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COMPARISON OF THE EFFECTIVENESS OF MORINDA CITRIFOLIA AND CHLORHEXIDINE AS IRRIGATION SOLUTIONS AGAINST ENTEROCOCCUS FAECALIS IN NONVITAL PRIMARY TEETH

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ABSTRACT

Objectives: One of the main goals of the root canal procedure is the removal of these germs from the root canal space. The current study's objective was to assess the effectiveness of Morinda citrifolia juice (MCJ) as an irrigant solution against E. faecalis and compare it to chlorhexidine (CHX), the gold-standard irrigant solution for root canals of primary teeth that are not vital. **Materials and Methods:** Children from both genders in Egypt between the ages of 4 and 7 were recruited for this clinical trial. A total of 32 mandibular primary molar teeth that needed pulpectomies were included in this study and were divided into two equal groups (n=16) based on the type of irrigant solution used: group (A) received 6% MCJ, while group (B) received 2% CHX. After access was opened, the microbiological samples (before and after irrigation) from the root canals were taken with sterile, wet paper points of size 35 that were pushed into the root canals for one minute. Samples were then cultured on Enterococci agar media, and microbiological analysis was carried out. **Results:** Significant potential was shown by the 6% MCJ irrigant solution against E. faecalis microorganisms. However, when compared to the 6% MCJ solution, a 2% CHX is more effective against E. faecalis microorganisms. **Conclusion:** The 6% MCJ solution works well as a herbal irrigant. When treated primary teeth with endodontics, the standard and most reliable irrigant solution against E. faecalis bacteria is 2% CHX.

KEYWORDS: Chlorhexidine, Enterococcus faecalis, Irrigant solution, Morinda citrifolia, Nonvital, Primary teeth.

INTRODUCTION

The ideal space maintainers are primary teeth, thus they should be kept for as long as feasible[1]. One of the effects of dental caries or trauma is necrotic pulp, which can create an abscess, a fistula, or a sinus. The complicated root shape of primary molars makes it difficult to completely eradicate microorganisms with routine pulpectomy[2].

As a durable root canal system organism, E. faecalis is commonly isolated from pulpal infections in the root canals[3]. It is present in 22–77% of cases of root canal failure and plays a significant role in the pathogenesis of periradicular lesions after root canal therapy[4]. The root canal must be sufficiently cleaned and sealed during canal obturation for endodontic therapy to be effective. Instrumentation and irrigation procedures utilizing chemomechanical methods are essential for root canal disinfection in those clinical situations[5].

Irrigation is a crucial component of root canal debridement because it enables cleaning that is not

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possible with root canal instrumentation alone[6]. The optimum root canal irrigant should be systemically non-toxic when it is in direct contact with vital tissues, non-harmful to periodontal tissues, and have a broad antimicrobial spectrum. It should also be able to dissolve traces of gangrenous pulp tissue and inactivate endotoxins. Additionally, shouldn't have much potential to result in an allergic shock [7].

A broad-spectrum antibiotic agent that works well against E. faecalis is CHX. Chlorhexidine can be adsorbed into tooth tissues and then released from them, producing substantive antibacterial activity, or "substantivity," in addition to its rapid effect on germs[8,9]. However, some limitations to its therapeutic application such as its inability to disintegrate organic materials[10]. Moreover, in vitro canine embryonic fibroblasts were killed by bactericidal doses of CHX diacetate, according to the findings of a prior investigation, while non-lethal quantities allowed for considerable bacterial survival[11]. Additionally, a lower concentration of CHX is linked to apoptosis while a higher dosage causes necrosis [12].

A member of the coffee family of trees, Morinda citrifolia is often referred to as Noni. Juice from the Morinda citrifolia seems to be the first to be recognized as a potential substitute for sodium hypochlorite [13]. It has antibacterial properties as well as antiviral, antifungal, and immune-boosting properties, indicating the possibility of using it as an endodontic irrigant [14, 15]. None of the irrigants now available is of perfect quality. The goal of this study was to compare MCJ to CHX, the gold-standard irrigant solution for non-vital root canals in primary teeth, in terms of how well it worked against E. faecalis.

MATERIALS AND METHODS

After receiving approval from the Ethical Committee of the Faculty of Dental Medicine at Al-Azhar University (Boys, Cairo) with approval reference (EC Ref No.379/1799) and obtained a signed informed consent, children from both sexes in Egypt between the ages of 4 and 7 were recruited for this clinical experiment. 32 non-vital, restorable primary mandibular molars required pulpectomy were included. Based on the type of irrigant solution employed, group (A) received 6% MCJ, while group (B) received 2% CHX (JK Dental Co., Egypt), the study's participants were split into two equal groups (n=16).

A sample size of 16 in each group, based on a prior study by Podar et al. [14], has 80% power to identify a difference between means of 3.34 with a significance level (alpha) of 0.05. (two-tailed). The results will be regarded as "statistically significant" if the p-value is less than 0.05 (two-tailed) in 80% (the power) of those experiments.

Preparation of MCJ extract

Pieces of fresh fruit were cut up and dried for 24 hours at room temperature. To eliminate moisture content, the air-dried fruits were placed in a hot air oven (LHAO-104 Series, Labocon, UK) at 40°C for 24 hours. The fruits were then ground into a powder using a mortar and pestle and combined in a 1:5 ratio with the solvent ethanol. The extraction process took place over the course of 48 hours in a shaker water bath at 40°C. The extract was concentrated to dryness, filtered through Whatman No. 1 filter paper, and then dissolved in 10% dimethyl sulfoxide, as needed. To create 6% MCJ, 6 grams of Morinda Citrifolia powder were dissolved in 100 ml of 10% dimethyl sulfoxide and then stirred for an hour with a magnetic stirrer. After being prepared, the solution was kept in the refrigerator and consumed within two weeks [14, 16].

Pulpectomy Procedures

The treatments began with the application of topical anesthetic (20% benzocaine, Keystone Industries, USA) for 1 minute to all involved teeth. Using aspirating dental syringes and 27-gauge needles, a further nerve block injection was given using an anesthetic solution (Mepecaine L,

Alexandria Co. for Pharmaceuticals, Alexandria, Egypt). A rubber dam was used to isolate the implicated side. Then, under extensive irrigation, caries was eliminated using a large, slow-speed round bur. Then a carbide fissure bur was used to get access to the pulp chamber. The lack of bleeding in the canal ultimately proved that the pulp was necrotic. A preapical radiograph was used to estimate the working length, which was fixed at 1 mm less than the radiographic apex. Barbed broaches were used to remove the pulp from the pulp chamber and root canals. In both groups, manual endodontic files up to size 35 were used to form the root canals [16,17,18].

Irrigation protocol

During the preparation of the canal, periodic watering for 30 seconds was used to remove organic material and sterilize the roots. In this investigation, 5 mL of each tested solution was utilized for standardization. For irrigation, a syringe was utilized with a 30-G side-vented needle with a soft tip. During the canal preparation, canals were irrigated with 5 mL 6% MCJ or 2% CHX solutions using a syringe and a 30-G positioned 1-2 mm short of the working length. All tooth samples had their root canals irrigated for five minutes with various test substances, then saline was used as the last irrigant [16].

Microbiological sample collection

After access was opened, the baseline microbiological samples (S1) from the root canals were taken with sterile, wet paper points of size 35 that were pushed into the root canals for one minute. Similar to how the first microbiological samples were taken, the second microbiological samples (S2) were taken right after the various irrigation techniques. Each paper point that had been collected was then immediately placed in a labeled screw-capped vial that contained 2 ml of peptone water liquid media as transfer media, acting as a bacterial diluent media and able to maintain the survival of the bacteria [19].

Restoration procedures:

The root canals were dried with paper points after irrigation with the two different testing and control irrigant solutions. The prepared roots were then filled with lentulo spirals and obturated with zinc oxide eugenol. After that, glass ionomer cement was used to seal the pulp chamber. After that, stainless steel crowns were used to restore the dental crowns [16, 19].

Microbiological investigation

All acquired microbiological samples were transported as soon as possible to the microbiology lab at the regional center for microbiology and biotechnology (Al-Azhar University, Cairo, Egypt), under strict aseptic conditions, for cultivation on the appropriate media. Using cell spreaders, the samples were streaked on Enterococci agar media. The plates were incubated for 7 days at 37°C in an anaerobic atmosphere. With the use of a digital colony counter, the total number of colonies on the incubated plates was counted after 7 days and expressed as the total number of colony-forming units per ml (CFU/ml). Based on colony form, gram stain appearance, and typical biochemical reactions, the organisms were identified [16, 19].

Statistical analysis

SPSS statistical version 21 was used for statistical analysis. The mean age of the two groups was compared using a student t-test. The cutoff for significance was chosen at p 0.05.

RESULTS

According to the findings, there was no statistically significant difference between the two MCJ and CHX-treated groups involved in root canal E. faecalis counts at baseline. However, the outcomes demonstrated that the MCJ and CHX-treated groups' E. faecalis counts before and after irrigation differed statistically significantly. Additionally, the outcomes demonstrated a

statistically significant difference in the E. faecalis counts between the affected root canal in the two study groups following irrigation. (Table 1)

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

Variable	Baseline	After irrigation	p-value
MCJ	981.56±135.50	591.56±91.76	< 0.0001*
CHX	990.88±116.10	385.94±56.46	< 0.0001*
p-value	0.8360 ns	<0.0001*	

**; significant at p < 0.05. ns= non-significant.*

DISCUSSION

The persistence of bacteria and their reinfection is one of the main reasons root canal therapy failure[16,20]. Because the architecture of the root canal system is so intricate, cleaning it with mechanical instrumentation alone is unsuccessful [21]. The selection of an irrigant is crucial since they can differ in how well they lubricate surgical instruments and remove bacteria, smear layer, and debris from canals [16]. In order to find out whether two root canal irrigations could successfully remove E. faecalis from the primary molars' prepared root canals, the current clinical trial was carried out.

Many different kinds of bacteria were found in unsuccessful root canal instances, however, E. faecalis was chosen for testing in the current investigation. This is due to the discovery that E. faecalis was the most common bacteria discovered in unsuccessful root canal patients [16]. Additionally, E. faecalis was selected for this investigation based on its significance in endodontic treatment failure and the fact that it was found in 55% of necrotic primary maxillary molars [22].

A 2% solution of CHX was chosen for the study's control irrigating solution to be used during root canal therapy. This is due to CHX's reputation as one of the most powerful irrigation agents [23]. Additionally, CHX is both biocompatible and antibacterial[16]. However, the use of CHX as an endodontic irrigant is typically limited due to the potential for tooth discoloration. Additionally, some patients may experience adverse effects including taste loss, an oral mucosal burning sensation, subjective dryness of the oral cavity, and tongue darkening [16, 21].

There is a need for an alternate disinfection method because of the ongoing rise in strains of bacteria that are resistant to antibiotics and the negative effects of synthetic medications [16]. As of right now, there aren't any or very few natural fruit juices that might be employed in place of CHX as a successful root canal irrigating solution [23]. Because it exhibits antibacterial and therapeutic benefits, MCJ was selected in this study as a tested natural irrigant solution as an alternative to CHX. This implies its potential to be utilized as an endodontic irrigant to overcome the drawbacks of currently recognized CHX irrigants.

The minimum inhibitory concentration of MCJ against E. faecalis was determined to be 6% in Murray et al.[23] investigation of this topic. The present study used a 6% concentration of MCJ as a tested concentration to evaluate the 6% MCJ as an endodontic irrigant during pulpectomy of primary molars based on this preliminary finding. Additionally, DMSO was employed in this study's production of MCJ as a solvent for the MC fruit extract. This is due to the fact that it is a pure, secure, highly polar, aprotic solvent that aids in bringing out all of the constituents of the plant that are being dissolved [16].

The only primary molars engaged in pulpectomy treatment in this study were the mandibular first molars. This is because the investigator can more easily visualize them, and because lower primary molars have less of a furcation and less overlap of permanent tooth buds on roots than maxillary molars, which makes it easier for them to be visualized [24]. A rubber dam was used just during pulpectomy procedures in the current investigation.

This is because it minimizes microbial contamination and safeguards the soft and hard tissues, making it the gold standard for isolation during endodontic treatment [25].

All tooth samples were used in the current investigation, and the root canals of each one were irrigated for 5 min with various test agents before being finished with saline. This was done to standardize the groups and cut down on each irrigant's extended contact time. As a result, it also prevented antibacterial activity would transfer to the culture medium [16]. Additionally, a culture-dependent method was used in the current study as a test method for counting E. faecalis. This is due to the fact that it can explain the significance of this method in identifying live bacteria, particularly when samples are taken right away following antibiotic treatment [26].

After irrigation, the E. faecalis count significantly decreased according to the MCJ results in the current study. This might be explained by the fact that the MCJ includes antibacterial substances included L-asperuloside and alizarin, which may be what gives it its antibacterial properties [16, 23, 27]. These findings supported the findings of Murray et al.[23] and Divia et al.[16], who came to the same conclusion about the MCJ's antibacterial activity on E. faecalis.

The current study's findings also showed that considerable suppression of E. faecalis after irrigation was achieved when a 2% CHX solution was used. This might be due to CHX's bactericidal activity, which kills bacteria by destroying their permeability barriers. Furthermore, cytoplasm condensation and denaturation were brought about by the study's usage of high concentrations (2%), which led to sterilization[28]. The 2% bactericidal dosage and increased medication diffusion into the dentinal tubules may be the cause [27].

The results of this study also demonstrated, with a statistically significant difference, that using 2% CHX as an irrigant solution was the most efficient way to get rid of E. faecalis. This could be explained by the fact that the 6% MCJ exhibited an inhibitory impact on E. faecalis that varied with concentration and contact time[16,29]. As previously mentioned, Murray et al.[23] state that the MCJ concentration utilized in the current investigation, which was 6%, was below its minimum inhibitory concentration (MIC), which results in a lesser inhibitory impact against E. faecalis bacteria.

However, the fact that CHX was used in this investigation at a higher concentration (2%) may be responsible for the higher antibacterial outcomes[28]. As reported, the concentration of CHX has a significant impact on its antibacterial action. These findings concur with those of earlier research by Murray et al.[23] and Divia et al.[16], which found that CHX has more antibacterial activity against E. faecalis when compared to MCJ.

CONCLUSION

As a herbal irrigant, the 6% MCJ solution performs admirably. The standard and most trustworthy irrigant solution against E. faecalis bacteria when treated primary teeth with endodontics is 2% CHX.

REFERENCES

1. Ahmad AJ, Parekh S, Ashley PF. Methods of space maintenance for premature loss of a primary molar: a review. Eur Arch Paediatr Dent. 2018; 19:311-20.
2. Bashar AKM, Akter K, Chaudhary GK, Rahman A. Primary molar with chronic periapical abscess showing atypical presentation of simultaneous extraoral and intraoral sinus tract with multiple stomata. BMJ Case Rep. 2019;12: 229-39.
3. Alghamdi F, Shakir M. The influence of enterococcus faecalis as a dental root canal pathogen on endodontic treatment: a systematic review. Cureus. 2020; 12:7257-64.
4. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006; 32:93-98.
5. Regan JD, Fleury AA. Irrigants in non-surgical endodontic treatment. Journal of the Irish Dental Association. 2006; 52:84-92.

6. Ali A, Bhosale A, Pawar S, Kakti A, Bichpuriya A, Agwan MA. Current trends in root canal irrigation. Cureus. 2022;14: 24833-42.
7. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004; 30:559-67.
8. Paquette L, Legner M, Fillery ED, Friedman S. Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo. J Endod. 2007; 33:788-95.
9. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. International endodontic journal. 2009; 42:288-302.
10. Gomes BP, Vianna ME, Zaia AA, Almeida JF, Souza-Filho FJ, Ferraz CC. Chlorhexidine in endodontics. Brazilian dental journal. 2013; 24:89-102.
11. Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. Iran Endod J. 2008; 2:113-25.
12. Li YC, Kuan YH, Lee SS, Huang FM, Chang YC. Cytotoxicity and genotoxicity of chlorhexidine on macrophages in vitro. Environment Toxicol. 2014; 29:452-58.
13. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, et al. Morinda citrifolia (Noni): a literature review and recent advances in Noni research. Acta pharmacol Sinica. 2002; 23:1127-41.
14. Podar R, Kulkarni GP, Dadu SS, Singh S, Singh SH. In vivo antimicrobial efficacy of 6% Morinda citrifolia, Azadirachta indica, and 3% sodium hypochlorite as root canal irrigants. European journal of dentistry. 2015; 9:529-34.
15. Zhang Q, Zhao Y, Li Y, Guo X, Guo Y, Ma G, Liang X, Yan S. Dietary polysaccharide-rich extract from noni (morinda citrifolia l.) fruit modified ruminal fermentation, ruminal bacterial community and nutrient digestion in cashmere goats. Animals (Basel). 2023;13:221-29.
16. Divia AR, Nair MG, Varughese JM, Kurien S. A comparative evaluation of Morinda citrifolia, green tea polyphenols, and Triphala with 5% sodium hypochlorite as an endodontic irrigant against Enterococcus faecalis An in vitro study. Dent Res J (Isfahan). 2018; 15:117-22.
17. Yousry Y, Abd Al Latif A, Abd Al Gawad R. Clinical and Radiographic Evaluation of Vital Pulpotomy in Primary Molar using Antioxidant Mix as a Novel Pulpotomy Medication versus Formcresol: A Randomized Clinical Trial. Advanc Dent J. 2021; 3:12-23.
18. Sayadizadeh M, Shojaeipour R, Aminizadeh M, Horri A, Raeisi Estabragh S, Aminizadeh M, et al. Comparing a Combination of Saline and Chlorhexidine with Saline as Root Canal irrigation solutions in pulpectomy of the primary molars in 6-9 years old children, a double-blind clinical trial. J Dent Mater Techn. 2019; 8:174-81.
19. Choudhary E, Indushekar KR, Saraf BG, Sheoran N, Sardana D, Shekhar A. Exploring the role of Morinda citrifolia and Triphala juice in root canal irrigation: An ex vivo study. J Conserv Dent. 2018; 21:443-49.
20. Topçuoğlu G, Topçuoğlu HS, Delikan E, Çalişkan S. The effect of two different irrigation needles on post-operative pain after pulpectomy in primary molar teeth: A randomized clinical study. Int J Paediatr Dent. 2020; 30:758-63.
21. Metzger Z, Solomonov M, Kfir A. The role of mechanical instrumentation in the cleaning of root canals. Endodontic Topics. 2013; 29:87-109.
22. Tulsani SG, Chikkanarasaiah N, Bethur S. An in vivo comparison of antimicrobial efficacy of sodium hypochlorite and Biopure MTAD™ against enterococcus faecalis in primary teeth: A qPCR study. J Clin Pediatr Dent. 2014; 39:30-4.
23. Murray PE, Farber RM, Namerow KN, Kuttler S, Garcia-Godoy F. Evaluation of Morinda citrifolia as an endodontic irrigant. J Endod. 2008; 34:66-70.
24. Mohammad G, Jerin F, Jebin S. Pulpal Diagnosis of Primary Teeth: Guidelines for Clinical Practice. Bangladesh J Dent Res Educ. 2013; 2:65-68.
25. Anabtawi MF, Gilbert GH, Bauer MR, Reams G, Makhija SK, Benjamin PL, et al. Rubber dam use during root canal treatment: findings from The Dental Practice-Based Research Network. J Am Dent Assoc. 2013; 144:179-86.
26. Donyavi Z, Arabestani MR, Dastan D, Esmaeilzadeh M, Shahsavand N. Comparison of Antimicrobial Effect of Berberine as an Endodontic Irrigant with that of Other Common Root Canal Irrigants on Three Microorganisms Involved in Persistent Endodontic Infections. J Molecu Biolg Res. 2018; 8:153.
27. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. Int Endod J. 2010; 43:419-23.
28. Chen X, Daliri EB, Kim N, Kim JR, Yoo D, Oh DH. Microbial etiology and prevention of dental caries: exploiting natural products to inhibit cariogenic biofilms. Pathogens. 2020; 9: 569-74.
29. Barani K, Manipal S, Prabu D, Ahmed A, Adusumilli P, Jeevika C. Anti-fungal activity of Morinda citrifolia (noni) extracts against Candida albicans: an in vitro study. Indian J Dent Res. 2014; 25:188-90.