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EFFICACY OF MORINDA CITRIFOLIA ASSOCIATED WITH MANUAL DYNAMIC AGITATION ON SMEAR LAYER REMOVAL: (IN VITRO SCANNING ELECTRON MICROSCOPIC STUDY)

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ABSTRACT

INTRODUCTION: Endodontic treatment requires chemo-mechanical preparation of root canal to minimize the intracanal bacterial load. However, mechanical debridement results in smear layer formation containing infected debris requiring its removal before obturation.

AIM OF THE STUDY: To assess the efficacy of Morinda Citrifolia juice (MCJ) as final irrigation protocol associated with manual dynamic agitation on smear layer removal by using scanning electron microscope.

MATERIALS AND METHODS: Forty-five human mandibular permanent premolar teeth with single canals were decoronated to a standard length of 15mm and instrumented using ProTaper Next rotary files (X3). Roots were divided into 3 equal groups (n=15) according to the final irrigant used: Group I: 6% MCJ, Group II: 6% MCJ + 17% EDTA, Group III (control group): 2.5% NaOCl + 17% EDTA. Manual dynamic agitation (MDA) was used to activate the irrigants in all groups. Teeth were then prepared for scanning electron microscopy evaluation, and smear layer removal was assessed.. Comparisons between the study groups were done using Kruskal Wallis test, while comparisons of different regions within each group were done using Friedman test.

RESULTS: Along complete canal length, no significant difference was found between 6%MCJ+17% EDTA and 2.5% NaOCl + 17% EDTA (p=0.29). However, a significant difference was noted between 6%MCJ and 2.5% NaOCl + 17% EDTA (p=0.001). In all groups, the coronal third showed the best results (P = 0.02) and the apical third showed the worst (P = 0.007).

CONCLUSION: The use of 6%MCJ+17% EDTA associated with MDA could be an alternative to 2.5% NaOCl + 17% EDTA for smear layer removal, however, MCJ alone isn't effective in smear layer removal.

KEYWORDS: EDTA, Morinda citrifolia juice (MCJ), smear layer, sodium hypochlorite.

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INTRODUCTION

To achieve successful endodontic therapy, the root canal system must be disinfected to reduce microbial load. This goal is accomplished through chemo-mechanical debridement, in which mechanical instrumentation along with chemical irrigant, are considered the key principles for a favourable prognosis (1).

Dentine chips are formed during canal preparation by the action of endodontic instruments, which are added to the organic material remnants and irrigating solutions, forming an amorphous layer called smear layer, which adheres to the canal walls. (2).

This layer is composed of two zones: the first, which is 1-2 µm thick and contains dentine particles and organic material, and the second; extend to about 40 µm into the dentinal tubules and primarily composed of dentine chips, its known as smear plug (3). Smear layer prevents irrigant, medication, and filling materials from penetrating dentinal tubules and may even prevent them from contacting the canal walls (4). This compaction, according to researchers, is harmful because it reduces dentine permeability by 25-

49%, which would protect bacteria that were previously present inside the dentinal tubules (5).

The clinical implications of the smear layer's detrimental effects during root canal instrumentation highlight the importance of removing it effectively. Because no single solution can dissolve organic tissues while demineralizing the inorganic component of the smear layer, it is recommended that organic and inorganic solvents be used sequentially (6).

Irrigation is an important step in root canal preparation because it allows for more thorough cleaning than instrumentation alone (7). To remove the smear layer, various organic acids, ultrasonic instruments, and lasers were used. Sodium hypochlorite is still commonly used to dissolve the organic substance in the smear layer. NaOCl is the most commonly used endodontic irrigant during chemo-mechanical preparation of root canals due to its remarkable antimicrobial action and capacity for dissolving organic materials, (8).

The best organic tissue-dissolving property of NaOCl, however, is non-selective, which means that at high concentrations, it may dissolve both vital and necrotic pulp remnants indistinguishably. Additionally, it has high toxicity to the periapical tissues in the event of accidental extrusion through the apical foramen to the periradicular space. NaOCl also has a drawback in that it weakens collagen and proteoglycan, which lowers dentin's mechanical resistance. When NaOCl was utilized for root canal therapy, there have also been reports of mishaps and allergic reactions (9). It is also claimed that when exposed to tissue debris, this solution soon loses its action. To maintain this irrigation solution's full effectiveness and potency, regular refilling is necessary (10).

Currently, the recommended regimen and gold standard for removing the inorganic and organic components of the smear layer is 17% ethylenediaminetetraacetic acid (EDTA) and 2.5 %NaOCl (11). At neutral pH, the calcium ions in dentin react with EDTA in a concentration of 15–17% to create soluble calcium chelates (12), therefore, larger concentrations could result in erosion, changing the microhardness of the dentin. Additionally, it might result in apoptosis, necrosis, inflammation, and cytotoxic effects if it is extruded into the periapical tissues (13).

Researchers are looking for safer, more patient-friendly natural alternatives as a result of the ongoing rise in microbial strains that are resistant to antibiotics, the negative effects of medications generated synthetically, and the dangers related to synthetic chemicals used as irrigants. If these natural alternatives can remove smear layer properly, are cost-effective to use, and are compatible with biological systems, they would be extremely ideal. Several natural organic products were tested as root canal irrigant, and some of them demonstrated good antimicrobial properties such as the herbal product Morinda citrifolia juice (MCJ). MCJ is gaining popularity for its natural remedy effect and have been recently used as an alternative irrigant in root canal preparation (14).

A wide variety of therapeutic actions of MCJ are present, including antibacterial, antiviral, antifungal, anticancer, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-stimulating activities (15). L-asperuloside and alizarin, two antimicrobial substances, are present in MCJ. Additionally, acetone extracts from MCJ showed some antibacterial action (16).

Several methods can be used for irrigant activation including sonics, ultrasonic and laser. However, manual dynamic agitation (MDA) introduced by Machtou (17) in 1980 has proved to be an effective simple method for activation eliminating the vapor lock effect that hinders the flow of irrigants to the apical third (18).

Accordingly, the aim of the present study was to assess the effect of morinda citrifolia associated with manual dynamic agitation on smear layer removal by using scanning electron microscope for evaluation. The null hypothesis of this study was that there would be no difference between the three tested final irrigation protocols for removing smear layer.

MATERIALS AND METHODS

This study was approved by the ethics committee of Faculty of Dentistry, Alexandria University (0114-01/2020). It was conducted at the Faculty of Dentistry and Faculty of Science, Alexandria University, Egypt.

Sample size estimation

Based on a previous study (18), the minimal required sample size was determined to be 15 teeth per group (number of groups = 3) (total sample size = 45) by using a power of 80% and a level of significance of 95% (=0.05). (19). GPower version 3.1.9.2 was used to calculate the sample size (20).

Preparation of the specimens

Forty-five mandibular premolar teeth that were extracted for orthodontic treatment were the subject of the current investigation. Single rooted teeth with completely formed apices that were devoid of calcifications, fractures, root caries, attrition, or external resorption were chosen. After extraction, teeth were scaled with an ultrasonic scaler o remove debris, calculus, and organic tissues before being placed in 0.9% saline solution for storage. Buccal and proximal view radiographs were done to ensure the presence of a single canal (Vertucci type I). Access cavity was carried out in all teeth using round and Endo Z burs (

Dentsply Maillefer, Ballaigues, Switzerland). Teeth were decoronated using a diamond disc with a straight handpiece and finished using a diamond stone with high speed handpiece to standardize the whole length of the root to 15 mm. A #10 K file (Dentsply Maillefer, Ballaigues, Switzerland) was then introduced in the canal until it was just visible at the apex to ensure patency of the canal and the working length was maintained at 1mm shorter than this length. Root apices were blocked with utility wax to prevent irrigant extrusion through the apical foramen.

For shaping the root canals, ProTaper Next rotary system (Dentsply, Maillefer, Ballaigues, Switzerland) was used up to X3 file (apical diameter 0.3 mm, taper 0.07). Glide path was established using file #15 K-file till the working length. X1, X2 and X3 files mounted on X-Smart plus rotary motor (Dentsply, Maillefer, Ballaigues, Switzerland) were used till the working length according to the preprogrammed settings recommended by the manufacturer. After each instrument change, 2 mL of saline was used for irrigation using side-vented needle (PPH CERKAMED, Stalowa Wola, Poland) and patency was maintained using # 10 K-file.

Specimen grouping

After completing mechanical instrumentation, canals were rinsed with 5 mL of saline using a side-vented needle and dried with absorbent paper points X3 (Dentsply, Maillefer, Ballaigues, Switzerland). The permuted block technique was used to randomize the data (21). Specimens were randomly divided into three groups of 15 teeth each based on the final irrigant. The randomization scheme was created using the website (http://www.Randomization.com):

Group I: 6% MCJ (Tahitian Noni International Inc, Orem, UT), Group II: 6% MCJ + 17% EDTA (CalixE EDTA 17%, DHARMA RESEARCH, Miami, USA) and Group III (control group): 2.5% NaOCl (Clorox for Chemical Industries, A.R.E) + 17% EDTA

The specimens in all groups were finally irrigated with a total volume of 10ml of each of the tested irrigants for 10 minutes to ensure even distribution of the solutions. Irrigation was done with 30-gauge side vented needle 1 mm shorter than working length in an apical-and-coronal direction and the irrigating solution was refreshed every 1 min.

Manual Dynamic Agitation

Following the final rinse of the main irrigating solution; the apical 2mm of the X3 cone was cut using a scalpel blade and introduced with a rapid pumping motion up and down the canal (100 strokes) for 1 minute period (22). Five ml of distilled water was then used to rinse the canals from any surplus solution of MCJ (groups I and II) and NaOCl (group III). One ml of 17% EDTA was used as a final flush in groups II and III using a 30-gauge side vented needle 1 mm shorter than working length for 1 minute without activation. Canals were then dried using absorbent paper points.

Scanning electron microscopy evaluation

After creating grooves along the roots' long axis using a water-cooled diamond disc, the roots were sliced longitudinally in a buccolingual orientation to reveal the whole canal extension.

Canals were tested under a SEM (Joel Scanning electron microscope (JSM-5300)) at the scanning electron microscopy unit in the Faculty of Science, Alexandria University. The samples were mounted using silver paint on the specimen holder then coated with gold for SEM examination (23). The effectiveness of the final irrigation protocols was evaluated using the scoring system suggested by Hulsmann et al (24) as follows:

Score 1: completely opened dentinal tubules.

Score 2: more than 50% of dentinal tubules are opened.

Score 3: less than 50% of the dentinal tubules are opened.

Score 4: dentinal tubules are almost completely covered with smear layer.

All specimens were observed and examined under magnification of 2000x along the complete canal length (coronal, middle and apical thirds) of all root canal wall.

Statistical analysis

Data were analyzed using IBM SPSS for Windows (Version 23.0) and significance was inferred at p value <0.05. Frequencies, percentage, means, standard deviation (SD), median and interquartile range (IQR) were calculated. Comparisons between the three study groups were done using Kruskal Wallis test, while comparisons of different regions within each group were done using Friedman test. Both tests were followed by multiple pairwise comparisons (for significant results) using Bonferroni adjusted significance levels. Calibration on smear layer scoring was done for three examiners; intra- and inter-examiner reliability were assessed, and kappa ranged from 0.67 – 0.99 indicating moderate to excellent agreement between examiners.

RESULTS

Figure 1 shows the percentage of smear layer scoring at different canal thirds in the three study groups. In the coronal third, score 1 (best results) was recorded in 66.7% of specimens in Group III (NaOCl+EDTA), followed by group II (MCJ+EDTA) (33.3%), and only 20% of specimens in group I (MCJ) had score 1. Score 3 was not recorded in group III, while 13.3% of specimens in groups I and II had score 3. In the middle third, Group III also revealed the best results with 40% of the specimens showing score 1, followed by group II (26.7%) and finally group I (6.7%). In the apical

third, score 1 was only recorded in group III (26.7%), while Group I showed the worst results where 66.7% of specimens had score 3. Score 4 was not recorded in any specimen in all study groups.

Table 1 shows the mean smear layer scores in the three canal thirds and along complete canal length. Regarding different canal thirds, group III (NaOCl+EDTA) showed the best results (lowest mean score) in the three canal thirds. A statistical significant difference was noted between groups I (MCJ) and III (NaOCl+EDTA) in the coronal and apical thirds (P = 0.02 and 0.01, respectively) while no significant difference was noted in the middle third between the three study groups (P=0.11). In addition, no significant difference was noted between groups II (MCJ+EDTA) and III in the three canal thirds. On comparing the mean smear layer scores in the three canal thirds within each study group, a statistical significant difference was only found in group I (P= 0.002), while no difference was found in the other groups (P=0.10 and P=0.06 in groups II and III, respectively). Along the complete canal length, a significant difference was only shown between groups I (MCJ) and III (NaOCl+EDTA) (P < 0.001) while no difference was shown on comparing groups II and III (figures 2-4).

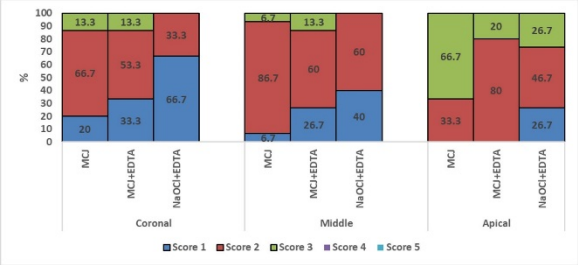

Figure (1): Percentage of smear layer scoring at different canal thirds in the three study groups

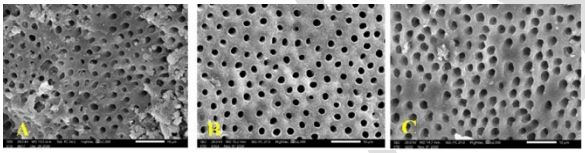

Figure (2): Representative images of scanning electron microscope photomicrographs showing coronal thirds of root canal walls in different study groups with assigned scores: (A) MCJ with score 2, (B) MCJ+EDTA with score 1, (C) NaOCl+EDTA with score 1.

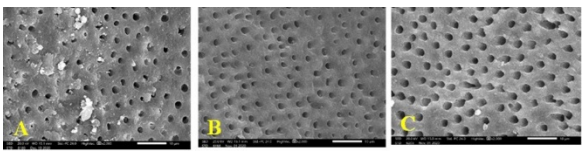

Figure (3): Representative images of scanning electron microscope photomicrographs showing middle thirds of root canal walls in different study groups with assigned scores : (A) MCJ with score 2 , (B) MCJ+EDTA with score 2, (C) NaOCl+EDTA with score 1.

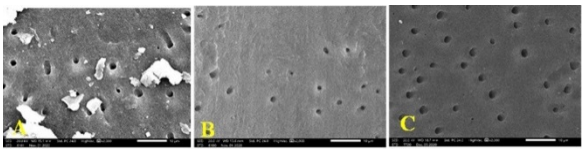

Figure (4): Representative images of scanning electron microscope photomicrographs showing apical thirds of root canal walls in different study groups with assigned scores : (A) MCJ with score 3, (B) MCJ+EDTA with score 3, (C) NaOCl+EDTA with score 2.

Table (1): Mean smear layer scores in the three canal thirds and along complete canal length

		Group I MCJ (n=15)	Group II MCJ + EDTA (n=15)	Group III NaOCl + EDTA (n=15)	KWT P value
Coronal	Mean ± SD	1.93 ± 0.59 a	1.80 ± 0.68 a,b	1.33 ± 0.49 b	H= 7.63 P= 0.02*
	Median (IQR)	2.0 (2.0, 2.0)	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)	
Middle	Mean ± SD	2.00 ± 0.38	1.87 ± 0.64	1.60 ± 0.50	H= 4.46 P= 0.11
	Median (IQR)	2.0 (2.0, 2.0)	2.0 (1.0, 2.0)	2.0 (1.0, 2.0)	
Apical	Mean ± SD	2.67 ± 0.49 a	2.20 ± 0.41 a,b	2.00 ± 0.76 b	H= 8.74 P= 0.01*
	Median (IQR)	3.0 (2.0, 3.0)	2.0 (2.0, 2.0)	2.0 (1.0, 3.0)	
Total	Mean ± SD	2.20 ± 0.25 a	1.96 ± 0.42 a,b	1.64 ± 0.32 b	H= 15.52 P <0.001*
	Median (IQR)	2.33 (2.0, 2.33)	2.0 (1.67, 2.33)	1.67 (1.33, 2.00)	
Friedman test		X^2= 12.76 P= 0.002*	X^2= 4.75 P= 0.10	X^2= 5.69 P= 0.06	

KWT: Kruskal Wallis test was used, IQR: Interquartile range
*statistically significant at p value <0.05
ab: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level

DISCUSSION

Three crucial steps can be used to summarise the success of root canal therapy: chemo-mechanical cleaning the root canal system to get the most bacterial reduction possible; getting a stable root canal filling to encapsulate any remaining bacteria and separate the endodontic system from the periapical tissues; and getting a long-lasting coronal restoration(25,26). This is achieved through mechanical instrumentation accompanied by irrigating solutions to ensure that the intracanal surface is clean and to eradicate infection (27). However, during mechanical instrumentation a smear layer is formed harboring infected dentin chips and bacteria which may have negative feedback on the treatment outcome.

It has been proposed that the smear layer should be removed from the root before the ultimate obturation for a number of reasons. In the beginning, the smear layer can serve as a substrate for the bacteria to live on and develop. Second, pulp tissue leftovers that are still present in the smear layer may lead to problems in the future. Third, the smear layer is less adherent and can hinder the sealer's successful bonding to the tubular dentin, increasing the risk of

microleakage. Fourth, they can prevent irrigant solutions and intra canal medications from entering dentinal tubules. These behaviors may eventually lead to unsuccessful treatment outcomes. Others, argue that the smear layer should stay in place and have their assumptions supported by several factors. Smear layer formation first isolates organisms, irritants, and toxins by preventing their inward or outward migration. The second benefit is that the smear layer blocks the dentinal tubular structure and can be modified appropriately to offer a long-lasting, reliable seal that is resistant to dissolution. (28).

The present study was done to investigate smear layer removal from different root canal sections using 6% MCJ and compare it to 6%MCJ+17% EDTA and 2.5% NaOCl + 17% EDTA that is considered the gold standard regimen for irrigation using SEM for evaluation.

Several precautions were suggested for standardization and balancing in the present study which included: teeth selection, total root length, mechanical preparation technique, gauge of the irrigation needle and its depth of penetration and activation method of the irrigants used.

In the current study, mandibular single rooted premolars with single straight canals extracted for orthodontic treatment were used as these teeth tend to have the same cross section with oval outline; also, they were taken from the same age group with almost the same number and width of dentinal tubules. Teeth with straight canals facilitate the flow and penetration of the irrigating solution to reach the apical part as curvatures may hinder the irrigating solution from reaching further apically. This is in accordance with Saghiri et al (29), Attur et al (30) and Jagzap et al (27) who used mandibular premolars in their study. Moreover, teeth were cut to 15mm length for standardization of the specimens' length.

For the mechanical preparation procedure, all root canals were prepared till X3 PTN file (size 30, 7% taper) to preserve the radicular dentin structure and to allow the irrigants used in this study to reach apical part as stated by Branson et al (31).

In the present study, a thirty-gauge side vented needle (0.31mm diameter) was used for the delivery of the irrigating solution to reach the apical third as the canal was instrumented to iso size 30. The needle was inserted 1mm shorter than the working length to ensure adequate flow of the irrigant in the apical third as it was proved that the irrigant can be delivered 1mm deeper than the tip of the irrigating needle (32).

Manual dynamic agitation was used in the present study following the final rinse of the irrigating solutions inside the root canal for irrigant activation as it is simple, fast and cost effective method for irrigant activation (17). It also allows the fresh mixed solution to reach the apical stagnant solution in the apical few millimeters of the canal (33). This is in agreement with Mandke et al (34) who mentioned that manual dynamic agitation technique allowed the irrigant to reach the apical portion of the canal and dislodged the vapor lock effect as well as sterilization of the gutta-percha cone before canal obturation.

Several irrigating solutions have been suggested in the literature for smear layer removal. The combined use of 17% EDTA and NaOCl (control) is considered the gold standard regimen for smear layer removal. EDTA has the ability to chelate calcium ions found within the dentin. It does, however, exhibit cytotoxicity that may elicit an inflammatory response in periapical lesions (27). In addition, according to Moon et al (35) the prolonged use of EDTA could cause a decrease in dentin microhardness and peritubular dentin decalcification. Consequently, it was necessary to search for other biocompatible irrigants rather than EDTA with less hazards. Moreover, the hazards associated with synthetic chemicals used as irrigants have prompted researchers to look for safer and natural alternatives. One of these is the juice of the Morinda citrifolia plant, which has a variety of medicinal benefits, including analgesic, hypotensive, anti-inflammatory, and immune-enhancing ones (36).

Scanning electron microscopic evaluation was performed to analyze the efficacy of smear layer removal. This method was chosen as it was proven that SEM has a high magnification power which enables the precise observation of dentinal tubules and is thus widely used for evaluating smear layer removal (37). However, SEM has several limitations as it cannot give an overall detailed view at low magnification and artifacts may be produced during preparation of the samples which may lead to interpretation mistake (38). The magnification utilized in this study was 2000x and all the specimens were observed and examined in coronal, middle and apical parts of their root canal wall. This is in agreement with Chandrasekhar et al (2021) (39) who evaluated the efficacy natural chelating agents in smear layer removal.

Following the mechanical preparation, a final rinse of the irrigating solution fills the canal, and a well-fitting master gutta-percha cone is introduced in the canal. The rapid pumping up and down 3mm motion with a snugly fitting gutta-percha cone generates higher intra canal pressure, carrying the irrigating solution to the "untouched canal walls" (22).

In the current study, 6% MCJ+ 17%EDTA was shown to be as effective as 2.5% NaOCl+ 17% EDTA in the removal of smear layer along the entire root length while 6% MCJ alone was not effective in smear layer removal. This might be due to that MCJ has no chelating properties to remove the inorganic

component of the smear layer while EDTA has a chelating action facilitating the removal of this inorganic component .This is in accordance with Murray et al (18) who evaluated the use of Morinda citrifolia as an endodontic irrigant and concluded that MCJ might have a positive impact on smear layer removal up to 80% when combined with EDTA to avoid the adverse effects of NaOCl. Accordingly, the null hypothesis of the present study was rejected.

The best results for smear layer removal in this study was seen in the coronal third in all study groups. This might be due to that the dentinal tubules are wider in this region while in the middle and apical thirds they are narrower with more peritubular and intertubular dentin. In addition, it is easier for the irrigants to achieve their maximal effect in the coronal third due to the absence of the vapour lock effect which hinders the optimum action of irrigants in the middle and apical thirds. This is in accordance with Madhusudhana et al (40) who studied the efficacy of Morinda Citrifolia in smear layer removal and concluded that MCJ showed better results in the coronal third than the middle and the middle also showed better outcome than the apical region.

Given the numerous negative properties of NaOCl; MCJ may be advantageous as a herbal alternative. However, before they are recommended for clinical use, the preparation of fresh irrigating solutions, standardization, toxicity, antimicrobial activity and their effect on dentin mechanical properties should be further evaluated.

Based on the results of this study, it is worth mentioning that none of the tested irrigants was able to completely remove the smear layer along the complete canal length. This emphasizes that further research should be conducted to develop the optimum irrigating solution for smear layer removal.

CONCLUSION

Within the limitations of this in vitro study, it could be concluded that the use of 6%MCJ+17% EDTA associated with MDA could be an alternative to 2.5% NaOCl + 17% EDTA for smear layer removal. MCJ alone showed inferior results, therefore, the use of a chelating agent such as EDTA is essential for the successful removal of the smear layer..Based on the results of the current study that showed that none of the tested irrigants could completely remove the smear layer, it is recommended to conduct further research to reach the optimal smear layer removal protocol.

CONFLICT OF INTEREST

The authors deny any conflict of interests related to the current study.

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