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Evaluation of Antibacterial Efficacy of 6% Citric Acid Versus Chitosan Irrigant Solution For Primary Anterior Teeth Root Canal

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KEYWORDS

Antibacterial efficacy, Chitosan, NaOCl, 6% citric acid, Root canal irrigation

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ABSTRACT

Aim: To evaluate and compare the antibacterial efficacy of 6% citric acid versus chitosan irrigant solution against Enterococcus Faecalis bacteria in treatment of root canals of primary anterior teeth **Subjects and Methods**: This in-vivo study was carried out on thirty anterior primary teeth of children attending the outpatient clinic of Pediatric Dentistry and Dental public health department Faculty of oral and dental Medicine, South Valley University. Ranging from three to six years of age. The teeth were equally divided into six groups (5 teeth each), based on the irrigant used during chemo mechanical preparation of the root canals. **Results:** There was a significance difference between the action of 6% chitosan and 6% citric acid against Enterococcus faecalis. Chitosan as root canal irrigation showed a significant antimicrobial effect. Citric acid as root canal irrigation has a lower antimicrobial effect when compared to chitosan and NaOC1. The 1% NaOC1 as root canal irrigation has a higher antimicrobial effect. **Conclusion:** The use of 6% chitosan exhibited a comparable result to 1% NaOC1 clinically and microbiologically.

INTRODUCTION

The success of endodontic therapy in primary teeth strongly depends on achieving an adequate level of disinfection within their root canals, Evidence has shown that the mechanical instrumentation technique with files has limited disinfection efficacy because it tends to leave significant portions of the infected canal walls untouched; thus, an important number of viable pathogenic microorganisms persist, lodged together with dentin debris and necrotic pulp- tissue remnants inside the dentinal tubules, canal ramifications. Therefore, it is necessary to significantly reduce or to eradicate to the extent possible the microorganisms and their by-products present to the pulp canals by employing clinically effective and biocompatible irrigants, which also aid in dissolving organic debris. However, none of the available irrigating solutions has been regarded clearly an optimal solution because of their limited effectiveness especially in apical third of the root canal system. Use of endo-activator has been proposed as an irrigation facilitator that is based on sonic vibration which facilitates the irrigants penetration and its renewal within the canal.

Primary root canal infections are polymicrobial, typically dominated by obligatory anaerobic bacteria. The most frequently isolated microorganisms before root canal treatment include gram- negative anaerobic rods, gram-positive anaerobic cocci, gram-positive anaerobic, and facultative rods, Lactobacillus species, and Gram-positive Facultative Streptococcus species.⁽¹⁾

Enterococcus faecalis (E. faecalis): One among the facultative organism is E. faecalis which has increased attention in the endodontics, as it can regularly be seen in root canals in cases of failed root canal treatments.^(2,3) Failed root canal treatment cases are nine times more likely to contain E. faecalis than primary endodontic infections.⁽⁴⁾

Sodium Hypochlorite (NaOCl) is a powerful antibacterial agent that dissolves pulpal remains and organic components of dentin in concentrations ranging from 0.5 percent to 5.25 percent.⁽³⁾

Chitosan, a natural polysaccharide, is prominently used in dentistry because it is biocompatible, biodegradable, bioadhesive, and non-toxic, with broad-spectrum antimicrobial properties and chelating activity. Chitosan has the ability to remove the smear layer and unblock dentin tubules without promoting significant dentine erosion.⁽⁵⁾

Citric acid is a chelating agent which available in gels and liquids forms, it has less cytotoxic effect than ethylenediamine tetra-acetic acid (EDTA) on per apical tissue, Citric acid can effectively dissolve the inorganic components and smear layer with little or no effect on organic tissue.⁽⁶⁾

Aim of the study

The aim of this study was directed to evaluate and compare 6% citric acid and chitosan as irrigant

solutions against Enterococcus Faecalis bacteria in treatment of root canals of primary anterior teeth.

MATERIALS AND METHODS

This in-vivo study was carried out on children aged between 3 and 6 years. The involved children were selected from thirty patients attending the outpatient clinic of Pediatric Dentistry and Dental public health department Faculty of oral and dental Medicine, South Valley University. This study involved a total of thirty carious primary anterior teeth indicated for pulpectomy from thirty patients. Inclusion criteria: Cooperative children aged between 3 to 6 years, Children with no history of any drug medication that can affect their immunity or cooperation, Healthy children without history of any systemic disease, with history of spontaneous, unprovoked toothache with eating, deep carious primary teeth with uncontrolled bleeding after pulp amputation and 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: Uncooperative children with parents refused to participate in the study. Each parent's/ caregiver was signed an informed consent having details about the whole clinical procedure.

A total of 30 primary teeth requiring pulpectomy treatment were selected and randomly assigned to six groups (n=5) based on the received irrigant solution and method of irrigation as follow

Group A1: 6% citric acid irrigation without activation (n=5).

Group A2: 6% citric acid irrigation with activation (n=5).

Group B1: 6% chitosan irrigation without activation (n=5).

Group B2: 6% chitosan irrigation with activation (n=5).

Group C2: 1% sodium hypochlorite irrigation with activation (n=5)..

Preparation of chitosan solution:

The preparation of 6% chitosan solutions was performed using 6 gram 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, and the pH was determined by using a digital pH meter (pH537, WTW; Wellheim, Germany). The solution was saved in the refrigerator and used within two weeks after preparation.⁽⁷⁾

Preparation of citric acid solution:

Citric acid is a weak organic acid with the appearance of white crystalline powder at room temperature.⁽⁸⁾ A stock solution of citric acid (50% wt/vol) was prepared from pure citric acid powder (ACS Co., USA) mixed with distilled water at room temperature. This solution was further diluted with distilled water to prepare 6% (wt/vol) concentrations of citric acid. Citric acid solution was adjusted with sodium hydroxide (NaOH) to maintain a pH of 6 and the pH was determined by using a digital pH meter (pH537, WTW; Wellheim, Germany).⁽⁹⁾ The solution was saved in the refrigerator and used within two weeks after preparation.⁽⁸⁾.

Preparation of Sodium Hypochlorite:

Sodium hypochlorite (NaOCl) is the most widely used irrigant because of its antibacterial and tissue dissolving properties, 1% NaOCl is a more effective irrigant in debris removal in deciduous root canals.⁽¹⁰⁾ 8.5 ml of 6% medical sodium hypochlorite solution (DHARMA Co, USA) is diluted by 50 ml distilled water to prepare a 1% concentration.

Operative Procedures:

Administration of local anesthesia and application of a rubber dam, then caries were removed and, Access cavity was gained on the palatal surface of each tooth using sterile round bur No 2 or No 4 and long tapering diamond point in a high speed hand piece with water coolant. The working length of canal was determined by Apex locator. The root canal preparation by manual files up to file #35.⁽¹¹⁾

Irrigation protocol and activation:

Periodic irrigation with a 3 mL syringe containing 3cm of either solution with or without irrigation activation for 5minuts was used to remove organic material and sterilize the roots. After traditional irrigation, the supplied irrigant was passively activated with ultrasonic waves for the teeth in groups (A2, B2 and C2). The ultrasonic tip with U-files was passively inserted and actuated at 3 mm from the operating length. The ultrasonic tip moved up and down in short 2 mm excursions during activation to shake the irrigant. The activation cycle was carried out five times more. The irrigant was replaced with a fresh solution after each cycle to maintain the active component.⁽¹²⁾

Restoration procedures:

After irrigation with different irrigant solution the root canals were dried with paper points, the prepared roots were obturated with zinc oxide eugenol (ZOE) and the pulp chamber was sealed with glass ionomer cement (GIC). Antibiotics were administered to the children when the alveolar abscess was not drained.⁽¹³⁾

Microbiological Sample collection:

 Microbiological baseline sample (S1): The first microbiological sample (baseline) was obtained by inserting a sterile paper point with a diameter that corresponded to the canal's diameter. The paper points were left in the root canals for 60 seconds before being transferred to a screw-capped test tube containing5 ml peptone water liquid media as transfer media which act as bacterial diluent media and is able to keep the viability of the bacteria.⁽¹⁴⁾

 Second microbiological sample (S2): were taken immediately after the different irrigation protocols in a similar way as the first microbiological samples (S1), the exposed root canal is sealed with a sterile cotton pellet (without any medicament).⁽¹⁴⁾

Microbiological investigation:

All collected microbiological samples were transported, under complete aseptic condition, immediately to the microbiological lab at the regional center for microbiology and biotechnology (South Valley University, Qena, Egypt), for culture procedure on the selective media.

Using cell spreaders, the samples were streaked on Bile- esculin agar media (a selective media for E. faecalis). The plates were incubated at 37°C for 7 days in an anaerobic room. With the use of a digital colony counter, the total number of colonies on the incubated plates were counted after 7 days and expressed as total colony-forming units per ml (CFU/ml).

Colony shape, gramme stain appearance, and normal biochemical reactions were used to identify the organisms.

Data management and analysis:

The collected data were tabulated and statistically analyzed using SPSS program software, version 20. Pearson Chi-Square test was applied to gauge the difference between demographic data. Unpaired *t-test* and ANOVA test were used to compare between sample means for quantitative data with normal distribution. The results were considered statistically significant at p<0.05.

RESULTS

Comparison of E. faecalis count (CFU/ml) among the studied groups along the study:The involved root canals among the studied groups after irrigation without activation (A1, B1, and C1) and with activation (A2, B2, and C2) were compared regarding the E. faecalis count and represented as (CFU/ml), and then were statistically analyzed using One-way ANOVA test.

The statistical analysis results of the E. faecalis count (CFU/ml) after irrigation without activation and with activation, which showing (mean \pm SD) of the involved root canal among the studied groups.

There was a statistically significant difference in E. faecalis count (CFU/ml) between the involved root canal among the studied groups with level of significance of (p= 0.00000) as indicated by One-way ANOVA test.

Among the studied groups the post Tukey's test showed statistically significant difference in E. faecalis (CFU/ml) count with p-value of (p > 0.05) in-between the studied groups except for chitosan and NaOCl without activation groups (B1 and C1).

Table (1) *E. faecalis count (CFU/ml) along the study.*

Variable	Mean	S. D	<i>f</i> - ratio	<i>p</i> -value
Citric acid without activation (A1)	2670.00 ^A	148.32	- - 375.9074 -	0.00000*
Citric acid with activation (A2)	2510 ^B	89.44		
Chitosan without activation (B1)	1336.00 ^c	61.07		
Chitosan with activation (B2)	1000 ^D	50.00		
NaOCl without activation (C1)	1174.00 ^c	95.55		
NaOCl with activation (C2)	798 ^e	78.23		

*; The result is significant at p < 0.05. Different capital litters mean significantly different.





Fig. (1) Illustrated diagram showing means of E. faecalis count (CFU/ml) along the study.

DISCUSSION

The main goal of endodontic intervention is to recover the functional aspects of the affected tooth by healing or preserving the integrity of the periapical tissue, while eliminating the microorganisms from root canals to prevent subsequent reinfection.⁽¹⁵⁾ Pulpectomy technique was selected as it considered a conservative option compared with tooth extraction.⁽¹⁶⁾ During pulpectomy, irrigation is necessary as it rendering the canal system free of necrotic pulp tissues, biofilms, bacteria and bacterial products and also serves as a physical flush to remove dentinal debris. This creates an environment favorable to successful obturation, and ultimately to clinical success.⁽¹⁷⁾

The in vivo nature of this study makes isolation, access, working length determination and instrumentation more clinically relevant than in vitro studies. That is, in vitro studies can control for poor access, can determine working lengths accurately by visualizing files at the apical foramen and instrument teeth without regard to difficult access or clinical time constraints. Therefore in vivo studies may be more clinically relevant than in vitro studies.⁽¹⁸⁾

For primary molars, it was found that pulpectomy at older ages was more likely to fail than that at a younger age ⁽¹¹⁾ so, the age of 3-6 years was selected for all samples of the study.

However, 6% citric acid was chosen in the present study because its good chemical stability, shows anti-microbial effects against the facultative and obligative anaerobes and used in different concentrations,⁽¹⁹⁾ and has lower cytotoxicity when compared to other concentration as its toxicity is dose dependent.⁽¹²⁾

Chitosan was chosen as tested irrigant solution in this study because it is biocompatible, biodegradable, bio adhesive, and non-toxic, with broad-spectrum antimicrobial properties and chelating activity.⁽⁵⁾

In this study NaOCl was chosen to evaluate the antimicrobial efficiency due to its popularity in the market among endodontists. It has low viscosity allowing easy introduction into the canal architecture, an acceptable shelf life easily available and inexpensive. By far, it is the gold standard, as it has the unique property of tissue dissolution ability.⁽²⁾

Overflow of irrigating solution through the apical region in primary teeth could damage the underlying permanent tooth due to probable resorption zones.⁽²⁰⁾ In case reports, the cytotoxicity of 5.25 percent NaOCl toward periapical tissues has been mentioned. As a result, 1 percent NaOCI was chosen as the optimal concentration for primary teeth root canal therapy with the lowest cytotoxicity in the current investigation.⁽²¹⁾

The ultrasonic activation is the activation of an endodontic irrigation by an ultrasonic device placed inside the root canal. This promotes mechanical agitation of a chemical substance without instrument contact with the root canal wall.⁽²²⁾.

Selection of E. faecalis in this study was based on their important role in failure of endodontic treatment as well as it detected in 55% of necrotic primary maxillary molars and etiology of chronicity of periapical lesion.⁽²³⁾

In The present study, Selection of ZOE as root canal filling material because it permits resorption of the primary tooth root and hence it permits normal eruption of the succedaneous tooth.⁽²²⁾

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In this study a culture-dependent method was relied as a test method for counting *E*. faecalis. Because it can account on the importance of this method in identify the viable bacteria, especially once the samples are taken immediately after antimicrobial treatment $^{(24)}$.

The results of this study detected no statistically significant differences in the number of E. faecalis in the infected root canals prior to the use of different irrigation protocols among the all tested groups. This could refer to standardization for bacterial counts in all groups.⁽²⁵⁾

The results of the use of 6% citric acid as irrigant solution in this study resulted in decrease in E. faecalis count. This could be due to the elimination the inorganic part of smear layer and its E. faecalis content after first rinsing of root canal. This results in agreement with Hariharan et al.⁽²⁶⁾ as they reported that; Citric acid may have an antibacterial impact, but no in vitro or in vivo research have been done to compare it to other root canal cleaning treatments.

The results of this study revealed that chitosan exhibited significant effect in reduction of E. faecalis count. This because chitosan exhibits antimicrobial property due to the electrostatic interactions between NH³⁺ which binds to the components of bacterial cell surface thereby altering the cell permeability and which results in the leakage of intracellular components and cell death ⁽⁵⁾. Moreover, chitosan attaches to DNA and inhibits mRNA synthesis by passing to the microorganisms nuclei and interfering with mRNA and proteins synthesis.⁽²⁷⁾

Furthermore, the results of the current study found that the use of ultrasonic activation significantly increases the activity of chitosan against E. faecalis. This may be because chitosan has effectively removed smear layer from the root canals after instrumentation,⁽²⁸⁾ and the use of ultrasonic activation increasing the dentinal permeability via removal of smear layer and hence increase its effectiveness in penetrating deeper into the dentinal tubules, thus contributing to decontamination of root canal system.⁽²⁵⁾ The saponification, amino acid neutralization, and chlor-amination reactions of NaOCl that occur in the presence of microorganisms and organic tissue lead to the antimicrobial effect and tissue dissolution process.⁽⁵⁾

When comparing the three irrigant materials used in the present study regarding to its activity against *E*. faecalis it was found that 1% NaOCl has the higher effect followed by 6% chitosan without statistically significant. This may be due to the higher concentration of chitosan (6%) which used in the present study. As the previous study revealed that the efficacy of chitosan against *E*. faecalis is concentration dependent.⁽²⁷⁾

In this study both of NaOCl and chitosan have significantly higher effect in reduction of *E*. faecalis count when compared to citric acid. This because the both of NaOCl and chitosan have mechanisms against *E*. faecalis.^(2,29)

However, the effectiveness of citric acid in decreasing the number of E. faecalis depends only on the removal of its content in the smear layer without direct effect on the microorganism itself.⁽³⁰⁾

CONCLUSION

Based on the results of this study the following conclusion can be drawn:

- 1. Chitosan as root canal irrigation showed a significant antimicrobial effect.
- 2. Citric acid as root canal irrigation has a lower antimicrobial effect when compared to chitosan.

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Evaluation of Antibacterial Efficacy of 6% Citric Acid Versus Chitosan Irrigant Solution For Primary Anterior Teeth Root Canal

النشر الرسمي لكلية طب الأسنان جامعة الأزهر أسيوط مصر

الأنه_ر محلة أسبوط لطب الأسنان



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تقييم التأثير المضاد للبكتيريا لمحلول ٪٦ حامض الستريك مقابل محلول الكيتوزان كغسول لقنوات جذور الأسنان اللبنية الأماميهً

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(لملخص:

الهدف: تقييم ومقارنة الفعالية المضادة للبكتيريا بمحلول حمض الستريك بنسبة ٪6 مقابل محلول غسول الشيتوزان ضد بكتيريا ENTEROCOCCUS FAECALIS في علاج قنوات جذر الأسنان اللبنيه الأمامية.

المواد والاساليب: أجريت هذه الدراسة في الجسم الحي على ثلاثين من الأسنان اللبنيه الأمامية للأطفال الذين يترددون على العيادة الخارجية لقسم لطب أسنان الأطفال والصحة العامةللفم و للأسنان بكلية طب الفم والأسنان . جامعة جنوب الوادي. تتراوح أعمارهم من ثلاث إلى ست سنوات. تم تقسيم الأسنان بالتساوي إلى ست مجموعات (5 أسنان لكل منها) . بناءً على الغسول المستخدم أثناء التحضير الميكانيكي الكيميائى لقنوات الجذر.

النتائج: كان هناك فرق معنوي بين تأثير ٪6 من الكيتوزان و ٪6 حامض الستريك ضد ENTEROCOCCUS FAECALIS. الشيتوزان كغسول لقنوات جذورالاسنان أظهر تأثيراً معنوياً كمضاد للميكروبات. حمض الستريك كغسول لقننوات الجذر له تأثير مضاد للميكروبات أقل عند مقارنته بالكيتوزان و NAOCL. إن ٪1 NAOCL كغسول لقناة الجذر له تأثير مضاد للميكروبات أعلى

الخلاصة: . أظهر استخدام 6 ٪ من الشيتوزان نتيجة مماثلة لـ 1 ٪ NAOCL سريريًا وميكروبيولوجيًا

الكلمات المفتاحية: فعاليه مضاده للبكتريا، الشيتوزان . ٪6 حامض الستريك . غسول قناة الجذر صوديوم هيوكلوريد .

