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INVESTIGATING THE POTENTIAL OF 2% CHLORHEXIDINE AS A VEHICLE FOR ENHANCING THE ANTIBACTERIAL EFFECT OF CALCIUM HYDROXIDE AND TRIPLE ANTIBIOTIC PASTE INTRACANAL MEDICAMENTS IN PRIMARY TEETH

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ABSTRACT

Objectives: The objective of this research is to investigate the antibacterial effect of mixing 2% Chlorhexidine with calcium hydroxide or triple antibiotic paste intra-canal medicaments.

Materials and Methods: Forty-five single rooted primary teeth were utilized in this study, after the basic chemico-mechanical root cleaning, the roots were sorted randomly into four groups (I- IV) according to the intra-canal medicament used such that; Group I: calcium hydroxide, Group II: triple antibiotic paste, Group III: calcium hydroxide in a 2% chlorhexidine solution and lastly Group IV: triple antibiotic paste in a 2% chlorhexidine solution.

Results: The least antibacterial effect was observed in the pure calcium hydroxide group, the antibacterial effect of calcium hydroxide and triple antibiotic paste is enhanced when mixing the powder components in chlorhexidine solution instead of distilled water, yet the increase of the antibacterial activity is not statistically significant.

Conclusions: Multidrug intracanal medicaments are more effective than over single drug medicaments to counteract resistant bacteria in necrotic root canals of primary teeth, in addition chlorhexidine appears to be a viable vehicle for calcium hydroxide and triple antibiotic paste.

KEYWORDS: intra-canal medicaments, necrotic primary teeth, antibacterial.

INTRODUCTION

The most significant factor for the success of endodontic treatment in teeth with infected pulps is the elimination of the bacterial load inside the pulp system [1] and despite the important role of the biomechanical preparation, it is not capable alone of complete eradication of microorganisms in the root canals as bacteria in the deeper layers of infected root dentin may occasionally remain and cause periapical impediment even after conventional root canal treatment [2,3]. Management of chronically

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infected primary teeth is even more challenging owing to the inherent limitations including the complex internal antomy, root resorption, difficult mechanical debridement and instrumentation in addition to the polymicrobial nature of infection which would further complicate the procedure and make entire desinfection almost impossible [4,5]. Hence, intracanal medicaments placed in the canals and left there in between appointments had been introduced in order to acheive better root canal sterilization which in turn improves the treatment outcomes [6].

Calcium hydroxide (CH) is traditionally used as the intracanal medication of choice for permanent teeth with necrotic pulps, mainly because its highly alkaline pH (around 12.5) which provides an excellent antibacterial activity [7,8] and capacity to inactivate bacterial endotoxin [9,10]. However it was not found to be very effective against some bacterial species, especially Enterococcus faecalis (E. faecalis). This bacterial strain occurs to be one of the most prevalent and resistant bacterial species in both deciduous and permanent teeth [8,11], it is also capable of tolerating starvation and high pH. Its ability to penetrate into dentinal tubules and creation of resistant species to antibiotics has made it very difficult to eradicate from root canals [12,13].

Other antibacterial intracanal medicaments have been introduced like chlorhexidine 2% (CHX), either alone or in combination with CH. Chlorhexidine (CHX) is a potent antiseptic with a broad antimicrobial spectrum and high substantivity. It is commonly used as interappointment root canal medication. Furthermore, it has been added to CH to improve its antibacterial properties and to produce synergistic effects [14-16].

Triple antibiotic paste (TAP) has been also experimented in necrotic pulp canals with varying degrees of success [11,17]. However, it was asserted that TAP yielded better results when it comes to root canal sterilization while being enclosed in the infected canals for a period of 2 weeks compared to single agents as CH [6, 18] also, better clinical results were achieved on medicating the root canals of primary molars with TAP instead of the conventional pulpectomy procedures using formocresol [6]. Given the paucity of data in the primary dentition, this study was carried out in order to investigate the antibacterial effect of chlorhexidine based intra canal medicament combinations against E. faecalis.

MATERIALS AND METHODS

Study Design

An in-vitro experimental design was adopted to conduct the current study.

Sample Size Estimation:

A power analysis was designed to have adequate power to apply a two-sided statistical test of the research hypothesis (null hypothesis) that there is no difference between the tested intra-canal medications regarding the antibacterial activity against E. faecalis. According to the results of Kaur et al. [19], using an alpha (α) level of 0.05 (5%) and a Beta (β) level of 0.20 (20%) i.e. power=80%; the predicted sample size was estimated to (N=10) samples per test group.

Methodology

Forty-five (N=45) freshly extracted single rooted primary teeth were included in the study. The selected teeth had complete sound roots, showed no cracks or any sign of resorption [20]. Teeth with curved roots, canal calcifications and root caries were excluded from the study. Any soft tissues on the root surfaces were slightly removed by means of a periodontal curette. Collected teeth were placed in 5.25% NaOCl for 1 hr. in order to disinfect the root surfaces and the samples were stored in physiologic saline at room temperature [8].

All the teeth were decoronated at the cemento-

enamel junction, using a diamond disc mounted on a high-speed handpiece using copious irrigation [21]. Then root canals were mechanically prepared using hand files (k-type) *, and enlarged to size #40 to standardize the diameter of the root canals, during cleaning and shaping, 2 ml sterile distilled water was used after each instrument size. Finally, the canals were flushed with 5 mL of distilled water to remove any debris [22]. The apical foramina were then sealed with light-cured composite resin** to prevent bacterial leakage [15].

Prior to bacterial inoculation, all the specimens are sterilized individually in the autoclave under 121 °C and 15 PSI pressure for 20 minutes, then they were immersed in a 24-hour pure culture suspension of E-faecalis grown in Brain Heart Infusion (BHI) broth and incubated at 37 °C in sealed vials. This procedure was repeated every second day using a 24-hour pure culture suspension for a period of 21 days. In the end of the inoculation period, the canal contents were aspirated, and each canal was rinsed with 5 ml saline using a sterile endodontic needle, then dried with sterile paper points [23]. (N=5) teeth were used as the negative control, they were not medicated to ensure a suitable environment for bacterial growth. The rest of teeth were sorted at random into four experimental groups (N=10) and the test medicaments were applied to the infected roots as follows:

Group I: CH*** powder was mixed with distilled water in a ratio of 1.5:1 (weight/volume) to obtain a paste-like consistency [24].

Group II: Triple antibiotic powder (Triple antibiotic powder was prepared by mixing equal proportions of Metronidazole 500 mg, Ciprofloxacin 500 mg and 100 mg Doxycycline) mixed with distilled water until a creamy mix is obtained [25].

Group III: CH powder was mixed with 2% CHX gluconate solution**** in a ratio of 1.5:1 (weight/volume) to obtain a paste-like consistency.

Group IV: Triple antibiotic powder was mixed with 2% CHX gluconate solution until a creamy mix is obtained.

Later, all samples were kept in labelled vials in a humid environment at 37 °C for one week in the incubator. After the 7-day period, all the samples irrigated with 20 ml sterile saline solution to remove the root canal contents. An endodontic file #15 H was placed into the canal and was circumferentially filed for 10 seconds before sterile absorbent paper points could absorb the transport fluid and transferred it to a test tube containing 1.0 ml of sterile saline [26].

Two sterile absorbent paper points were used for each root to absorb the irrigation fluid and transferred to a test tube containing 1.0 ml of saline and vortexed for twenty seconds and 10-fold dilutions were prepared in saline. Aliquots of 0.1 ml were spread plated onto BHI agar plates, incubated at 37°C for 48 hours. Visible colonies of E. faecalis were counted in every plate and the number of colonies/plate was multiplied by the corresponding dilution factor and by 10 to determine the total colony forming units (CFU) per ml of sample [27].

Statistical analysis was performed with IBM*****® SPSS******® Statistics Version 26 for Windows. The results of colony-forming units per millilitre from all test groups were transformed at decimal logarithms for data standardization. Descriptive statistics, including means and standard deviations were calculated for each group. Intergroup comparisons were done using one-way ANOVA followed by Tukey's post hoc test. Probability values of $P < 0.05$ were set as the reference for statistically-significant results.

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RESULTS:

The negative control group (inoculated and not medicated) displayed mean and standard deviation of (M = 55.43, SD = 13.4) CFU/mL. As presented in (Table 1 and Fig.1) all the test groups showed significant reduction in *E. faecalis* counts when compared to the control group. Group I (CH+ distilled water) showed the least antibacterial effect. Furthermore, test groups containing TAP (group II and IV) showed statistically significant lower values compared to test groups containing CH (I and III), thus reflecting better antibacterial effect. The lowest counts of bacterial colonies was obtained in group (IV) (TAP+CHX) when compared to other medicaments. Although CHX based formulations (group III and IV) showed more antibacterial effect against *E. faecalis* compared to their distilled water-based formulations (group I and II) counterparts, yet the difference between them was not statistically significant.

TABLE (1) Antibacterial effect of different intracanal medicaments described as the mean and standard deviation of (CFU/mL) of *E. faecalis*

Groups	Mean	SD
(I)	23.40 [a]	2.70
(II)	3.20 [b]	1.3
(III)	18.15 [a]	4.5
(IV)	2.16 [b]	1.2
p-value	<0.001*	

Different superscript letters indicate a statistically significant difference within the same column

** significant ($p \leq 0.05$)*

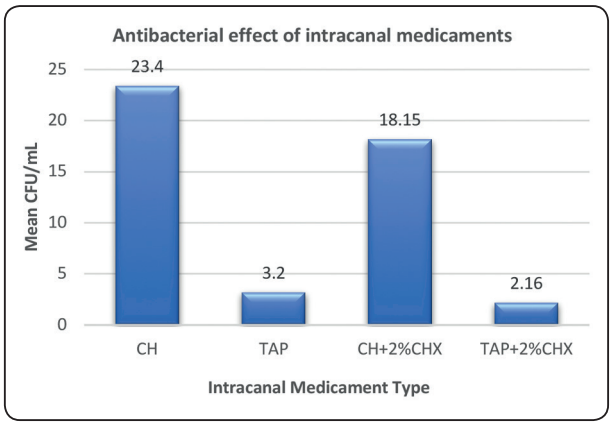

Fig. (1) Antibacterial effect of the intracanal medicaments against E. faecalis

DISCUSSION

Considering the fact that the ribbon-shaped pulp spaces of primary teeth can not be adequately disinfected following the routine mechanical debridement and even chemical irrigation, an intra-canal medication is required is such conditions to eliminate residual infection and bacterial load [18]. To test the efficacy of various intracanal medicaments, *E. faecalis* was considered as the test strain since it is usually isolated from resistant apical lesions, and it was shown to penetrate dentinal tubules which renders it more challenging to eliminate through various endodontic maneuvers [28,29].

In attempt to eliminate infection in primary teeth and reduce the incidence of recurrence, several medicaments have been investigated with diverse success rates. Calcium hydroxide (CH) has been considered the gold standard of intracanal medicaments, and despite of that, *E. Faecalis* displayed some resistance to CH, this has prompted widespread search for a more effective intracanal medicament. Following that, several studies described that single component intra-canal dressing is unlikely to be effective in sterilizing the root canals, therefore, multi-agent medicaments are considered more successful [18, 30,31].

Water-based vehicles (distilled water and CHX solution) were utilized in all the study groups, since it was postulated by Silva et al. [32] that water-based medicaments yielded better penetration of the canal walls compared to more viscous formulations (gel) with the same constituents. In addition, CHX with high concentration of 2% was used since it is more bactericidal as it results in precipitation of the bacterial cells cytoplasmic contents and hence cell death when compared to lower concentrations [33].

Given the change in the ecosystem of infected root canals, and apparent mutation of the existing bacterial genetic makeup, it is unlikely that a single antimicrobial agent could effectively sterilize the infected root canals, for this reason a combination of these agents is preferred through possible additive or synergistic effect to counteract this diverse and resistant microflora [18], it has been also proposed that these combination are more effective in eliminating the development of resistant strains [34].

Based on this finding, triple antibiotic paste (TAP) was used to achieve complete disinfection. The combination that appears to be of best results consists of metronidazole, ciprofloxacin, and minocycline [35]. Not only has it proved to be microbicidal and biocompatible thus optimal in regenerative procedures since it's not toxic to stem cells but also it has been considered as the most successful in achieving complete disinfection of infected root canals [36].

In an attempt to find the most potent antibacterial combination, we considered using CHX as a vehicle for either CH or TAP to find out whether it could achieve better antibacterial effect compared to a neutral vehicle thus achieving better disinfection.

The results of the current study showed that the antibacterial effect of TAP was significantly higher than CH whether being mixed with distilled water or CHX solution. This goes in accordance with the results obtained by Adl el al. [37], who also concluded that TAP yielded better antibacterial effect compared to CH, as the former intracanal medicament has been able to penetrate deeper in dentinal tubules and eradicate the bacterial load in even a shorter duration compared to CH, as reported by the same authors. Furthermore, Zancan et al. [38], reported that TAP had better antibacterial effect against E. faecalis than CH or even CH mixed with double antibiotic paste (DAP), the authors argued that despite the high OH^- (hydroxyl ions) released by both CH and CH/DAP and hence high alkalinity, it has however adversely affected the biological effect of the DAP by hampering its penetration into the bacterial cells, the authors further advised against such combination.

Although, it has been stated that the combined use of CHX and CH may generate excessive reactive oxygen species, which may potentially kill various root canal pathogens and yet, the alkalinity of CH mixed with CHX remains unchanged [39]. Therefore, an additive or synergistic effect between the two medications is expected. Our results, however, show that despite the antibacterial effect of the combination is slightly higher than CH alone, yet no statistically significant difference was noted. The additive/synergistic effect was also refuted by Onçag et al. [11], as they indicated that although the combination was significantly better than CH alone, yet a 2% CHX gel utilized as a single component intracanal medicament was the most effective against E. faecalis.

Haenni et al. [40] disapproved of the additive or synergistic effect between CH and CHX in combination and they justified this by possible deprotonation of CHX at the high pH generated by CH which alters the interaction of the former medication with the bacterial cells. A lower concentration of CHX (0.5%) was utilized in the previous study, so this could be the reason why the researchers could not trace any improvement with CH+CHX combination, which is not the case in the present study or that of Onçag et al. [11] as the maximum concentration of CHX was used instead.

Although the maximal antibacterial effect was noted in TAP+CHX combination group, yet the difference in the antibacterial effect between this combination and TAP in a neutral carrier was not statistically significant. Dutta et al. [18] in their in-vivo study, utilized similar medication combinations as intracanal medicaments that were placed for 7 days after mechanical debridement of the infected root canals, the microbial samples obtained from their patients showed a significant reduction in *E. Faecalis* counts in all the study groups including the test group of TAP+CHX, and likewise the CH group displayed the least antibacterial effect. The authors inferred that any drug combination will be effective in addressing even resistant bacteria commonly seen in infected primary root canals rather than a single medicament.

Apparently, TAP can perform efficiently on its own or in combination, as it was also mixed with CH in one of the test groups that were inspected by Hanin et al. [41] in their in-vitro study conducted in 2020. Based on their results, the researchers concluded that combining both medicaments result in a better antibacterial efficiency that was depicted by having the widest inhibition zones on agar plates contaminated with *E. Faecalis* compared to those of each medicament alone, they further claimed that both medications could have potentiated the bactericidal activity of each other to achieve better antimicrobial activity.

CONCLUSIONS

Based on the results of the present study, it can be concluded that, multidrug intracanal medicaments could be considered to be more efficient than pure calcium hydroxide in counteracting resistant infections in necrotic primary teeth and that Chlorhexidine could be considered as a successful vehicle for intracanal medicaments as CH and TAP.

Moreover, the best antibacterial effect against *E. Faecalis* could be achieved by utilizing an intracanal medicament combination of TAP mixed in 2% CHX solution in the infected canals of primary teeth.

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