighly expensive and is contraindicatedin pregnancy.[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.Pavlovicet al[29] and Arslan et al[30]concluded no significant difference inthe antibacterial efficiency between 5%NaOCl and 2%CHX solutions on E. faecalis. E.L.Pashleyet al[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 tissuescauses severe immediate pain, swelling and bleeding. Sodium hypochlorite also causes deleterious effect on root dentinereducing its elastic modulus and flexural strength.[32]Improper use of 2%CHX solution as root canal irrigant causescontact dermatitis, desquamative gingivitis, discoloration of teeth and tongue.[33]

Invitro studies definitely cannot duplicate the environment that exists invivo. However, these invitroevaluations 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 aganist E. faecaliscompared to all other root canal irrigants used. Triphala,5% sodium hypochlorite and 2% chlorhexidine gluconate solutionsexhibited higher and similarantibacterial efficiency against E. faecalis. Using triphala as a safe herbal alternative root canal irrigant proves tobe advantageous considering several undesirable characteristics of commonly used chemical root canal irrigants(NaOCl, CHX) in endodontic treatment.Further

research is needed to conclusively recommendQMix 2in1 solution and Triphala as root canal irrigants in routine endodontic therapy.

REFERENCES

1. Tabassum S, Khan FR. Failure of endodontic treatment: The usual suspects. Eur J Dent 2016;10(1):144-147.
2. Paridhi Garg, Shashi PrabhaTyagi, Dakshita Joy Sinha, UdaiPratap Singh, Vibha Malik, Edgar Richard Maccune. Comparison of antimicrobial efficacy of propolis, morindacitrifolia, azadirachtaindica, triphala, green tea polyphenols and 5.25% sodium hypochlorite against Enterococcus faecalis biofilm. Saudi Endodontic Journal 2014;4(3):122-127.
3. Jerin Jose, Shoba Krishnamma, FaizalPeedikayil, ShibuAman, NithyaTomy, JithinPulickalMariodan. Comparative evaluation of antimicrobial activity of QMix 2in 1 solution, 2.5% sodium hypochlorite, 2% chlorhexidine, guava leaf extract and aloe vera extract against Enterococcus faecalis and Candida albicans. An in-vitro study. Journal of Clinical and Diagnostic Research 2016;10(5):20-23.
4. Zehnder M. Root canal irrigants. J Endod 2006;32(5):389-398.
5. Sinha DJ, Vasudeva A, Jaiswal N, Garg P, Tyagi SP, Singh J. Antibacterial efficacy of Malaleuca alternifolia(Tree tree oil), Curcuma longa(Turmeric), 2% chlorhexidine and 5% sodium hypochlorite against Enterococcus faecalis. An invitro study. Saudi Endodontic Journal 2015;5(3):182-186
6. Torabinejad M, Khademi A. Ali, Jalil B, Yongbum C, William B. Johnson, Krassimir B, et al.Effect of MTAD on the surface of instrumentedroot canals. J Endod 2003;29(3):170–175
7. Veeramachaneni Chandrasekhar, Gayathri Cherukuri, SoujanyaElakanti, Venkateswara G Rao, Anitha S Rao, MuralidharTummala. Comparative evaluation of antimicrobial efficacy of QMix 2in1, sodium hypochlorite and chlorhexidine against Enterococcus faecalis and Candida albicans. J Conservative Dentistry 2015;18(2):128-131.
8. J.Prabhakar, M.Senthikumar, M.S.Priya, K.Mahalakshmi, P.K.Sehgal, V.G.Sukumaran. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala& Green Tea Polyphenols), MTAD and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate: An In-vitro study. J Endod 2010;36(1):83-86.
9. MadhuPujar, SaleemMakandar. Herbal usage in endodontics - A review. International Journal of Contemporary Dentistry 2011;2(1):34-37.
10. Bhargava K, Kumar T, Aggarwal S, Zinzarde S, Sanap A, Patil P. Comparative evaluation of the antimicrobial efficacy of neem, green tea, triphala and sodium hypochlorite: An in vitro study. Journal of Dental Research and Review 2015;2(1):13-16.
11. Joana Galvao, Benjamin Davis, Mark Tilley, Eduardo Normando,Michael R. Duchen, Francesca Cordeiro.Unexpected low-dose toxicity of the universal solvent DMSO.FASEB J 2014;28(3):1317-1330.
12. G.P.V.Srikumar, K.S.Sekhar, K.G.Nischith. Mixture tetracycline citric acid and detergent-A root canal irrigant. A review.Journal of Oral biology and Craniofacial research 2013;3(1):31-35

13. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod 2007;33(12):1473-1476.
14. Collins CH, Lyne PM, Grange JM, Falkinham JO. Collins and Lyne's Microbiological Methods. Chapter no.10. Estimating microbial numbers. 8th edition. Oxford, London: Butterworth Heinemann; 2004. Page no. 144-155.
15. Haapasalo M, Orstavik D. In vitro Infection and Disinfection of Dentinal Tubules. J Dent Res 1987;66(8):1375-1379.
16. Basrani B, Santos JM, Tjaderhane L, Grad H, Gorduysus O, Huang J, et al Substantive antimicrobial activity in chlorhexidine treated human root dentin. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2002;94(2):240-245.
17. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J. Endod 2006;32(5):417-420.
18. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of novel irrigant; QMix. IntEndod J 2012;45(5):363-371
19. Gijo John, K. Pavan Kumar, S.Sujatha Gopal, Surya Kumari, Bala Kasi Reddy. Enterococcus faecalis, a nightmare to endodontist: A systemic review. Afr. J. Microbiol.Res 2015;9(13):898-908.
20. Ma J, Wang Z, Shen Y, Haapasalo M. A new non invasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod 2011;37(10):1380-1385.
21. ShirurKrishnarajSomayaji, NidamburVasudevBallal, Shobha KL, Mohandas Rao. Comparision of antimicrobial efficacy of triphala, withaniasomnifera and sodium hypochlorite against E. faecalis. An Invitro Study. International Journal of Pharmacy and Pharmaceutical Sciences 2014;6(2):808-811.
22. de la Torre JC. Biological actions and medical applications of dimethyl sulfoxide.Ann NY AcadSci 1983;411:1–404.
23. Suchi Agarwal, ParimalaTyagi, Ashwini Deshpande, Saurabh Yadav, Vipul Jain,Kuldeep Singh Rana. Comparison of antimicrobial efficacy of aqueousozone, green tea, and normal saline as irrigantsinpulpectomy procedures of primary teeth. Journal of Indian Society of Pedodontics and Preventive Dentistry 2020;38(2):164-170.
24. Lee P, Tan KS. Effects of epigallocatechin gallate(EGCG) againstEnterococcusfaecalisbiofilm and virulence. Arch Oral Biol2015;60(3):393-399.
25. AbdulkareemJ.Al-Azzawi. The antibacterial effect of herbal alternative, green tea and salvadorapersica (siwak) extracts on Enterococcus faecalis. Journal of Baghdad College Dentistry 2015;27(2):1-5.
26. Thomas R. Dunavant, John D. Regan, Gerald N. Glickman, Eric S. Solomon. Comparative evaluation of endodonticirrigants against Enterococcus faecalis biofilms. J Endod 2006;32(6):527–531.

27. Johal S, Baumgartner JC, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/Biopure MTAD to 5.25% NaOCl/15%EDTA for root canal irrigation. J Endod 2007;33(1):48-51.
28. TasanFugen. Surface tension of root canal irrigants. J Endod 2000;26(10):586-587.
29. Pavlovic V, Zivkovic S, Chlorhexidine as a root canal irrigant – Antimicrobial and scanning electron microscopic evaluation. Serbian Dental J 2010;138(9):557-563.
30. Arslan S, Ozbilge H, Kaya EG, OzgurEr. Invitro antimicrobial activity of propolis, Biopure MTAD, sodium hypochlorite and chlorhexidine on E.faecalis and candida albicans. Saudi Med J 2011;32(5):479-483.
31. E.L. Pashley, N.L. Birdsong, K. Bowman, D.H. Pashley. Cytotoxic effects of sodium hypochlorite on vital tissues. J Endod 1985;11(12):525-528.
32. Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochloriteand calcium hydroxide on its flexural strength and elastic modulus. IntEndod J 2001;34(2):113–119.
33. SistlaDatta Prasad, Prem Chand Goda, KunamSashidhar Reddy, Chennuru Sunil Kumar, MelpatiHemadri, DappiliSwamyRanga Reddy. Evaluation of antimicrobial efficacy of neem and aloe vera leaf extracts in comparision with 3% sodium hypochlorite and 2% chlorhexidine against E. Faecalis and C.albicans. Journal of Dr.NTR University of health Sciences 2016;5(2):104-110.