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ANTIBACTERIAL EFFECT OF FOUR IRRIGATING SOLUTIONS COMPARED TO THAT OF SODIUM HYPOCHLORITE (A COMPARATIVE IN-VITRO STUDY)

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ABSTRACT

Aim : The aim of the present study was to evaluate the antimicrobial activity of four irrigating solutions against *Enterococcus faecalis* compared with sodium hypochlorite 5.25% namely: propolis extract, QMix, Chloroxylenol and CHX-Plus.

Materials and methods: Enterococcus faecalis (batch no. 10541) thawed and incubated under aerobic condition. The harvested bacterial colonies are then spectrophotometrically calibrated to 5.9 x 104 (CFU/mL-1). Fifty specimens instrumented conventionally, reaching master apical file size #50. Twenty µL of the bacterial culture transferred to the canal lumen using sterile micropipette. All specimens were then incubated for 48 hours at 37 ˚C.

Results : Intracanal sample collection by paper points showed an insignificant difference between different irrigation solutions tested and positive control Group at p=0.001. Group 1 showed a median of $8.9*10^4$(CFU/ml), where group 2, 3, 4 and positive control groups showed mean values of $2.2*10^4$, $7.3*10^4$, $8*10^4$ and $3*10^4$ (CFU/ml) respectively. Negative control group showed the highest significant values for colony forming units ($118.4*10^4$CFU/ml).

Conclusion : All groups except the saline produce of significant reduction of bacteria. Although QMix, NaOCl and chlorhexidine plus are capable of reducing bacterial count their effect is statistically insignificant.

KEYWORDS: Enterococcus faecalis,QMix, propolis extract, CHX-Plus, Chloroxylenol, NaOCl 5.25%.

INTRODUCTION

Bacteria and their products play an essential role in the development and perpetuation of pulpal and periradicular diseases. Although the root canal flora is dominated by obligate anaerobic bacteria, some facultative strains, e.g. *Enterococcus faecalis*, have been involved in persistent infections, influencing the prognosis of the root canal treatment. Once bacteria are established in the root canal, they cannot easily

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be reached by the defense mechanisms of the host. Hence, irrigation during an endodontic treatment serves several purposes such as canal lubrication; dissolving pulp tissue, mechanical washing out of debris created by canal instrumentation, eradication of microorganisms from the root canal. Sometimes an infection is resistant to normal treatment, and the therapy cannot be successfully completed. Therefore, a search for better root canal irrigant continues.

MATERIAL AND METHODS

Selection and preparation of teeth

Fifty recently extracted human lower single canalled first premolars were used in this study. Access cavity was prepared to gain access. Canals were then instrumented, coronal one third of the canals was enlarged with Gates Glidden drills sizes # 2, 3 and 4.Teeth were then prepared up to size 50 k-file 1 mm shorter from the apical foramen. During instrumentation the root canals were irrigated with saline between successive files. The apical foramen was sealed with resin restoration to prevent bacterial leakage.

Teeth were horizontally decapitated at the level of cemento-enamel junction. Sterilization of the samples after complete root canal cleaning and shaping was achieved by autoclaving for 30 min at 121 °C pressure 15 Psi.

Selection and preparation of bacteria

A pure bacterial culture of *Enterococcus faecalis* (ATCC 10541) was obtained , the frozen bacterial samples (-20°C) were thawed and incubated for 24 hours on a solid culture medium (Brain Heart Infusion Agar, supplemented with 7% sheep blood) 37 °C under aerobic conditions. The grown bacterial colonies were then harvested, placed in Mueller-Hinton nutrient broth and incubated for an additional 24 hours at 37 °C under aerobic conditions.

The *Enterococcus faecalis* cultures were then calibrated to 5.9 x 10^4 colony forming units per ml (CFU ml) spectrophotometrically in Muller-Hinton broth. Twenty μl of the bacterial culture were transferred to the canal lumen of the mechanically enlarged root canals using sterile micropipette and then stored for 48 hours at 37°C in the incubator.

Specimens classification

After the 48 hours incubation period the specimens were then divided into five groups; 10 specimens each;

Group 1: Specimens *(n=10)* were irrigated with propolis (2 ml per specimen).

Group 2: Specimens *(n=10)* were irrigated with QMix (2 ml per specimen).

Group 3: Specimens *(n=10)* were irrigated with chloroxylenol (2 ml per specimen).

Group 4: Specimens *(n=10)* were irrigated with chlorhexidine plus (2 ml per specimen).

Group 5: Positive control: Specimens *(n=5)* were irrigated with 5.25% sodium hypochlorite (2 ml per specimen). **Negative control:** Specimens *(n=5)* were irrigated with physiological saline (2 ml per specimen).

Specimens irrigation

All procedures were conducted inside a laminar flow chamber using sterile instruments to avoid contamination. The irrigating solutions were kept inside the canals for a period of 1 min then irrigated with saline.

Thereafter, a sterile paper point # 50 was inserted into each canal and maintained for 1 min. for sample collection. The paper point of each group was individually transported to test tubes containing 2 ml of sterile saline then vortexed for 20 seconds.

A calibrated loop 10 μL of each tube was adjusted and placed in Mueller-Hinton agar culture plates and incubated at 37ºC for 48 hours. A classical bacterial counting technique was used for

each group for the recovery of viable Enterococcus faecalis on Mueller-Hinton agar plates. The mean value of CFU for the plates of each group was then calculated.

Statistical analysis

Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 21 for Windows. Colony forming units were transferred using log transformation before statistical analysis. A non-parametric one-way ANOVA (Kruskal–Wallis) test followed by paired group comparisons using the Mann–Whitney U test at a 5% significance level were used to analyze the effect of different irrigant types on colony forming units. Mann–Whitney U test was used to test the difference between negative and positive controls.

RESULTS

Difference between different irrigations used and positive and negative control groups on Biofilm Susceptibility:

A non-parametric one-way ANOVA (Kruskal–Wallis) test showed an insignificant difference between different irrigation solutions tested and positive control Group at p=0.001. Group 1 showed a median of $8.9*10^4$(CFU/ml),where group 2, 3, 4 and positive control groups showed mean values of $2.2*10^4$, $7.3*10^4$,$3.5*10^4$ and $3*10^4$ (CFU/ml) respectively. Negative control group showed the highest significant values for colony forming units ($118.4*10^4$CFU/ml), as shown in Table 1.

TABLE (1) Comparison between different irrigating groups and the control groups.

		Group						
		Group 1 (Propolis)	Group 2 (Qmix)	Group 3 (Chloroxylenol)	Group 4 (Chlorhexidine plus)	Group 5 (+Ve Control) (5.25% NaOCl)	Group 6 (-Ve Control) (Saline)	p-value
Colony forming unit (CFU/ml)	Mean	$8.9*10^4$	$2.2*10^4$	$7.3*10^4$	$3.5*10^4$	$3*10^4$	$118.4*10^4$	0.001*
	SD	$8*10^4$	$6.9*10^4$	$15*10^4$	$2.3*10^4$	$6.7*10^4$	$19.6*10^4$	
	Minimum	0.0	0.0	0.0	0.0	0.0	$94*10^4$	
	Maximum	$21*10^4$	$22*10^4$	$40*10^4$	$45*10^4$	$15*10^4$	$147*10^4$	
	Rank	A	A	A	A	A	B	

Means with the same letters within each row are not significantly different at p=0.05. ****= Significant***

DISCUSSION

The results obtained from this study indicated that there was a statistically significant reduction in the mean values of the bacterial count after the application of the allocated treatment for each group when compared with saline (negative group). The physiological saline lacks any antibacterial properties. In addition it serves as an indicator to the incubation process and the ability of the microorganisms to grow. Also it provides a standard in which the performance of other irrigants are compared.

Results of the present study showed that the QMix has the highest percentage of mean value of bacterial count reduction, with no significant difference between other groups except saline. QMix irrigant and its modifications containing a mixture of a bisbiguanide antimicrobial agent, a polyaminocarboxylic acid calcium-chelating agent, saline, and a surfactant have been found to be more effective than BioPure MTAD against bacterial biofilms [1]. A surface-active agent decreases the surface tension of solutions and increases their wettability [2]. Also, it enables better penetration of an irrigant into the root canal that coincide with Stojicic et al [3], Morgental et al [4] and Wang et al[5]. Abidal et al [6] demonstrated that the addition of a surfactant to EDTA and CHX in the composition of QMix may have accounted for its potent antimicrobial efficacy. Arias-Moliz*etal* [7] showed that the association of a surfactant (cetrimide) to CHX provided better results than their application as single agents against *E. faecalis*. Furthermore, EDTA is regarded as a potentiator of the activity of other antimicrobial agents [8].

Followed by the QMix, NaOCl showed high percentage of bacterial count reduction while the difference between 5.25% NaOCl and QMix was not statistically significant that corroborate with Ma et al [9], Sodium hypochlorite also has a potent antibacterial efficiency due to its excellent organic tissue solvent [10] and sodium also effective in aiding the mechanical flushing of debris from root canal [11]. Previous study investigated that sodium hypochlorite has been recommended as an irrigant solution in the treatment of infected root canals, because of its well-known bactericidal action[12,13]. On the contrary, Lekshmy et al [14], Menezes et al [15] and Ahangari et al [16] observed that NaOCl is a weak antibacterial irrigant in comparison with the other irrigants, this discrepancy might be related to the difference in the concentration as this study was done at concentration of 5.25% while others used 2.5% concentration.

Chlorhexidine, came next to NaOCl with also no statistically significant difference with both QMix and NaOCl as mentioned with Heling and Chandler[17]. The antimicrobial effect of CHX is mediated by several mechanisms. It binds electrostatically to negatively charged sites on bacteria. CHX causes the osmotic balance to be lost, resulting in leakage of intracellular material. It also binds to hydroxyapatite and soft tissues, changing their electrical field to compete with bacterial binding [17]. Surfactant have been added, as it increases the efficiency of an endodontic irrigant by reducing its surface tension and increasing the fluid flow over the debris on the root canal walls[18]. In other words, if a root canal irrigant can easily spread over a dentine surface, irrigation efficiency may improve. Also, once a given dentine surface has been treated with one irrigant, the spreading of another irrigant would change [19]. However, Wang et al [20] reported that six percent NaOCl and QMix had stronger antibacterial effects against young and old E. faecalis biofilms in dentin than 2% NaOCl and 2% CHX. The difference between the results of Wang et al [20] and the present investigation can be explained by the fact that the present investigation was done on planktonic bacteria whereas their studies were done on bacterial biofilms.

Next in effect came chloroxylenol showing a statistically significant reduction in the mean values of the bacterial count but was statistically insignificant with other groups except saline. Chloroxylenol efficient antimicrobial effect is due to the mechanism of antimicrobial action which occurs by the disruption of cell membranes by preventing the uptake of essential amino acids [21]. Also likely, this agent, as for other phenolic compounds, may act on the cytoplasmic membranes, producing leakage and disruption of membrane transport [22]. Although the difference between chloroxylenol and other irrigants was statistically insignificant but it was weaker than QMix, NaOCl and chlorhexidine plus. This was consistent with Schafer and Bossmann[23] who demonstrated that there were no

significant differences between the antimicrobial activity of chlorhexidine 2% and chloroxylenol 10%. In contrast Aly and Maibach[24] observed that the use of chlorhexidine achieved significantly greater adjusted mean log bacterial count reduction than chloroxylenol at all samples. This discrepancy might be related to the different bacterial type with different media and technique.

The present results led to the conclusion that propolis is effective against *E.* faecalisafter biomechanical preparation. However, it was weaker than other groups though statistically insignificant. It is a potent antimicrobial, antioxidant, and anti-inflammatory agent. The main chemical elements present in propolis are flavonoids, phenolics, and various aromatic compounds. Flavonoids are well-known plant compounds that have antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties[25]. Some components present in propolis extract, like flavonoids (quercetin, galangin, pinocembrin) and caffeic acid, benzoic acid, cinnamic acid, probably act on the microbial membrane or cell wall site, causing functional and structural damage. Many properties have been described for propolis, including antibacterial, antiviral, antifungal, and antiprotozoan activities[26-29]. The antibacterial activity of propolis is variable, which depends primarily on its origin due to its flavonoids contents [30, 31, 32]. However, irrigation with propolis glycolic extract was not effective enough to neutralize the endotoxins [33]. For this reason, it is not possible to report the exact microbial activity of very well defined composition of propolis theoretically [32]. The present study coincides with Carbajal Mejia [34] who demonstrated that there was no significant difference between CHX and propolis in reducing *E. faecalis*. *On the contrary* Kandaswamy[35] observed that chlorhexidine has better antimicrobial efficacy than propolis. This controversy can be explained by the fact that there is difference in time of the irrigation as he kept the irrigant in the canals for one, three and five days.

CONCLUSIONS

All groups except the saline produce significant reduction of bacteria. Although QMix, NaOCl and chlorhexidine plus are capable of reducing bacterial count their effect is statistically insignificant.

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