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Antimicrobial Efficacy of Saline, Chlorhexidine, and Zataria Multiflora and Mentha Piperita Essential Oils in Root Canal Irrigation of Primary Molars

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ABSTRACT

Objectives: This study aimed to compare the antimicrobial efficacy of saline, 0.5% and 2% Zataria multiflora (Z. multiflora) essential oil, 0.5% and 2% Mentha piperita (M. piperita) essential oil, and 0.2% chlorhexidine (CHX) as root canal irrigants for primary molar teeth.

Materials and Methods: A total of 64 primary molars were used in this in vitro study. The teeth were randomly assigned to six groups (N=10). The root canals were prepared up to file #35, and all teeth were sterilized before contamination with Enterococcus faecalis (E. faecalis; ATCC 29212) suspension. After 48 hours of incubation, the root canals in each group were irrigated with the respective irrigants. Sterile paper points were then used to collect microbial samples from the root canals. A colony counter was used to count the number of colony-forming units (CFUs). Data were analyzed by SPSS version 20 (alpha=0.05).

Results: The colony count was significantly different among the groups (P<0.001), and 2% M. piperita (P=0.009), 0.5% Z. multiflora (P=0.021), and 0.2% CHX (P=0.002) were significantly more effective than saline in elimination of E. faecalis. The ascending order of microbial count after irrigation was as follows: saline > 0.5% M. piperita > 0.2% CHX > 2% M. piperita > 0.5% Z. multiflora.

Conclusion: The current study showed the optimal antibacterial activity of 0.5% Z. multiflora essential oil and 2% M. piperita essential oil against E. faecalis, and indicated their possible efficacy for use as an irrigant for root canal irrigation of primary molars.

Keywords: Root Canal Irrigants; Tooth, Deciduous; Root Canal Therapy; Herbal Medicine

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INTRODUCTION

Many investigations have shown that bacteria and their products are the primary cause of pulpal and periapical diseases [1, 2]. The severity of pulpal and periapical inflammation is directly correlated with the number of microorganisms present in the root canal system, and duration of tissue exposure to microorganisms [3]. Debridement of the root canal system for physical and chemical removal of microorganisms and their products is among the most essential steps of endodontic treatment [1]. This step has a significant impact on the success and prognosis of endodontic treatment [4]. This process is particularly important in primary teeth due to the complex morphology and biology of their root canal system. The root canals of primary molars are often small and hardly accessible. Secondary dentin deposition also changes the root canal morphology over time, altering the size and number of canals. Other differences of primary and permanent root canals include higher frequency of lateral and apical ramifications and fins in primary teeth [5].

The previously examined antimicrobial agents/modalities for endodontic purposes include sodium chloride (NaCl), chlorhexidine (CHX), potassium iodide, a mixture of doxycycline, citric acid, and a detergent (MTAD), calcium hydroxide, laser irradiation, photodynamic therapy, and ozone therapy [6,7]. The success rate of removing root canal microorganisms is not acceptably high even with the application of canal clearing techniques [8]. An effective antibacterial irrigant should have some certain mechanical and chemical features. It should be able to debride the canal and dissolve debris, and must possess low surface tension to reach inaccessible and hard-to-reach areas, the ability to flow in the canal, and optimal substantivity.

Saline is commonly used as a control irrigant since it cannot properly clean unclean or necrotic wounds, and lacks antibacterial properties [9]. CHX has a broad-spectrum antibacterial activity and a slow, steady release at therapeutic concentrations. However, long-term use of CHX can cause discoloration, hypersensitivity reactions, and significant changes in the salivary flora [10]. CHX is more effective than saline as an endodontic irrigant [11-13]; however, inability to dissolve the pulp tissue is a major drawback of CHX as an endodontic irrigant [14].

Mentha piperita (M. piperita) or peppermint essential oil has antibacterial, antifungal, antiviral, and larvicidal properties [15,16]. It is harmless to humans with a lethal dose (LD50) of 2000 mg/kg for various microorganisms [17]. Zataria multiflora (Z. multiflora) or thyme is another medicinal plant, and the antimicrobial activity of its essential oil has been studied in an attempt to find a solution with similar or more desirable antimicrobial properties and fewer side effects than sodium hypochlorite (NaOCl) [18]. The essential oil of Z. multiflora has two phenolic isomers of thymol and carvacrol. The strong antibacterial properties of Z. multiflora have been attributed to the presence of thymol and carvacrol in its composition. Also, it has a more favorable taste and smell than NaOCl [19].

Although the dental literature is rich on irrigants, the efficacy of herbal irrigants for primary teeth especially primary molar teeth has not been adequately addressed in the literature. Therefore, the current study aimed to compare the antimicrobial efficacy of saline, 0.5% and 2% Z. multiflora essential oil, 0.5% and 2% M. piperita essential oil, and 0.2% CHX as root canal irrigants for primary molar teeth.

MATERIAL AND METHODS

This study was conducted in continuation of a previous study on 2% Z. multiflora, 2.5% NaOCl, and saline with the exact same methodology [20]. The study was ethically approved by the Research Ethics Committee of the university.

The current in vitro study was performed on 64 primary molars. The teeth were disinfected by immersion in 0.5% (w/v) chloramine T solution (MF aqua, Tehran, Iran) for one week at 3°C and were then stored in saline until the experiment.

The inclusion criteria were primary molar teeth that met the clinical and radiographic criteria for pulpectomy, and the exclusion criteria were primary molar teeth with roots exfoliated by more than one-third of the root length, teeth that had undergone restorative or endodontic treatments, teeth with pathological external or internal root resorption defects, and teeth with fracture or resorption of more than one third of their root length. The molar teeth had been extracted for financial reasons, parental reluctance to retain the teeth, inefficiency, irreparability of the crown, and orthodontic reasons.

The sample size was calculated using the Bonferroni formula ($k=6$; $\alpha=0.05$; $n_i=10$; $n=60$) according to a study by Hasheminiya et al [21]. A total of 64 extracted human primary molars, including 35 first molars and 29 second molars, were selected. They were randomly assigned to six study groups using the simple randomization method; two teeth were considered as the positive controls, and two other teeth as the negative controls.

Tooth preparation:

The soft tissues of the teeth were cleaned by a hand instrument, and the teeth were placed in 0.5% NaOCl for 24 hours [22]. The teeth were then stored in sterile saline at room temperature [23]. Next, the crowns were cut at the cementoenamel junction. The pulpal residues were removed by barbed broaches proportionate to the diameter of the canal, and then a #15 k-file was introduced into the canal. The file length was measured when the

file tip was visible at the root apex, and 1 mm shorter than this length was considered as the working length [24]. The canals were then cleaned and instrumented with #15–35 files using the standard technique. The root canals were irrigated with 2 mL of saline followed by a final rinse with 5 mL of saline [25]. Following root canal preparation, the roots were sealed with cyanoacrylate glue to prevent bacterial microleakage [26]. Each root was individually wrapped in aluminum foil and autoclave-sterilized at 12°C and 15 psi pressure for 20 minutes. From this stage onward, all procedures were performed under sterile conditions using sterile instruments [25].

The root canals were then contaminated with standard and resistant strain of Enterococcus faecalis (E. faecalis; ATCC 29212). This Gram-positive coccus was obtained from the Microbiology Department of Tehran University of Medical Sciences. A bacterial suspension was prepared in a tube containing 10 mL of sterile saline, with 1 McFarland standard concentration containing 3 x 10^8 colony forming units (CFUs)/mL. Two teeth remained intact as negative controls to ensure the accuracy of sterilization. Next, all teeth were incubated at 37°C for 48 hours. To confirm the accuracy of intracanal contamination, 2 teeth were considered as positive controls. The remaining 60 samples were randomly assigned to six groups and irrigated with the following solutions: saline, 0.2% CHX (Shahredaru Products, Tehran, Iran), 2% Z. multiflora essential oil (Ebnemasouyeh Products, Tehran, Iran), 0.5% Z. multiflora essential oil (Ebnemasouyeh Products, Tehran, Iran), 2% M. piperita essential oil (Ebnemasouyeh Products, Tehran, Iran), and 0.5% M. piperita essential oil (Ebnemasouyeh Products, Tehran, Iran). The root canals were irrigated with 2 mL of the respective solution using a #20 syringe with a 28-gauge needle. After 15 minutes, the canals were rinsed with 2 mL of saline to remove the irrigants.

For microbial sampling, a paper point one number smaller than the largest file (the final file was #35 and the paper point was #30) was inserted into the canal, and remained there for 1 minute. The paper point was then placed on a culture plate containing blood agar [27,28]. All samples were then sent to the microbiology laboratory of the Microbiology Department of Tehran University of Medical Sciences for 48 hours of incubation at 37°C. After incubation, the number of grown colonies was counted by a colony counter (Funke-Gerber, Germany).

Statistical analysis:

SPSS version 20 (SPSS Inc., IL, USA) was used for data analysis. The Kolmogorov-Smirnov test was applied to analyze the normality of data distribution, which showed non-normal distribution of data. Thus, the Kruskal-Wallis test was used to compare the antimicrobial activity of the tested 5 irrigants. The Mann-Whitney test was applied for pairwise comparisons of the irrigants. $P<0.05$ was considered statistically significant.

RESULTS

The positive control group showed bacterial growth, while the negative control group showed no sign of bacterial growth. The number of colonies was significantly different among the groups ($P<0.001$). Pairwise comparisons of the irrigants revealed significantly higher antibacterial activity of 2% M. piperita ($P=0.009$), 2% Z. multiflora ($P<0.001$), 0.5% Z. multiflora ($P=0.021$), and 0.2% CHX ($P=0.002$) compared to saline. Table 1 shows the measures of central dispersion for the colony count in the study groups.

All irrigants decreased the colony count significantly more than saline ($P<0.05$). Table 2 presents pairwise comparisons of the irrigants.

Table 1. Measures of central dispersion for the colony count in the study groups

Group	Mean ± std. deviation	95% confidence interval	P value
Saline	8.123 ± 0.085	(8.062, 8.123)	0.0001
0.5% M. piperita	4.120 ± 0.703	(3.598, 4.643)	
Chlorhexidine	3.159 ± 0.534	(2.777, 3.541)	
2% M. piperita	2.863 ± 0.558	(2.464, 3.262)	
0.5% Z. multiflora	2.850 ± 0.602	(2.419, 3.281)	
2% Z. multiflora	1.813 ± 0.3185	(1.858, 2.041)	

Table 2. Pairwise comparisons of irrigants based on the log number of colonies

Compared groups	P-value*
2% Z. multiflora-Saline	<0.001
0.5% Z. multiflora -Saline	0.009
2% Z. multiflora - 2% CHX	0.018
2% Z. multiflora - 0.5% M. piperita	0.015
0.5% M. piperita -Saline	0.009
2% M. piperita -Saline	0.009
2% CHX-Saline	0.002

*Mann-Whitney test

DISCUSSION

Bacteria are the main causes of pulpal and periapical diseases, and the severity of inflammation is directly related to the number of microorganisms in the root canal system [3,18]. Therefore, elimination of bacteria from the root canal system is the basis of a successful endodontic treatment with a good long-term prognosis [29]. The use of herbal medicines, due to their organic nature and fewer side effects, has attracted the attention of many medical researchers [30,31]. The present study assessed the antibacterial activity of saline, 0.2% CHX, 2% and 0.5% Z. multiflora essential oil, and 2% and 0.5% M. piperita essential oil as root canal irrigants. The results showed that 0.5% Z. multiflora and 2% and 0.5% M. piperita had the same efficacy as CHX while 2% Z. multiflora was significantly more effective than 2% CHX.

The present study is a continuation of a previous study on 2% Z. multiflora, 2.5% NaOCl, and saline [20] which indicated that 2% Z. multiflora may be used as an organic irrigant but it had no significant difference with saline. However, in the present study, all irrigants were significantly more effective than saline in elimination of E. faecalis.

Ravanshad et al. [18] demonstrated that 2 mL of 1% Z. multiflora and 2 mL of 2% Z. multiflora were as effective as 2 mL of 2.5% NaOCl in elimination of E. faecalis. On the contrary, Heidari et al. [20] showed that 0.5% Z. multiflora was not as effective as 2.5% NaOCl; thus, it appears that the efficacy of Z. multiflora is probably dose-dependent. In line with this statement, the present study showed that higher concentrations of Z. multiflora (2%) and M. piperita (2%) were more effective in elimination of E. faecalis than lower concentrations (0.5%).

Another study showed comparable efficacy of NaOCl (5.25% and 2.5%) and CHX (2% and 0.2%) in reduction of intracanal bacterial load [32]. However, a previous study reported that NaOCl was more effective than CHX [20]. Nonetheless, the present study focused on the efficacy of Z. multiflora and M. piperita, rather than NaOCl, for root canal irrigation and showed their optimal antibacterial activity against E. faecalis, comparable to that of CHX.

Another study on 60 mandibular premolars assessed the efficacy of NaOCl and Z. multiflora essential oil as irrigants in Candida albicans-infected root canals. They discovered that Z. multiflora essential oil had the same antifungal activity as NaOCl when used with a concentration twice the minimal fungicidal concentration [33]. Mathew et al. [34] compared the ex vivo effectiveness of an indigenously prepared herbal extract called "EndoPam," which contained Syzygium aromaticum, Eucalyptus globules, Cinnamomum zeylanicum, and M. piperita, with 2% CHX, 5.25% NaOCl, and saline for disinfection of root canals contaminated with E. faecalis. They reported that the diameter of the growth inhibition zones observed was as follows: 2% CHX > EndoPam > 5.25% NaOCl > saline [34]. Their results regarding the higher efficacy of 2% CHX and M. piperita than saline in reducing the biofilm count after root canal irrigation were in line with the present findings. Phenolic components, especially carvacrol and thymol, rosmarinic acid, and flavonoids present in Z. multiflora are known to possess antimicrobial activity [35]. Previous reports have shown that flavonoids, essential oils, and active mint compounds are responsible for the antimicrobial properties of plant extracts [36,37].

Several studies are available regarding the antimicrobial efficacy of 2% CHX [38,39]. The present study also showed the optimal antibacterial efficacy of 0.2% CHX against E. faecalis. CHX in 2% concentration has higher toxicity and causes greater skin irritation than its 0.2% concentration, which is commonly used [40].

The main strength of the current study was to evaluate the antibacterial activity of two different organic compounds at different concentrations under in vitro conditions.

One limitation of the present study was that the antimicrobial properties of irrigants may

vary in the clinical setting and the current results need to be verified and confirmed in clinical trials. Future studies are recommended to evaluate other properties of Z. multiflora and M. piperita essential oils, including their biocompatibility and tissue solubility, which are important for their application as root canal irrigants. Also, future investigations should evaluate the antimicrobial properties of Z. multiflora and M. piperita essential oils against other microbial species involved in endodontic infections. Ultimately, such findings should be verified clinically.

CONCLUSION

The present results revealed that Z. multiflora and M. piperita essential oils had favourable antimicrobial activity against E. faecalis in vitro, and may have the potential for future use as root canal irrigant in primary teeth instead of saline or even CHX due to their possibly fewer side effects.

CONFLICT OF INTEREST STATEMENT

None declared.

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