ORIGINAL PAPER

ANTI-BACTERIAL ACTIVITY OF APPLYING CHITOSAN AND PROPOLIS DRESSING AGAINST *ENTEROCOCCUS FAECALIS* IN PRIMARY TEETH: *IN VITRO* STUDY

Bushra M. Shamma[1], Ettihad Abo Arrag[1], Anas Rajab[2], Saleh Al Kurdi[1]

[1]Department of Pediatric Dentistry, Faculty of Dentistry, Damascus University, Syria
[2]Department of Organic Chemistry, Faculty of Pharmacy, Syrian Private University (SPU), Syria

ABSTRACT

Introduction: Endodontic treatment in infected primary teeth is obligated to reserve teeth until normal exfoliation. However, achieving disinfection by bio-mechanical preparation is difficult due to internal complex nature of the root canal system. Thus, the use of intra-canal medicaments is necessary.

Objectives: This study was designed to evaluate the efficacy of chitosan and propolis dressing as intra-canal medicament against Enterococcus faecalis in primary root canal.

Material and methods: Seventy-two extracted primary second molars with two third of their roots length undamaged were collected. Teeth preparation was completed to size 30 K-file. Teeth were randomly divided into 3 groups, such as A: chitosan + propolis dressing; B: calcium hydroxide dressing; C: negative control group. The tooth specimens were inoculated with *Enterococcus faecalis*. Tested materials were applied for all groups in accordance with the groups each tooth belonged to. Bacterial colonies were counted after 24 hours, 72 hours, and 1 week after applying dressing materials. One-way ANOVA test was used to analyze differences between experimental groups. *P*-value < 0.05 was considered statistically significant and Bonferroni test was used to analyze differences between binary groups.

Results: Group A exhibited the highest anti-bacterial effect after 24 hours of applying dressing material compared to groups B and C, with statistically significant difference. However, group A and group B showed similar anti-bacterial effects after 72 hours and 1 week, with no statistically significant differences.

Conclusions: Incorporating chitosan and ethanol extract of propolis may be a promising medicament in reducing *Enterococcus faecalis* in primary root canal treatment.

Key words: chitosan, propolis, primary molars, *Enterococcus faecalis*, intra-canal medicament.

J Stoma 2022; 75, 1: 36-43
DOI: https://doi.org/10.5114/jos.2022.114496

INTRODUCTION

Primary teeth with pulpal and periapical problems should be retained until their normal time of physiologic exfoliation to reserve chewing, pronunciation, aesthetics, and eruption of permanent teeth [1, 2]. Therefore, preserving primary teeth by performing endodontic treatment is necessary [3]. This procedure is considered a major therapeutic challenge in pediatric dentistry [4] because of internal complex nature of the root canal system in primary teeth that makes it difficult to achieve proper disinfection by bio-mechanical preparation of root canal [1].

JOURNAL OF STOMATOLOGY
CZASOPISMO STOMATOLOGICZNE

Address for correspondence: Dr. Bushra Munzer Shamma, Department of Pediatric Dentistry, Faculty of Dentistry, Damascus University, Syria, e-mail: bushrashamma93@gmail.com

Received: 11.06.2021 • Accepted: 20.12.2021 • Published: 15.03.2022

One of the main keys in successful root canal treatment is disinfecting the root canal system prior to application of final filling material [5]. Although most bacterial species are eliminated after mechanical instrumentation and irrigation of canals, some of them may stay active leading to undesirable complications of the treated root canals, including periapical or root bifurcation lesions, root resorption, and alveolar abscess [2, 6, 7]. However, these microbial organisms may persist even after bio-mechanical preparation; hence, the use of intra-canal medicaments is necessary [8].

Most commonly used materials are calcium hydroxide $Ca(OH)_2$-based materials [5]. The anti-microbial activity of $Ca(OH)_2$ is related to a release of these hydroxyl ions, damaging cytoplasmic membranes and DNA of microbial cells [9].

Chitosan is a natural bio-material, derived mainly from chitin through the process of deacetylation. The main source of chitin is the exo-skeleton of shrimp and crab.

Chitosan has been recognized as an anti-microbial agent, with a broad anti-bacterial spectrum covering Gram-negative and Gram-positive bacteria as well as fungi [10-12]. In addition, it has bio-compatibility, non-toxicity, anti-inflammatory, and wound healing properties [11]. Therefore, chitosan has been used in a bio-dental application [12].

Several studies have shown the efficacy of chitosan in removing the smear layer from the root canal [13]. Moreover, chitosan was found superior to EDTA 17% in removing smear layer in the apical third of root canal [14]. The effectiveness of chitosan in reducing bacterial counts of *Enterococcus faecalis* when used as a root canal irrigation in permanent teeth, was also evaluated [15], and a significant increase in this efficacy when it was combined with chlorhexidine was observed.

Propolis or bee glue is a natural product, collected from plant by honeybees. It has many biologic activities, including anti-bacterial, anti-inflammatory, anti-oxidant, and healing properties [16, 17]. The main chemical composition of propolis are phenolics and flavonoids; they present anti-oxidant, anti-bacterial, anti-fungal, anti-viral, and anti-inflammatory proprieties [18].

Due to its' anti-bacterial properties, propolis has been used in a wide range of medical and dental applications as root canal irrigation [19], in direct and indirect pulp capping [18], reduction of dentin hypersensitivity [17], and in preventing dental caries [20], in addition to a storage media for avulsed teeth [21].

Several studies have investigated the microflora in root canal infections. *Enterococcus faecalis* are a selective anaerobic Gram-positive cocci [7]. They are the most common species that has been detected in the root canal [22, 23], and their ability to invade dentinal tubules may be attributed to their high resistance to alkalinity [7].

Chitosan has been suggested for root canal irrigation in both primary and permanent teeth by several *in vitro* studies [13, 15, 24]. On the other hand, propolis has shown various anti-bacterial activity against *Enterococcus faecalis* [25, 26]. However, the combination of chitosan and propolis was not assessed as dressing materials in primary teeth.

OBJECTIVES

This study was designed to evaluate the synergistic efficacy of the combination of chitosan and propolis against *Enterococcus faecalis* in primary root canal as intra-canal medicament.

MATERIAL AND METHODS

STUDY SAMPLE

Study sample included 72 primary second molars extracted for orthodontic reasons, with two third of their roots staying undamaged without any physiological or pathological resorption, and they were not subjected to any treatment before extraction. Teeth were stored in sterile saline until using in the study. Samples were distributed randomly into 3 groups, including chitosan + propolis, calcium hydroxide, and saline.

CHITOSAN AND PROPOLIS DRESSING PREPARATION

Chitosan (≥ 75% deacetylated) was acquired from Sigma-Aldrich Chemicals, Germany, and propolis was obtained from Sakka Amini Company, Syria.

2 g of chitosan was dissolved in 100 ml of acetic acid (2%), with continuous stirring for 24 hours to form chitosan 2% [27, 28]. 5 g of crude propolis was dissolved in ethyl alcohol (80%) in a ratio of 1 : 15, and then the solution was evaporated under reduced pressure, using a rotary evaporator (at 40-45°C); it was then filtered using filtering paper (Whatman paper) [29]. 1.5 g of ethanol extract of propolis (EEP) was mixed with the resulting 2% chitosan solution (CH) using constant stirring. 1 g of sodium alginate 1% was added until reached the required thickness. The mixture was steamed on Petri dishes in an oven at a temperature of 37°C until all water evaporation.

TEETH SAMPLES PREPARATION

Teeth were cleaned with a gentle curettage to remove residual periodontal ligament, and scaled to remove any calculus. An access opening was prepared using a high-speed diamond bur, with working length 1 mm shorter than the root apex, and the root canals were prepared to size 30 K-File. 10 ml of a 2.5% sodium hypochlorite solution was used to irrigate the root canals during canal preparation. Apical foramen was sealed with flowable composite resin, and the teeth were placed in an acrylic

resin block to allow handling the teeth during the experiment. Teeth were sterilized using moist heat at 121°C for 30 minutes, using a pressure of 15 pounds (lb.) [30].

PREPARING BACTERIAL SUSPENSION

Enterococcus faecalis was isolated clinically from the necrotic pulps of patients treated in the Department of Pediatric Dentistry, Damascus University, and the phenotype was determined using BD PHOENIX™ M100 (BD Company, USA). *Enterococcus faecalis* was cultured on Petri dishes with nutrient medium (Mueller-Hinton agar) at 37°C for 24 hours, in a 5% CO_2 incubator. Bacterial suspension was formed and adjusted to 4.5×10^8 colony-forming units per ml (No. 1.5 McFarland Standard), using a BD PhoenixSpec™ nephelometer (BD Company, USA). 50 µl of bacterial suspension 4.5×10^8 (CFU/ml) was applied into the prepared root canals using a sterile micropipette [30]. The suspension was activated into the canal using a sterile file size 20 for the same period for each sample. All samples were incubated at 37°C for 24 hours under aerobic conditions [30].

EXPERIMENTAL PROCEDURE

Samples were divided randomly into 3 groups to apply the tested materials. Randomization was performed using an online service (www.randomization.com).

Group A: CH + EEP (n = 24): 2% chitosan + 10% ethanol propolis extract dressing was applied using a sterile syringe with disposable plastic needles to inject the dressing into the root canal.

Group B: Positive control group $Ca(OH)_2$ (n = 24): calcium hydroxide dressing was applied using lentulo spirals at low speed after mixed with sterile saline solution in a volume ratio of 1 : 1.

Group C: Negative control group (n = 24): samples without any dressing in root canals application.

Each canal was filled with its' dressing before sealing the access cavity with glass ionomer cement (Fuji IX GP, GC Corporation, Tokyo, Japan). Finally, specimens were incubated at 37°C/CO_2 in 5% environment.

EVALUATION OF ANTI-BACTERIAL EFFECT

The bacterial colonies were counted after 24 hours, 72 hours, and 7 days of dressing application, as the study groups were divided into 3 sub-groups according to the three periods studied:

- 24 hours (n = 24): (A1 = 8, B1 = 8, C1 = 8),
- 72 hours (n = 24): (A2 = 8, B2 = 8, C2 = 8),
- 7 days (n = 24): (A3 = 8, B3 = 8, C3 = 8).

All the root canals were rinsed with 1 ml of sterile saline, and the solution was left inside the canals with a peripheral filing using a sterile H file for a minute. Then, a paper point was inserted over the entire length of the canal for 60 seconds to absorb the content of the canal, whereas the saline solution acted as a medium to transfer the bacteria to agar plate. The paper point was transferred to a sterile tube containing 2 ml of sterile saline, and the swab was repeated three times for each root canal to get a true microbial reality of the canal. The tubes, which contained the paper points were shaken using a vortex device for one minute to ensure homogeneity of the solution. Then, 20 µl of the solution was taken by a sterile micropipette and inoculated on blood agar plates, and these plates were incubated with the same conditions as before for 24 hours (Figures 1-3). Bacterial colonies in colony forming units/ml (CFU/ml) were calculated, and were converted into logarithmic numbers to facilitate statistical analysis [30-32].

RESULTS

The distribution of bacterial counts after disinfection procedures is presented in Table 1 and Figure 4.

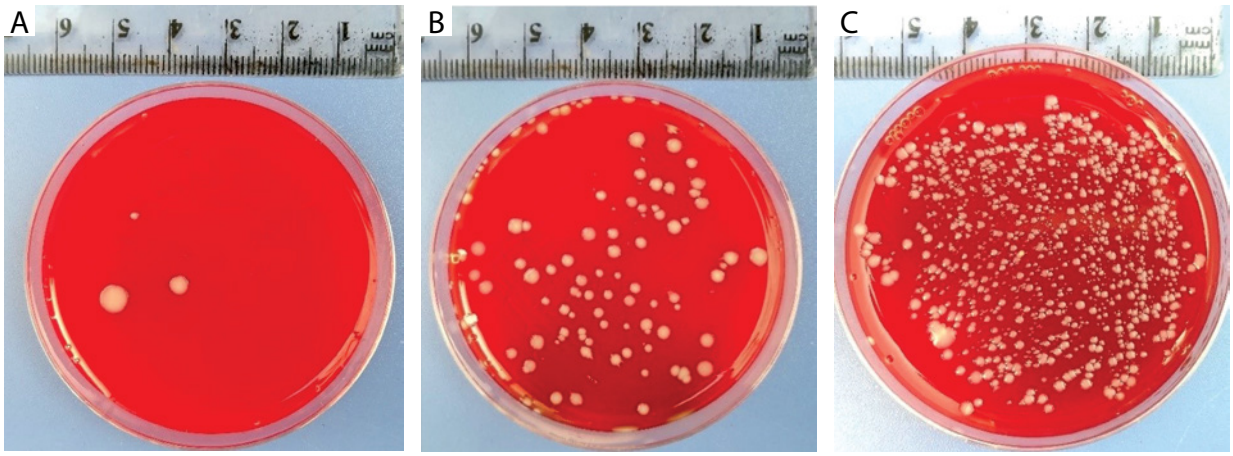

FIGURE 1. Growth of *Enterococcus faecalis* after 24 hours of disinfection with respective tested materials. **A**) Chitosan + propolis. **B**) Calcium hydroxide $Ca(OH)_2$. **C**) Saline

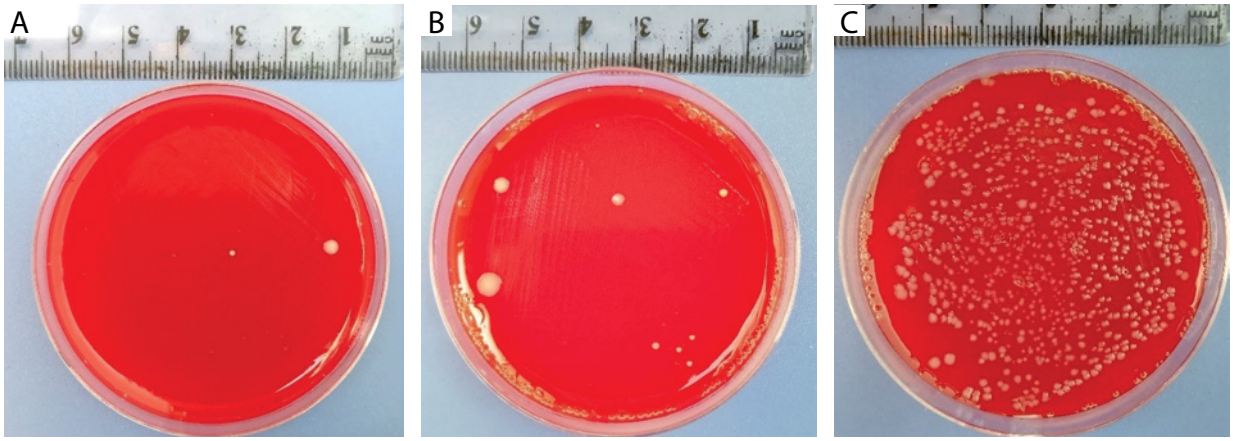

FIGURE 2. Growth of *Enterococcus faecalis* after 72 hours of disinfection with respective tested materials. **A**) Chitosan + propolis. **B**) Calcium hydroxide $Ca(OH)_2$. **C**) Saline

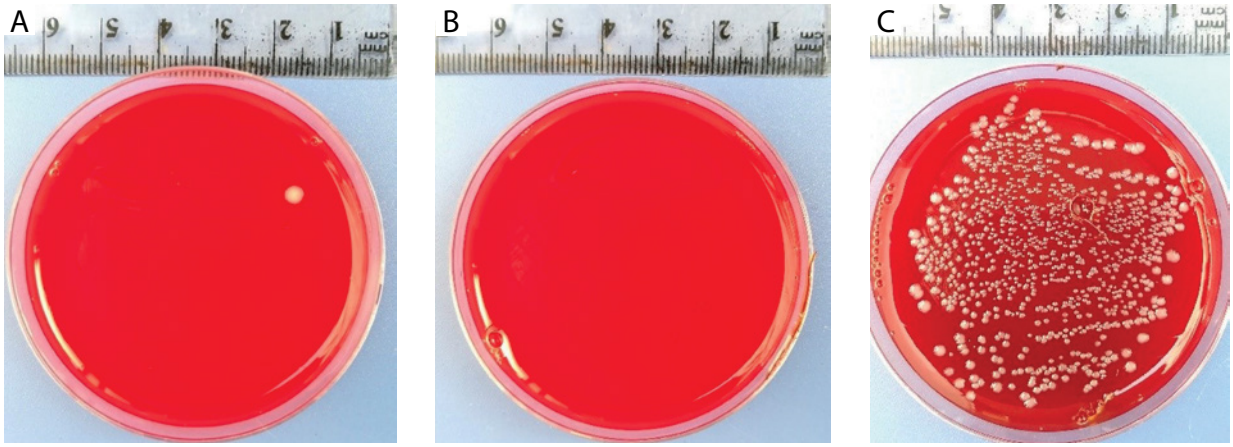

FIGURE 3. Growth of *Enterococcus faecalis* after 1 week of disinfection with respective tested materials. **A**) Chitosan + propolis. **B**) Calcium hydroxide $Ca(OH)_2$. **C**) Saline

TABLE 1. Mean and standard deviation of bacterial colony count (CFU/ml, mean ± SD) before and after (24 hours, 72 hours, 7 days) application of dressing materials, average percentage of the bacterial decrease, and anti-microbial efficacy of each dressing in study groups. The effectiveness of chitosan + propolis was the highest after 24 hours of applying the dressing, while the effectiveness of calcium hydroxide and chitosan + propolis was similar after 72 hours and 7 days

Groups	Samples	Mean and standard deviation	Percentage of bacterial reduction	Percentage of residual bacteria
Bacterial count before	72	8.653 ± 0.000		
Bacterial count after 24 hours				
Chitosan + propolis	8	1.997 ± 0.51	76.91	23.08
Calcium hydroxide	8	3.408 ± 0.28	60.60	39.39
Saline	8	3.927 ± 0.93	54.60	45.39
Bacterial count after 72 hours				
Chitosan + propolis	8	2.246 ± 0.40	74.04	25.95
Calcium hydroxide	8	2.543 ± 0.31	70.61	29.38
Saline	8	4.070 ± 1.47	52.96	47.03
Bacterial count after 7 days				
Chitosan + propolis	8	0.158 ± 0.34	98.16	1.83
Calcium hydroxide	8	0.008 ± 0.01	99.90	0.09
Saline	8	4.476 ± 2.91	48.26	51.73

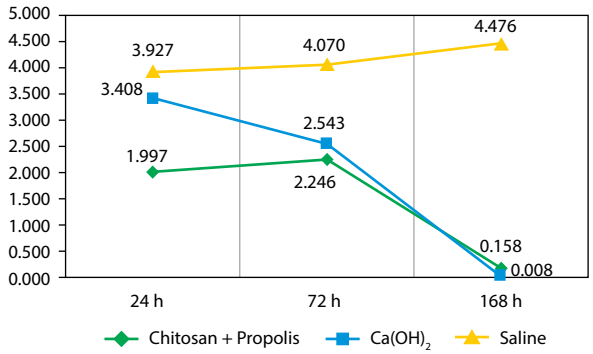

FIGURE 4. Mean of bacterial colony units (CFU/ml, mean ± SD) in the study groups during studied time periods

Differences between groups were analyzed using one-way ANOVA test. *P*-value less than 0.05 was considered statistically significant, and Bonferroni test was used to analyze differences between binary groups (Table 2). Wilcoxon test was used to evaluate the bilateral differences in the mean bacterial colony units between two stages before and after 24 hours, 72 hours, and 7 days of dressing application (Table 3). Statistical calculations were performed with SPSS Statistics for Windows software. As expected, the negative control group revealed the highest number of bacteria (mean, 3.927 ± 0.936). Chitosan + propolis exhibited the highest anti-bacterial effect (1.997 ± 0.515) after 24 hours of applying the dressing material, with statistically significant difference ($p < 0.05$) compared to calcium hydroxide group and saline group (3.408 ± 0.289 and 3.927 ± 0.936, respectively). Calcium hydroxide showed higher anti-bacterial effect than saline, with no statistically significant difference ($p > 0.05$). After 72 hours and 1 week, chitosan + propolis and calcium hydroxide demonstrated similar anti-bacterial effects, with no statistically significant difference ($p > 0.05$). Both showed statistically significant difference compared to saline ($p < 0.05$) (Table 2).

TABLE 2. Results of Bonferroni test to investigate the significance bilateral differences in the mean bacterial colony units (CFU/ml, mean ± SD) between the groups after 24 hours, 72 hours, and 7 days of dressing application

	(I) Groups	(J) Groups	Mean difference (I-J)	Standard error	Significance
24 hours	Chitosan + propolis	Calcium hydroxide	–1.410875*	0.479555	0.006
		Saline	–1.929750*	0.479555	0.000
	Calcium hydroxide	Saline	–0.518875	0.479555	0.287
72 hours	Chitosan + propolis	Calcium hydroxide	–0.296712	0.462225	0.525
		Saline	–1.823963*	0.462225	0.000
	Calcium hydroxide	Saline	–1.527250*	0.462225	0.002
7 days	Chitosan + propolis	Calcium hydroxide	0.150375	0.747940	0.842
		Saline	–4.318125*	0.747940	0.000
	Calcium hydroxide	Saline	–4.468500*	0.747940	0.000

**Mean difference is significant at the 0.05 level .*

TABLE 3. Results of Wilcoxon test to evaluate the significance bilateral differences in the mean bacterial colony units (CFU/ml, mean ± SD) between two stages before and after 24 hours, 72 hours, and 7 days of dressing application in the study groups. Mean difference is significant at *p*-value < 0.05

Bilateral comparisons of bacterial colony units between two stages before and after dressing application	Chitosan + propolis	$Ca(OH)_2$	Saline
		p-value	
Before			
24 hours	0.012*	0.012*	0.012*
72 hours	0.012*	0.012*	0.012*
1 week	0.011*	0.011*	0.012*
After 24 hours			
72 hours	0.401	0.012*	0.575
1 week	0.012*	0.012*	0.327
After 72 hours			
1 week	0.012*	0.012*	0.484

**Mean difference statistically significant.*

Table 1 and Figure 5 showed the average percentage of the bacterial decrease of each dressing in study groups.

DISCUSSION

An ideal intra-canal medicament should exhibit maximum anti-bacterial and minimum toxic effects. Nowadays, calcium hydroxide is the most commonly used intra-canal medicament in endodontic practice. Moreover, it is clear that the anti-bacterial effect of calcium hydroxide is related to a release of hydroxyl ions in aqueous environment, which in turn affect the bacterial cells. However, there are bacterial strains that are resistant to calcium hydroxide alkalinity. *Enterococcus faecalis* is the most common bacteria detected in secondary infections, even in primary infections in permanent [7] and primary teeth [23, 33].

The anti-bacterial efficacy against *Enterococcus faecalis* was preferred in this study as long as it was the most persistent bacteria strains of infected root canal; therefore, if the studied materials were effective against this type of bacteria, it would be effective against other species of bacteria in the root canal. The present study attempted to determine the effective duration of all intra-canal medications tested, as they were verified in three different time periods since the anti-bacterial effect is time-dependent, which is considered beneficial in clinical practice of root canal disinfection during endodontic treatment [34, 35].

The results showed reduction in the bacterial count after applying the dressings compared to the bacterial count before, with statistically significant difference in both study groups chitosan + propolis and $Ca(OH)_2$ as well as during the three time periods studied.

Chitosan and propolis dressing showed the highest anti-bacterial efficacy 24 hours after applying the dressing.

The anti-bacterial activity of chitosan is due to interaction of positively charged amino group in chitosan (exhibiting a polycationic nature), with negative charges of the bacterial cell wall resulting in a leakage of intra-cellular components that impart bacterio-static activity [10, 12].

Propolis also affects the bacterial cell wall, causing structural and functional damage [36]. Ethanolic extracts of propolis showed high anti-bacterial activity against anaerobic bacteria, including *Enterococcus faecalis* [25] and anti-bacterial efficacy of propolis extracts due to the presence of flavonoids content [37]. There is a high concentration of artepillin C in ethanolic extracts of propolis [38], which is one of the numerous phenolic compounds found in propolis. It has a bacterio-static activity with membrane bulging, in addition to cinnamic acid and pinocembrin, which present similar anti-microbial activity against *Enterococcus faecalis* [37].

After 72 hours and 1 week, calcium hydroxide showed high anti-bacterial effect similarly to chitosan and propolis medicament.

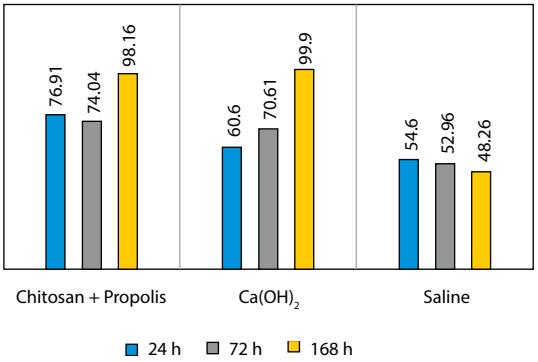

FIGURE 5. Average percentage of decrease in the number of bacterial colony units (CFU/ml, mean ± SD) for each studied dressing

This may be attributed to the fact that the anti-microbial efficacy of the calcium hydroxide dressing depends mainly on releasing hydroxyl ions and raising the level of pH, as studies reported that calcium hydroxide shows pH variation ranging from 8.1 to 11.8 after 72 hours of dressing application [39]. Bystrom *et al.* reported that *Enterococcus faecalis* is a resistant bacterium in root canals, surviving at pH 11.5. Therefore, a high pH level is desired to eliminate the bacteria [40].

These results are in accordance with Parolia *et al.* study, where calcium hydroxide was found to be less effective than chitosan and propolis nano-particle and 2% chlorhexidine on day one and day three. However, it was as effective as chitosan and propolis nano-particle on seventh day of application [35].

On the other hand, significant reduction in the number of bacterial colonies was found in the group A (chitosan + propolis) on the first, third, and seventh day after medicament application compared to the base counts of bacterial colonies, where the effectiveness of chitosan and propolis was highest one week after application, with significant differences compared to its' effectiveness 24 and 72 hours after application. This is due to the fact that chitosan acts as drug delivery that indicate continuous active release of components carried by chitosan even after prolonged times, therefore it is possible that chitosan resulted in a continuous release of propolis throughout the period of dressing application, which resulted in increased anti-bacterial efficacy. In a study by Balata *et al.*, chitosan chips containing propolis extract showed an initial release of 41.7% of loaded propolis, followed by continuous release up to 7 days [41].

Del Carpio-Perochena *et al.* also found that combining chitosan with calcium hydroxide dressing has increased chitosan anti-bacterial activity in short- and long-term exposure (day 7 and 14), and restrained bacterial re-colonization in root canal after endodontic treatment [42]. However, the bacterial inoculation in the present study was performed at a concentration of 4.5×10^8 cell/

ml, which was stronger than that used by Del Carpio-Perochena *et al*.

Cationic nature of chitosan, which react with negative charge of bacteria cells wall, bio-compatibility, compatibility to blend with other materials, and the potential use of chitosan as oral delivery drug, are the main factors to explain the findings mentioned previously. However, further studies are necessary to validate the anti-bacterial behavior of EEP and chitosan in in-vivo conditions.

CONCLUSIONS

Our study showed that the combined application of chitosan and ethanol extract of propolis had a potential anti-bacterial activity against *Enterococcus faecalis* in primary teeth as intra-canal medication.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Nagar G, Verma R, Sharma DS, Pathak AK, Sharma DS, Nagar G. Antibacterial efficacy of pastes against e faecalis in primary root dentin: a confocal microscope study. J Clin Ped Dent 2015; 39: 247-254.
2. Dai S, Xiao G, Dong N, Liu F, He S, Guo Q. Bactericidal effect of a diode laser on Enterococcus faecalis in human primary teeth-an in vitro study. BMC Oral Health 2018; 18: 1-7.
3. Carrotte PV, Waterhouse PJ. A clinical guide to endodontics – update part 2. Br Dent J 2009; 206: 133-139.
4. Pinheiro SL, da Silva JN, Gonçalves RO, Villalpando KT. Manual and rotary instrumentation ability to reduce enterococcus faecalis associated with photodynamic therapy in deciduous molars. Braz Dent J 2014; 25: 502-507.
5. Kriplani R, Thosar N, Baliga MS, Kulkarni P, Shah N, Yeluri R. Comparative evaluation of antimicrobial efficacy of various root canal filling materials along with aloevera used in primary teeth: a microbiological study. J Clin Pediatr Dent 2013; 37: 257-262.
6. Dean JA. Treatment of deep caries, vital pulp exposure, and pulpless teeth. In: McDonald and Avery's Dentistry for the Child and Adolescent: Tenth Edition; 2016. p. 221-242.
7. Sakko M, Tjäderhane L, Rautemaa-Richardson R. Microbiology of root canal infections. Prim Dent J 2016; 5: 84-89.
8. Nelson-Filho P, Assed L, Assed S, Assed R, Ito IY. Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, calen paste thickened with zinc oxide, Sealapex and EndoREZ. Braz Dent J 2009; 20: 290-296.
9. Mohammadi Z, Dummer PMH. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011; 44: 697-730.
10. Tanikonda R, Ravi RK, Kantheti S, Divella S. Chitosan: applications in dentistry. Trends Biomater Artif Organs 2014; 28: 74-78.
11. Kmiec M, Pighinelli L, Silva MM, Reis VO. Chitosan-properties and applications in dentistry. Adv Tissue Eng Regen Med Open Access 2017; 2: 00035.
12. Husain S, Al-Samadani KH, Najeeb S, et al. Chitosan biomaterials for current and potential dental applications. Materials (Basel) 2017; 10: 1-20.
13. Vallabhaneni K, Kakarla P, Avula SSJ, Venu Gopal Reddy N, Pratap Gowd MJS, Vardhan KR. Comparative analyses of smear layer removal using four different irrigant solutions in the primary root canals – a scanning electron microscopic study. J Clin Diagnostic Res 2017; 11: ZC64-ZC67.
14. Kamble AB, Abraham S, Kakde DD, Shashidhar C, Mehta DL. Scanning electron microscopic evaluation of efficacy of 17% ethylenediaminetetraacetic acid and chitosan for smear layer removal with ultrasonics: an in vitro study. Contemp Clin Dent 2017; 8: 621-626.
15. Jaiswal N, Sinha DJ, Singh UP, Singh K, Jandial UA, Goel S. Evaluation of antibacterial efficacy of chitosan, chlorhexidine, propolis and sodium hypochlorite on Enterococcus faecalis biofilm: an in vitro study. J Clin Exp Dent 2017; 9: e1066-e1074.
16. Agrawal V, Kapoor S, Agrawal I. Critical review on eliminating endodontic dental infections using herbal products. J Diet Suppl 2017; 14: 229-240.
17. Askari M, Yazdani R. Comparison of two desensitizing agents for decreasing dentin hypersensitivity following periodontal surgeries: a randomized clinical trial. Quintessence Int (Berl) 2019; 50: 320-329.
18. Parolia A, Kundabala M, Rao NN, et al. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. Aust Dent J 2010; 55: 59-64.
19. Kumar VM, Kumar PR, Richa K, Jyotsna A. The antimicrobial effectiveness of 25% propolis extract in root canal irrigation of primary teeth. J Indian Soc Pedod Prev Dent 2014; 32: 120-124.
20. Hayacibara MF, Koo H, Rosalen PL, et al. In vitro and in vivo effects of isolated fractions of Brazilian propolis on caries development. J Ethnopharmacol 2005; 101: 110-115.
21. Özan F, Polat ZA, Er K, Özan Ü, Değer O. Effect of propolis on survival of periodontal ligament cells: new storage media for avulsed teeth. J Endod 2007; 33: 570-573.
22. Gajan EB, Aghazadeh M, Abashov R, Milani AS. Microbial flora of root canals of pulpally-infected teeth: enterococcus faecalis a prevalent species. J Dent Res Dent Clin Dent Prospects 2009; 3: 24-27.
23. Cogulu D, Uzel A, Oncag O, Aksoy SC. Detection of Enterococcus faecalis in necrotic teeth root canals by culture and polymerase chain reaction methods. Eur J Dent 2007; 1: 216-221.
24. Roshdy NN, Kataia EM, Helmy NA. Assessment of antibacterial activity of 2.5% NaOCl, chitosan nano-particles against Enterococcus faecalis contaminating root canals with and without diode laser irradiation: an in vitro study. Acta Odontol Scand 2019; 77: 39-43.
25. Djauharie N, Kemala N. Antibacterial efficacy of 5% ethanolic extract of propolis (EEP) solution against enterococcus faecalis (laboratory experiment). J Int Dent Med Res 2017; 10: 19-23.
26. Roh J, Kim KR. Antimicrobial activity of korean propolis extracts on oral pathogenic microorganisms. J Dent Hyg Sci 2018; 18: 18-23.
27. Ballal NV, Kundabala M, Bhat KS, et al. Susceptibility of Candida albicans and Enterococcus faecalis to chitosan, chlorhexidine gluconate and their combination in vitro. Aust Endod J 2009; 35: 29-33.
28. Witedja U, Suwartini T, Prahasti AE, Widyarman AS. Comparing the effectivities of chitosan citrate and chitosan acetate in eradicating Enterococcus faecalis biofilm. Sci Dent J Comp 2018; 2: 1-7.
29. Woo SO, Hong I, Han S. Extraction properties of propolis with ethanol concentration. J Apic 2015; 30: 211-216.
30. Öter B, Topçuoglu N, Tank MK, Çehreli SB. Evaluation of antibacterial efficiency of different root canal disinfection techniques in Primary teeth. Photomed Laser Surg 2018; 36: 179-184.
31. Attiguppe PR, Tewani KK, Naik SV. Comparative evaluation of different modes of laser assisted endodontics in primary teeth: an in vitro study. J Clin Diagnostic Res 2017; 11: 124-127.
32. Camacho-Alonso F, Julián-Belmonte E, Chiva-García F, Martínez-Beneyto Y. Bactericidal efficacy of photodynamic therapy and chitosan in root canals experimentally infected with enterococcus faecalis: an in vitro study. Photomed Laser Surg 2017; 35: 184-189.
33. Cancio V, Ferreira DDC, Cavalcante FS, et al. Can the Enterococcus faecalis identified in the root canals of primary teeth be a cause of failure of endodontic treatment ? Acta Odontol Scand 2017; 75: 423-428.

34. Neelakantan P, Sanjeev K, Subbarao CV. Duration-dependent susceptibility of endodontic pathogens to calcium hydroxide and chlorhexidene gel used as intracanal medicament: an in vitro evaluation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104: e138-141.
35. Parolia A, Kumar HK, Ramamurthy S, Pau A. Effectiveness of chitosan-propolis nanoparticle against Enterococcus faecalis biofilms in the root canal. BMC Oral Health 2020; 20: 339.
36. RojaRamya KS, Vinay C, Uloopi KS, Chandrasekhar R. In vivo evaluation of zinc oxidepropolis mixture as root canal filling material in the primary molars: a 24-month follow-up randomized controlled trial. J Indian Soc Pedod Prev Dent 2020; 38: 171-176.
37. Przybyłek I, Karpiński TM. Antibacterial properties of propolis. Molecules 2019; 24: 11-13.
38. Veiga RS, De Mendonça S, Mendes PB, et al. Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and Baccharis dracunculifolia DC. J Appl Microbiol 2017; 122: 911-920.
39. De Andrade Ferreira FB, Silva E Souza PDAR, Do Vale MS, De Moraes IG, Granjeiro JM. Evaluation of pH levels and calcium ion release in various calcium hydroxide endodontic dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 97: 388-392.
40. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985; 1: 170-175.
41. Balata GF, Abdelhady MIS, Mahmoud GM, Matar MA, Abd El-Latif AN. Formulation of saudi propolis into biodegradable chitosan chips for vital pulpotomy. Curr Drug Deliv 2017; 15: 97-109.
42. del Carpio-Perochena A, Kishen A, Felitti R, et al. Antibacterial properties of chitosan nanoparticles and propolis associated with calcium hydroxide against single- and multispecies biofilms: an in vitro and in situ study. J Endod 2017; 43: 1332-1336.