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COMPARATIVE EVALUATION OF 6% CITRIC ACID AND CHITOSAN AS IRRIGANTS ON ROOT CANALS OF PULPECTOMIZED PRIMARY TEETH. (A CLINICAL, RADIO GRAPHICAL AND MICROBIOLOGICAL STUDY)

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ABSTRACT

Objectives: In primary teeth, the clinical success of pulpectomy depends on the eradication of pathogenic microorganisms from the infected canals. Thus, this study was directed to evaluate the antimicrobial effect of citric acid and chitosan in comparison with sodium hypochlorite (NaOCl) on E. faecalis. S**ubjects and Methods:** A total of 30 children aged between 4-7 years were included in the present study. The enrolled children had primary carious teeth that were indicated for pulpectomy treatment. The involved teeth were categorized into three main groups according to the type of irrigant solution; 6% citric acid (A), 6% chitosan (B), and 1% NaOCl (C). Then each main group was further subdivided into non-activated (A1, B1, and C1) or ultrasonic activated (A2, B2, and C2). *E. faecalis* count was carried out using a colony-forming unit (CFU). **Results:** All tested irrigants with and without activation showed a significant reduction in *E. faecalis* count. The ultrasonic activation significantly improves the antibacterial effect of the tested irrigant. In comparison between the tested irrigants, there was a significant difference. The higher reduction in *E. faecalis* count was recorded with 1% NaOCl followed by 6% chitosan. The use of 6% citric acid has a lower effect among the tested irrigants. **Conclusion:** Activation of irrigant solution significantly improves their antibacterial effect. chitosan has a significant effect on the reduction of *E. faecalis* count. Citric acid cannot be used alone as an irrigant solution.

KEYWORDS: Ultrasonic Activation, Citric Acid, Chitosan, Enterococcus faecalis, Primary Teeth

INTRODUCTION

The clinical success of pulpectomy treatment in primary teeth depends strongly on attaining prepared root canals with sufficient disinfection levels[(1)]. The clinical evidence revealed that mechanical preparation of root canal with hand or rotary instruments has a narrow disinfectant effect as it leaves the marked amount of infected debris or necrotic tissues inside the root canals [(2)]. Hence, there is an increased need for the employing of chemical or natural agents to effectively clean the pathogenic microorganisms from the infected root canals [(3)].

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NaOCl is a popular endodontic irrigant solution and is marked as the gold standard for root canal disinfection during pulp therapy [4]. This is because of its sufficient fluidity which allowing the smooth introduction into the complicated root canal architecture, as well as due to its acceptable storage time, feasibility, and economic price [4,5]. However, the antimicrobial action, as well as cytotoxicity of NaOCl, is concentration-dependent [6].

Citric acid is a weak organic acid used in endodontic therapy because of its effectiveness in dissolve the smear layer as well as the inorganic components of root dentine with no or little effect on organic components [7]. Citric acid has a chelating action similar to EDTA "ethylenediamine tetra-acetic acid" but with lower cytotoxicity on preapical tissues [8].

Chitosan, a natural biopolymer of polysaccharide, is used in dental practice recently due to its biocompatibility, biodegradability, and broad antimicrobial activity [9]. Chitosan also has chelating activity through its ability to eliminate effectively the smear layer and increase the dentin tubule's permeability without marked dentinal erosion [10].

However, the clinical evidence exhibited that no single irrigant solution can be considered as an ideal solution in root canal disinfection because of their limited performance in the complicated root canal system especially in the apical third [11]. Therefore, endo activator such as ultrasonic activators has been introduced to overthrown such limitations[12]. Ultrasonic vibration is used in combination with irrigant solution to facilitates the penetration of irrigant solution deeper into the dentine via increasing dentine permeability [11,12].

Thus, the present study was directed to evaluate clinically the efficacy of citric acid and chitosan as root canal irrigants against *E. faecalis* microorganisms, and compare it with NaOCl in primary teeth during pulpectomy.

SUBJECT AND METHODS

This randomized controlled clinical study was conducted on children aged from 5-7 years after approval of Ethical Committee, Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo) with approval reference No (EC Ref No.471/1715). The enrolled children were elected from outpatients of the Pedodontics and Oral Health Department, Faculty of Dental Medicine, Al-Azhar University (Cairo, Boys).

The current study involved thirty carious primary teeth registered for pulpectomy treatment.

Inclusion criteria:

1. Parents/caregivers acceptance and cooperation.
2. Children aged between 4 to 7 years.
3. Children with deep carious primary teeth with uncontrolled bleeding after pulp amputation.
4. Children with at least one primary tooth with symptoms and signs of irreversible pulpitis such as abscess, sinus tract, spontaneous pain, tenderness to percussion, and obvious radiolucency.

- **Exclusion Criteria:**

1. Children with parents refused to participate in the study.
2. Children with age less than 4 years and more than 7 years.
3. Children with a history of any systemic condition or drug medication that can affect their immunity or cooperation.
4. Children with mobile tooth that indicted for extraction.

The involved teeth were randomly divided into three equal main groups (n=10) according to the type of irrigant solution; group A; 6% citric acid, group B; 6% chitosan solution, group C; 1% NaOCl

as a control group. Then, each main group was further subdivided into non-activated (A1, B1, and C1) or ultrasonic activated (A2, B2, and C2).

The sample size for the current study was determined based on the results of the previous studies by Demirel et al (2019) [13] and del Carpio-Perochena et al (2015) [14].

Subject selection:

Before starting this study, all selected children and his/her parents/caregiver were informed about all the procedures used in this clinical study. Then, each parent's/care giver signed an informed consent having details about the whole clinical procedure.

Subjects enrolled in the present study should have no history of; any drug medication that can affect their immunity or cooperation, any systemic disease. However, children with a tooth that indicted for extraction due to tooth mobility or root resorption more than 1/3 of radiographic length were excluded from the present study [15].

Preparation of citric acid solution:

A 50% (wt./vol.) solution of citric acid was prepared from citric acid powder (Al-Gomhoria Co., Egypt) that was mixed with distilled water at room temperature. Then, further dilution with distilled water was done to prepare a concentration of 6% citric acid. A pH of 6 of the citric acid solution was adjusted with NaOH [16,17].

Preparation of chitosan solution:

The preparation of 6% chitosan solutions was performed using 6 grams of chitosan powder (Sigma Co., Egypt), diluted in 100 ml of 1% acetic acid, and the mixture was mixed by using a magnetic stirrer until the chitosan particles completely dissolved in the acetic acid liquid. The chitosan solution was adjusted with NaOH solution to maintain a pH of 3.5 [15].

Operative procedures:

Pulpectomy procedures:

Following confirming the dental examination along with periapical radiographs, the procedure was performed as follows: local anesthesia, followed by placement of a rubber dam. Then, caries was removed with a high-speed handpiece and diamond bur. After that, access cavity preparation by a sterile fissure bur. The elimination of the pulp from the pulp chamber and root canals was carried out with barbed broaches. The root canal instrumentation was carried out up to file size 35 and was shorter by 1mm than the radiographic apex [18].

Irrigation protocol and activation:

Root canal irrigation and sterilization were conducted with periodic irrigation for 30 seconds with a 5 mL sterile syringe (side-perforated needle with a soft tip) containing either 6% citric acid, 6% chitosan, or 1% NaOCl solutions with or without ultrasonic activation [18].

The ultrasonic activation using U-file [MANi medical company, Japan] mounted to E2 tip screwed to ultrasonic device [WOODPECKER UDS-P LED ultrasonic scaler, China](output power : 3W to 20W , Frequency : 28kHz 3kHz) conveyed irrigant was achieved after typical irrigation, for the teeth in groups (A2, B2, and C2). The ultrasonic tip was inserted into the root canals to 3 mm away from its working length and was activated. Then, the activated ultrasonic tip was moved up and down to shake the solution in repeated cycles for 5 times at low power (3W), while the irrigant solution was renewal after each cycle [19].

Restoration procedures:

After ending the irrigation protocols, the root canals were dried with sterile paper points. Then, the prepared roots were obturated with zinc oxide eugenol using a Lentulo spiral filler and the pulp chamber was sealed with glass ionomer cement. Finally, the involved teeth were restored with stainless steel crowns [18].**(Figure 1)**

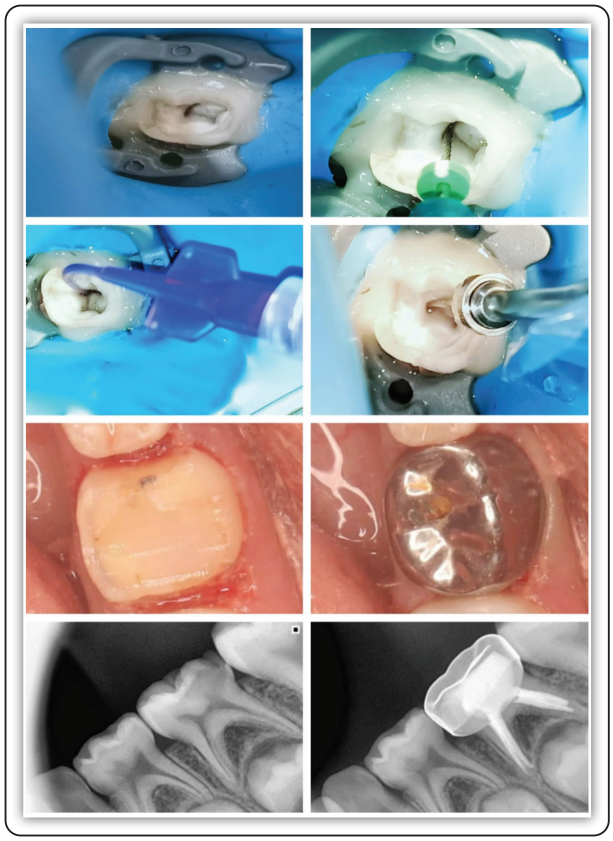

FIG (1) Full case pulpectopmy (Access cavity, mechanical preparation, irrigation. Activation, Extra-coronal Restoration, Pre & Postoperative x-ray)

Microbiological Analysis:

The base-line bacteriological samples (S1) were taken from the full working length of the involved root canal after access opening by introducing of sterile wet paper point of size 35 for 1 minute. After that, each collected paper point was placed immediately in a tight screw sterile container with 2-ml of bacterial diluent media (peptone water liquid media) as transfer media that was able to keep the microorganism viability. Then, similarly, the second bacteriological samples (S2) were immediately collected after finishing the irrigation protocols [20].

All collected bacteriological samples were transported, under complete aseptic condition, as soon as possible to the microbiological lab at the regional center for microbiology and biotechnology (Al-Azhar University, Cairo, Egypt), for culture procedure on the selective media (Enterococci agar media). The plates were incubated in an anaerobic chamber for 7 days at 37°C. Then, the *E. faecalis* organisms were identified and the total number of colonies on the incubated plates were counted with the help of a digital colony counter and expressed as CFU/ml.

Statistical analysis:

The collected data were tabulated and analyzed statistically using ANOVA test for quantitative data with normal distribution to compare between groups and Paired t-test to compare the same group means before and after different treatments. The results were statistically significant at $p<0.05$.

RESULTS

There was a no-statistically significant difference at the baseline counting of *E. faecalis* along with the study. However, there was a significant difference in *E. faecalis* counting after irrigation with and without ultrasonic activation. However, in groups (B1 and C1) there was no statistically significant difference (Table 1).

The statistical results of paired t-test showed a significant decrease in *E. faecalis* counting after the use of all irrigant solutions with and without ultrasonic activation (Table 1). However, the use of ultrasonic activation significantly decreases the *E. faecalis* counting when compared to the non-activated groups except for citric acid irrigant solution (Table 2).

Table (1) Comparison of *E. faecalis* count (CFU/ml) before and after irrigation with and without ultrasonic activation.

Variable Mean ± SD		Base-line	After irrigation	p-value
		Mean ± SD		
Without ultrasonic activation	Citric acid (A1)	2904.00±117.18	2670.00^{A} ±148.32	0.0031*
	Chitosan (B1)	2910.00±167.33	1336.00^{B} ±61.07	<0.0001*
	NaOCl (C1)	2856.00±111.49	1174.00^{B} ±95.55	<0.0001*
	p-value	0.78833^{ns}	<0.00001*	
With ultrasonic activation	Citric acid (A2)	2926.00±126.02	2510.00^{A} ±89.44	0.0074*
	Chitosan (B2)	2920.00±125.50	1000.00^{B} ±50.00	<0.0001*
	NaOCl (C2)	2920±130.38	798.00C ±78.23	<0.0001*
	p-value	0.9963 ^{ns}	<0.00001*	

**; significant at p < 0.05.* *; non-significant at p >0.05.*

Table (2) Comparison of percentage of reduction in *E. faecalis* count (CFU/ml) after irrigation with and without ultrasonic activation.

Variable		% of reduction	*t*-value	*p*-value
Citric acid	Without activation (A1)	8.07±2.87	2.0614	0.0732^{ns}
	With activation (A2)	14.04±5.80		
Chitosan	Without activation (B1)	53.92±4.33	5.3848	0.0007*
	With activation (B2)	65.70±2.27		
NaOCl	Without activation (C1)	58.93±2.15	8.5597	<0.0001*
	With activation (C2)	72.64±2.86		

**; significant at p < 0.05.* *; non-significant at p >0.05.*

DISCUSSION

The ultrasonic activation is the use ultrasonic device to promotes the mechanical agitation of the irrigant solutions inside the root canal without direct contact with the root canal wall [21]. The use of ultrasonic activation as an auxiliary tool in the root canal disinfection in the present study because it is a technique able to improve cleaning and disinfection of the root canal system [22].

The selection of *E. faecalis* as a tested microorganism in this study was based on their marked role in the failure of the endodontically treated teeth as well as it has a significant role in the incidence and persistence of the periapical lesion even after endodontic treatment [23].

In the present study, NaOCl was used as a control group because of its proven antimicrobial action with sufficient fluidity which allowing the smooth introduction into the complicated root canal architecture [4,5]. Furthermore, in the present study 1%, NaOCI was chosen for appropriate concentration at primary teeth root canal treatment with the lower cytotoxicity [6].

Citric acid is was selected as a tested irrigant solution in this study because of its effectiveness in dissolve the smear layer as well as the inorganic components of root dentine with no or little effect on organic components[7]. However, a concentration of 6% citric acid was chosen in the present study because of its lower cytotoxicity when compared to other concentrations as its toxicity is dose-dependent [24].

Also, chitosan was chosen as a tested irrigant solution in the current study due to its biocompatibility, biodegradability, chelating activity, and broad antimicrobial activity [9,10]. Moreover, a concentration of 6% chitosan also was chosen in the current study because the antimicrobial activity of chitosan is concentration-dependent [10].

The results of this study detected no statistically significant differences in the counting of *E. faecalis* in the infected root canals before the use of different irrigation protocols among all tested groups with and without ultrasonic activation. This could refer to standardization for bacterial counts in all groups[25].

The results of this study showed that the use of NaOCl irrigation significantly decreases the count of *E. faecalis* in the infected root canals. This is because that the antimicrobial activity of NaOCl results from releasing the hypochlorous acid as well as by strong oxidation action of sulfhydryl groups against bacterial enzymes which result in an interruption to the metabolic process and bacterial death[26].

Also, the results of this study revealed that chitosan exhibited a significant effect in the reduction of E. *faecalis* count. This is because chitosan exhibits antimicrobial activity due to the electrostatic interactions between amino group (NH^{3+}) which binds to the bacterial cell surface and hence altering the cell permeability and results in the leakage of intracellular components and finally cell death [10]. Moreover, chitosan can attach to bacterial DNA by passing to the microorganisms' nuclei and interfering with proteins synthesis [27].

However, the results of the use of 6% citric acid as an irritant solution in this study resulted in a decrease in *E. faecalis* count. This could be due to the elimination of the inorganic part of the smear layer and its *E. faecalis* content after the first rinsing of the root canal [28].

Furthermore, the results of the current study found that the use of ultrasonic activation increases the activity of the irrigant solutions against *E. faecalis*. This is because the use of ultrasonic activation facilitates the penetration of irrigant solution deeper into the dentine via increasing dentine permeability as well as improves its performance through the formation of more active ingredients [11,12].

CONCLUSION

Based on the findings of the study, it could be explored that the use of 6% chitosan as an irrigant solution was effective in decreasing the *E. faecalis* count during pulpal treatment in primary teeth. However, citric acid cannot be used alone as an irrigant solution. Moreover, the ultrasonic activation improves the antimicrobial activity of the irrigant solution.

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