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Comparative evaluation of antibacterial efficacy of QMix, 2% chlorhexidine and curcumin against E. faecalis-An In-vitro Study

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Abstract

Background: Irrigation is one of the important steps to a successful endodontic treatment. The aim of irrigating solution is to achieve a root canal system free from damaging bacteria and other irritants without causing any periapical tissue damage. Newest irrigants and Herbs have renewed importance in this modern era for their antimicrobial properties and fewer side effects. Insufficient root canal disinfection is possibly one of the main reasons for failure of pulpectomy. Thus, for a successful endodontic treatment, irrigating solution with optimum antimicrobial properties is advocated in the present study.

Aim: To evaluate and compare the antibacterial efficacy of QMix, 2% Chlorhexidine Gluconate, Curcumin and Normal saline as endodontic irrigants against E. faecalis bacteria.

Materials and methods: Antimicrobial efficacy of QMix, 2% Chlorhexidine Gluconate and Curcumin as experimental groups, normal saline as control groups were assessed by using agar diffusion method. The statistical analysis was done.

Results: Intergroup comparison revealed significant difference amongst all the experimental groups. QMix endodontic irrigant had significantly higher zone of inhibition among all the groups.

Conclusion: Antibacterial efficacy against E. faecalis is more in QMix endodontic irrigants followed by 2% Chlorhexidine Gluconate irrigant and comparatively least in Curcumin herbal irrigant. So QMix can be used as a better alternative to other endodontic irrigants used in this study.

Keywords: Root canal irrigants, E faecalis, QMix, 2% Chlorhexidine Gluconate, Curcumin

Introduction

Endodontic irrigating solution is to achieve a root canal system free from damaging bacteria and other irritants without causing any periapical tissue damage particularly when it is used in primary teeth. Insufficient root canal disinfection is possibly one of the main reasons for failure of pulpectomy. Presence of curved canals and accessory canals allow microorganisms to penetrate deep into the dentinal tubules where they are seldom reached by conventional irrigants, making it difficult if not impossible to entirely eliminate microorganisms from this system.[1]

Enterococcus faecalis is the one of the predominant bacteria present in cases of endodontic failure. E. faecalis can able to persist in harsh environmental conditions such as nutritional deprivation, in the presence of medicaments like Calcium hydroxide, high pH etc.[2]

Chlorohexidine gluconate (CHX), which has antibacterial effects and no cytotoxicity, is another popular irrigants, for chemo-mechanical debridement. But the longterm use of the CHX might leads to resistance towards microbes. This drawback paved the path for herbal irrigants. The herbal irrigants like aloe vera, curcumin, neem etc, which also proved to be effective in eradicating microorganisms from the infected root canal without creating any cytotoxicity. But none of these irrigants can easily reach target areas within the intricate structure of the root canal system due to its high surface tension.To achieve deeper penetration of irrigants into the dentinal tubules and lateral canals surfactant have been added to the irrigants to reduce the surface tension.QMix is a novel one-step irrigating solution that contains a mixture of bisbiguanide antimicrobial agent, calcium-chelating agent, saline and surfactant. QMix has also have been proven to have effective antimicrobial activity for the disinfection of hydroxyapatite discs infected with E.faecalis. However, to date, little information is available on the antimicrobial ability of QMix in human teeth infected with E.faecalis.[3]

Therefore, the present study has been undertaken to evaluate the antibacterial efficacy of QMix, 2% Chlorhexidine gluconate and Curcumin as endodontic irrigants against E. faecalisbacteria.

Aim & objectives

To evaluate and compare the antibacterial efficacy of QMix, 2% Chlorhexidine gluconate and Curcumin as endodontic irrigants against E. faecalis bacteria.

Materials & method

A total of 60 wells in 15 mueller hinton agar plates were prepared for the study, which were divided in four groups having 15 wells in each group: Group 1: QMix irrigant (n= 15), Group 2: 2% Chlorhexidine gluconate irrigant (n= 15), Group 3: Curcumin herbal irrigant (n= 15) and Group 4: Normal saline (n=15). The antibacterial efficacy of endodontic irrigants used in primary teeth was evaluated against E. faecalis (ATCC® 29212™) by agar diffusion method. The standard bacterial strain of E. faecalis was obtained from Himedia labs.

The bacterial strain wereinoculated in BHI broth and incubated at 37°C for 24 hours. Following incubation, the cultures were centrifuged at 3000 rpm for 10 minutes. The precipitate containing microbial cells was re-suspended in saline and turbidity of this culture

suspension was adjusted until it was equivalent to the no.1 0McFarland Standard (Approximately 3 X 108 cells/mL). Several drops of this primary broth were inoculated on Trypticase soy agar with defibrinated sheep blood (Blood agar) using a micropipette. The inoculated blood agar plate was incubatedat 37°C for 24 hours. Rest of the primary broth was kept in the refrigerator at 2 - 8°C till the time the culture medium was prepared.

Confirmation of microbs was done After 24 hours of incubation by observing colonies on blood agar which appeared circular, entire, glistening, smooth, and low convex according to the ATCC product sheet andon Gram staining from the primary broth tube, gram positive cocci in short chains were observed under the 10X and 40X magnification of compound microscope also confirming the growth of the microbial indicator as E. faecalis(ATCC 29212).

The mueller hinton agar plates were inoculated with E. faecalis bacteria for agar well diffusion test to assess the antibacterial efficacy. The back side of each mueller hinton agar plate was labelled using a permanent marking pen of respective group, 0.1mL from the primary broth vial using sterile swab in one swiping motion all over the mueller hinton agar plate.Using the dropper, agar was punched out from the agar plates to create an agar well for the placement of irrigating solutions. The 4 wells (4mm of depth X 6mm of diameter) were made in each of the agar plates.

50 µL of the QMix, 2% CHX, Curcumin and saline were taken with the help of a micropipette and then poured into the labelled wells respectively. Likewise, all other 14 agar plates were prepared.The agar plates were kept for 2 hrs at room temperature for diffusion of the materials, and then incubated for 24 hours at 37°C.Most uniform outer diameter of the inhibitory zone(**Figure 1**) was recorded in millimetres using a vernier calliper.Data was collected, tabulated and sent for statistical analysis.

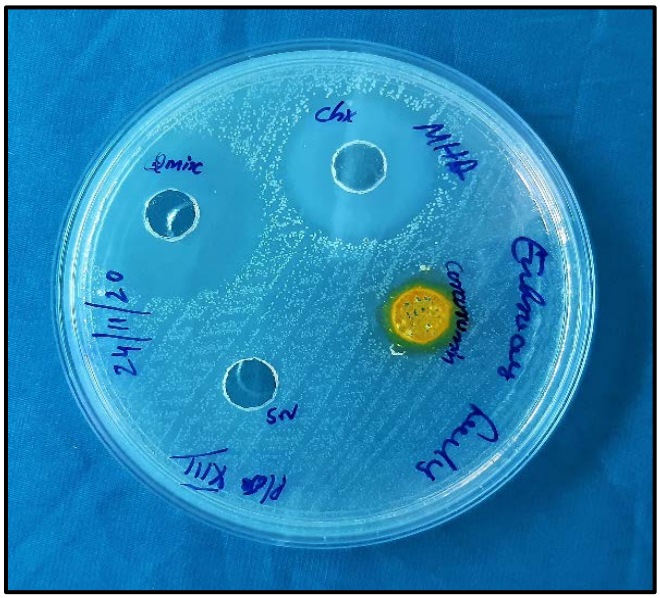

Figure 1: Inhibition zone

Results and observations

The statistical analysis was done using SPSS Version 15.0. The Analysis of Variance (ANOVA) and Post-Hoc Test (Tukey-HSD) were performed to know the effect of each variable and to reveal the statistical significance. The values were represented in number and mean ± SD. For the purpose of statistical interpretation p value of 0.05 was considered statistically significant.

The mean value of the inhibition zone for Group 1 (QMix), Group 2 (2% CHX), Group 3 (Curcumin) and Group 4 (saline) were 29.8, 25.33, 17.53 and 0 respectively. It was found that Group 1 had highest mean value followed by Group 2 and 3. The control group (saline) had the least value among the four groups. (Table 1, Graph 1)

On applying One Way ANOVA, we found that inhibition zone in all the groups have a significant difference as p value was 0.000 at 0.05 at 95% confidence interval. (Table 2)

When inter group comparison of various groups were done using Tukey's (2-sided) test (Post Hoc tests)

difference was statistically highly significant between all the four groups (Table 3)

Table 1: Mean values of inhibition zone in different groups

Groups	N (Sample Size)	Minimum	Maximum	Mean	Std. Deviation
Group I (Qmix)	15	29.00	33.00	29.80	1.26491
Group II (2% CHX)	15	24.00	27.00	25.33	.72375
Group III (Curcumin)	15	16.00	19.00	17.53	.91548
Group IV (Normal Saline)	15	.00	.00	.00	.00

Graph 1: Graphical representation of mean of antibacterial efficacy of all the groups

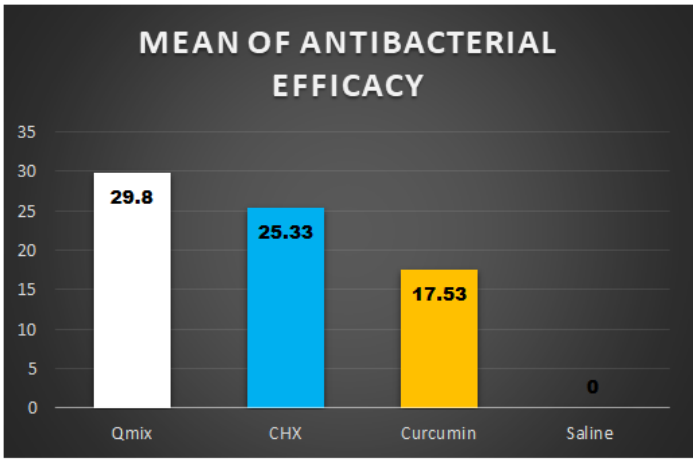

Table 2: Comparison of Means of inhibition zone in all groups

One way ANOVA					
	Sum of Squares	df	Mean Square	F	p value
Between Groups	7756.867	3	2585.622	3491.837	.000
Within Groups	41.467	56	.740		
Total	7798.333	59			
* p value < 0.05 Significant, ** p value > 0.05 Not Significant					

Table 3: Intergroup Comparison of means of inhibition zone by Tukey's (2sided) Post-hoc Test

Multiple Comparisons using Tukey HSD						
(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	4.46667*	.31421	.000	3.6347	5.2987
	Group III	12.26667*	.31421	.000	11.4347	13.0987
	Group IV	29.80000*	.31421	.000	28.9680	30.6320
	Group III	7.80000*	.31421	.000	6.9680	8.6320

Group II	Group IV	25.33333*	.31421	.000	24.5013	26.1653
Group III	Group IV	17.53333*	.31421	.000	16.7013	18.3653
p value < 0.05 Significant, ** p value > 0.05 Not Significant. *., The mean difference is significant at the 0.05 level.*						

Discussion

Preservation of the primary teeth has been given an undying effort in the field of pediatric dentistry, which facilitate the alignment of the succeeding permanent tooth. Pulpectomy is indicated if the teeth has become irreversibly infected or necrotic due to caries, trauma or other causes. The objective of pulpectomy is the removal of pathologic pulpal tissue, sterilisation of the canals and restoration with appropriate obturating material. sterilisation of the canals is achieved by thorough irrigation. However, the tortuous and complex nature of the root canal system of the deciduous teeth and change in their morphology with root resorption makes it difficult to achieve proper cleansing by mechanical instrumentation and irrigation of the canals which might lead to failure of the treatment due to growth of residual micro-organism. The fact that some micro-organisms are capable of survival under harsh, nutrient-limited conditions of the root-filled canal for so long is remarkable.[4]

Enterococcus faecalis is one of the most prevalent bacteria in secondary infected teeth. **Gajan EB et al (2009)[5]** stated that E. faecalis is a prevalent species in the failed root canals evaluated. **Rôças IN et al (2004)[6]** stated that the prevalence of E. Faecalis is 4 to 40% of primary endodontic infections and consistently higher percentages (67-77%) in secondary infections or persistent periradicular lesions. It is more resistant to mechanical instrumentation and irrigation with antimicrobial agents and has the ability to adapt to the harsh environmental condition in instrumented and medicated root canals. Thus, E. Faecalis was chosen as the microbial indicator for the present study.

Several studies have generally concurred that sodium hypochlorite has a broad-spectrum antimicrobial activity, though NaOCl is a commonly used root canal irrigant, it has an unpleasant odor, taste and is toxic when extruded into the periradicular tissues. It can damage permanent tooth follicles, peripheral tissues and oral mucosa. Another major drawback is its high surface tension, which limits it penetration into canal irregularities and the depth of dentinal tubules. So, it is proven that NaOCl is not safe to use as endodontic irrigants in primary teeth. To overcome these drawbacks of NaOCl they started to use 2% Chlorhexidine as an alternative.[7]

Chlorhexidine is a cationic bisbiguanide, it is most stable in the form of its salt like Chlorhexidine gluconate. Aqueous solutions of 2% is used for mechanical irrigation of root. It is highly antimicrobial especially at pH 5.5-7.0, and is known for its long-lasting effectiveness even after the removal of the solution and it does not provide any tissue dissolving properties.[8] 2% CHX has relatively low toxicity, substantivity and antibacterial properties against gram negative and gram positive bacterial. Commonly, CHX is used in conjunction with NaOCl as an irrigant as it raises effectiveness of the irrigation protocol. When NaOCl and CHX are mixed, an orangebrown precipitate known as para-chloroaniline is formed, which might be carcinogenic. The major advantages of CHX over NaOCl are its lower cytotoxicity and lack of foul smell and bad taste

A newly introduced irrigating solution used in our study, QMix™ 2in1(Dentsply a Dental Specialties, Tulsa, OK, USA), contains a mixture of a bisbiguanide antimicrobial agent (2% CHX), a polyamino carboxylic acid, calcium chelating agent (17% EDTA); saline; and a surfactant (cetrimide). This novel irrigant has been introduced to both remove smear layer and kill recalcitrant bacteria such as E. faecalis, in one application. Mixing of EDTA and CHX is known to produce a white precipitate. In QMix, this is avoided because of its chemical design. A study by **Al Khatani et al (2014)**[9] demonstrated its biocompatibility and also reported that compared to sodium hypochlorite, QMix is less aggressive and more acceptable to living tissues. **Stojicic et al (2012)**[10] demonstrated that this irrigant has antibacterial activity comparable to NaOCl and superior activity against Enterococcus faecalis in planktonic form and biofilm compared to MTAD.

Recently, herbs have shown promising results in dentistry. In recent endodontics because of the limitations of most of the commercial intracanal medicaments used such as cytotoxicity, unpleasant taste, odor and their inability to eliminate bacteria from dentinal tubules, trend of recent medicine to use biologic medication extracted from natural plants is drawing a lot of attention. Literature has shown that herbs can have a promising role as root canal irrigants. However, there is a need for evidence based research for use of herbal products in endodontics, particularly when it is used in children. Among the many herbs the most extensively researched herbs are turmeric extract "Curcumin". Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric, has been shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory, anti-cancer and antioxidant activities. **Araújo CC, and Leon LL (2001)**[11] stated that Curcumin is a major component in Curcuma longa L., being responsible for its biological actions. keeping this in mind our study was aimed to evaluate the antibacterial efficacy of the QMix, 2% Chlorhexidine gluconate, Curcumin and normal saline as endodontic irrigants against E. faecalis bacteria.

In the present study, the highest zone of inhibition was seen with Group I QMix against E. faecalis which was statistically higher than the other irrigants used in this study. This might be attributed to the premixed combination of 2% CHX and 17% EDTA present in the formulation of QMix, which enables it to not only remove the smear layer, but also kill bacteria within the tubules. The rationale of adding a surface active agent (cetrimide) in QMix is because of its ability to lower surface tension of solutions and increase their wettability.[12] Our result is validated by other studies which showed QMix to have superior antibacterial activity against E.faecalis microorganism. **Linu Y et al (2015)**[3] and **Jose J et al (2016)**[12] reported that QMix showed the best results among the tested solutions, thereby making it a potential alternative to existing root canal irrigants. However, contrasting result was obtained by **Ordinola-Zapata et al (2013)**[13], where QMix could not significantly remove nor kill biofilms developed on intra-orally infected dentin.

The antimicrobial efficacy of Group II 2% chlorhexidine gluconate was found to be statistically lower than that of QMix, but was statistically better than Curcumin herbal irrigants in reduction of E.faecalis. This might be due to the action of positively charged 0.2% CHX that binds readily to the negatively charged microbial cell surface, leading to disorganization of cytoplasmic membrane. Low concentrations of CHX ie., 0.2% allow cytoplasmic constituents to leak out, while a high concentration like 2% CHX (used in the present study) coagulates them.

Thereby inhibiting the membrane ATPase and anaerobic process, finally leading to cell lysis. In a studies conducted by **Gomes BP et al (2003)**[16] and **Goud S et al (2018)**[15] they have concluded that 2% chlorhexidine exhibited good antimicrobial efficacy against E. faecalis.

The antimicrobial efficacy of Group III Curcumin herbal irrigant was found to be statistically lower than Qmix and 2% CHX. Curcumin irrigant contains curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. So, the synergistic effect of all herbal contents might be responsible for its antimicrobial efficacy. But evidence suggests that out of all the contents present in Curcumin only diferuloylmethane, has been shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory, anti-cancer and antioxidant activities. This might be the reason why Curcumin herbal irrigants has significantly lower antimicrobial efficacy than QMix and 2% CHX. Inter-group comparison of CHX and Curcumin have been carried out by **Yadav RK et al (2018)**[16] which showed that Curcumin has lower antimicrobial efficacy compared to chlorhexidine irrigants. While contradictory results have been discussed in the study done by **Kumar H (2013)**[17] which showed that there is no significant difference between the antimicrobial efficacy of chlorhexidine gluconate and Curcuma Longa

Group IV Normal saline showed no antibacterial efficacy against E. Faecalis, thereby authenticating the validity of the present study.

Therefore, in the present study it can be concluded that QMix can be an alternate to 2% Chlorhexidine gluconate and curcumin as an endodontic irrigants for primary teeth. We recommend further studies to authenticate these results. Clinical trials should be made so as to check other properties in the intraoral environment.

Summary and Conclusion

Within the limitations of the present study, it can be concluded that antibacterial efficacy against E. faecalis is more in QMix endodontic irrigants followed by 2% Chlorhexidine gluconate irrigant and comparatively least in Curcumin herbal irrigant. So QMix can be used as a better alternative to other endodontic irrigants used in this study

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