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Cleanliness efficacy and effect on dentin microhardness of a novel plant extract irrigant

Maram E. Khallaf, Engy M. Kataia, Yousra Aly*, Nada Omar and M. Abdullah Mohamed

Abstract

Objective: This study was done to evaluate the effect of the prepared *Moringa oleifera*-based root canal irrigant compared to conventionally used irrigants on the microhardness of root dentin and smear layer removal.

Materials and methods: One hundred freshly extracted teeth with single root were divided into 5 groups according to the irrigant used: group 1, saline; group 2, *Moringa oleifera*; group 3, *Moringa oleifera* + chlorhexidine; group 4, chlorhexidine; and group 5, sodium hypochlorite. Root canal preparation was done using Protaper universal system till size F5 using one of the irrigants after each file. Fifty teeth were prepared to measure dentin microhardness, while the remaining fifty were prepared for evaluation of the remaining debris and smear layer using stereomicroscope and scanning electron microscope (SEM) in each third of the canal.

Results: Chlorhexidine alone and combined with *Moringa oleifera* showed higher microhardness values in apical and coronal parts. In the middle parts, chlorhexidine followed by *Moringa oleifera* showed the highest results. SEM examination showed that none of the used irrigants has the ability to completely remove smear layer. However, *Moringa oleifera* group showed the least amount of smear layer on canal wall.

Conclusions: *Moringa oleifera* is a promising final irrigant solution alone or combined with chlorhexidine throughout the instrumentation. *Moringa oleifera* was proved to have a high effect as a chelating agent.

Keywords: Irrigant, Microhardness, *Moringa oleifera*, Smear layer removal

Background

Proper cleaning and shaping of the canal prior to its obturation is the key of success of endodontic treatment (Muhammad et al. 2015). To achieve this purpose, different types of root canal irrigants and intracanal medicaments are used (Hasselgren et al. 1988). Sodium hypochlorite (NaOCl) is the most commonly used irrigant for this purpose. It has many advantages such as tissue dissolving, antibacterial, and lubricant action (Zehnder et al. 2002; Siqueira Jr et al. 1998). However, it causes severe reaction when it gets in contact with periapical tissues (Pashley et al. 1985; Zhu et al. 2013). That

is why researches are nowadays directed towards the use of non-toxic and more biocompatible herbal materials to be used as root canal irrigants. Examples of these new biocompatible materials are green tea, *Embllica officinalis*, *Psidium guajava*, neem, and *Moringa oleifera* (Ghonnade et al. 2013; Dubey 2016). *Moringa oleifera* (*M. oleifera*) is a plant that grows in many countries from South Asia to West and East Africa (Anwar et al. 2007). *Moringa oleifera* is a native Indian tree that showed an antiviral, antibacterial, antioxidant, antisclerotic, and anti-inflammatory properties. It has been used for treatment of malaria, malnutrition, colon cancer, and myeloma (Jung 2014).

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Materials and methods

Specimen selection

One hundred extracted single-rooted teeth were used in this study (National Research Centre project; Ethics Committee approval number 16/344).

Each tooth was radiographed to confirm the presence of a single canal. Teeth with previous root caries, cracks, curved canals, endodontic treatment, internal resorption, or calcification were excluded. After removal of calculus and soft-tissue debris, the teeth were placed in 2.5% sodium hypochlorite for 1 h to allow for surface disinfection and then stored in saline solution until instrumentation.

Specimen preparation

The teeth length was standardized to a length of 16 mm. Teeth decoration was done using a safe-sided diamond disk mounted in a low-speed handpiece under a water coolant. The working length was measured by subtracting 1 mm from the length recorded when the tip of a # 15 K file (Dentsply Maillefer, Switzerland) was visible at the apical foramen. Root canal preparation was done using Protaper (Dentsply, Maillefer) universal nickel titanium rotary system driven by x-smart endomotor (Dentsply, Maillefer) till size F5 file.

Grouping

Teeth were divided into 5 groups ($n = 20$) according to the irrigant used, as follows: group 1, saline; group 2, *M. oleifera* (extracts were obtained from the Egyptian Scientific Society of the moringa trees, National Research Centre, Dokki, Giza, Egypt); group 3, *M. oleifera* in addition to chlorhexidine (CHX); group 4, chlorhexidine; and group 5, sodium hypochlorite.

Irrigation was done by 3 milliliters (ml) of irrigant solution according to each group after each file size. A final rinse was performed with 3 ml distilled water.

Microhardness testing

Fifty teeth were used for this part. Teeth were sectioned into two halves using a carborundum disk to create a longitudinal groove on the buccal and lingual surfaces. The teeth were then split using a chisel and mallet. Teeth from each group were rinsed thoroughly with saline and mounted on acrylic cylinders with self-cure acrylic resin with the root canal facing up. The specimens' surfaces were finished using a silicon carbide paper in the following order: 400, 800, and 1200 grit. The microhardness measurements were done using a Vickers Diamond Microhardness Tester (Nexus 4000/60, INNOVATEST, Netherlands, Europe) in Vickers hardness units (VHN). At each root third, the microhardness measurements were taken at three different points at a depth of 500 μ m from the lumen. Each

measurement was carried out by using a 300 g load for 15 s dwell time.

Canal cleanliness and smear layer removal

Fifty teeth were used for this part of the study. Each tooth was divided into equal sections of coronal third, middle third, and apical third with a diamond disk. Each slice was marked as coronal, middle, and apical to identify its site. Images of each third of the canal were taken using a Canon digital camera with magnification $\times 25$ connected to a Zeiss stereomicroscope (Technival 2). The captured images were analyzed using ImageJ software (ImageJ 1.47 V, National Institute of Health, USA). The percentage of debris on the entire surface area was analyzed in relation to the whole area of each one third of the canal (Fig. 3).

The samples were then air-dried, sputter-coated with gold using a fine coat ion sputter JFC-1100 (fine coat ion sputter JFC-1100, JEOL Ltd., Tokyo, Japan), and then evaluated using scanning electron microscope (SEM) (Jeol JSM-6360 LV, JEOL Ltd.). Samples were examined with magnification $\times 50,000$ and $\times 100,000$.

Statistical analysis

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests; the data showed parametric (normal) distribution.

Repeated measures ANOVA was used to compare between more than two groups in related samples. Paired-wise sample *t* test was used to compare between two groups in related samples. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples.

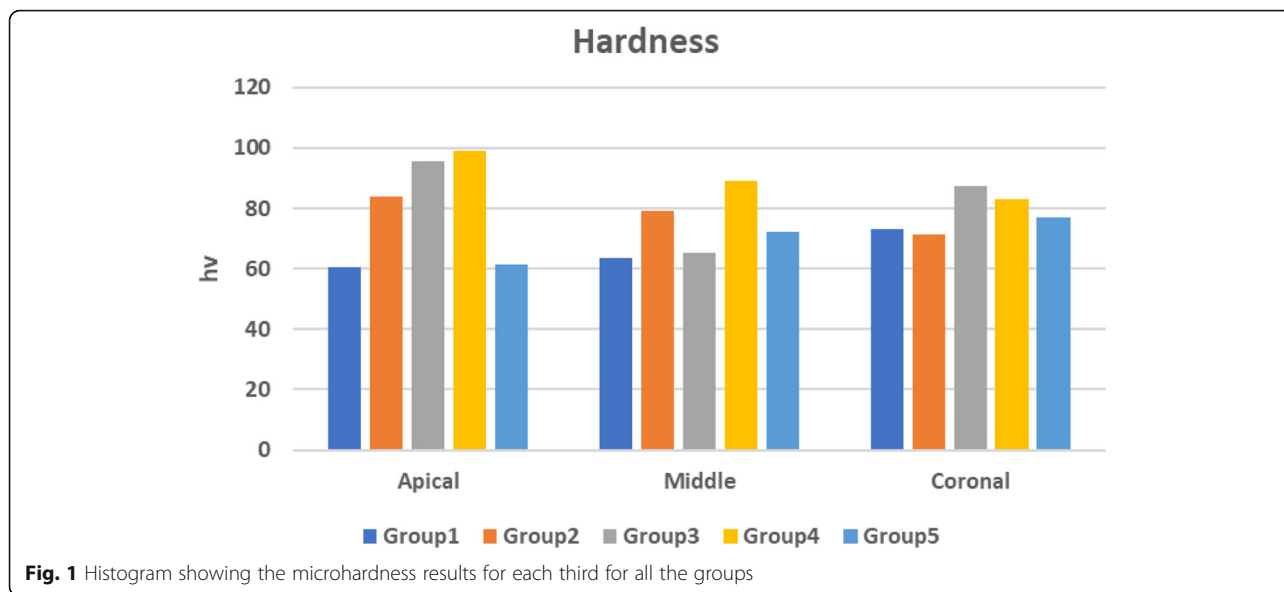
The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Table 1 The mean and standard deviation (SD) of hardness in each third in different groups

Variables	Hardness						p value
	Apical		Middle		Coronal		
	Mean	SD	Mean	SD	Mean	SD	
Group 1	60.57	3.16	63.73	2.85	73.10	12.74	0.292 ns
Group 2	83.90	5.01	79.03	9.92	71.30	3.02	0.304 ns
Group 3	95.60	7.61	65.33	5.10	87.33	7.15	0.025*
Group 4	99.17	2.36	89.23	6.22	82.87	12.97	0.295 ns
Group 5	61.37	2.95	72.30	2.15	76.77	3.24	0.028*
p value	< 0.001*		0.002*		0.228 ns		

ns non-significant ($p > 0.05$)

*Significant ($p < 0.05$)



Results

Microhardness results (Table 1 and Figs. 1 and 2)

No statistically significant difference was found between apical, middle, and coronal thirds in group 1 (saline), group 2 (*M. oleifera*), and group 4 (CHX). In group 3 (*M. oleifera* and CHX), a statistically significant difference was found between the three thirds, where the highest mean value was found in apical third followed by coronal, then middle. In group 5 (NaOCl), a statistically significant difference was found between apical and coronal thirds where the highest mean value was found in the coronal third.

A statistically significant difference was found among the different groups in the apical and middle thirds.

While no significant difference was found in the coronal third.

Remaining debris evaluation results (Table 2 and Figs. 3, 4, and 5)

In group 1, a statistically significant difference was found between the coronal and each of the middle and apical third where $p = 0.027$ and $p = 0.012$ respectively. In groups 2 and 3, a statistically significant difference was found between the apical and coronal thirds where $p = 0.042$. In group 4, a statistically significant difference was found between the apical and each of the middle and coronal thirds where $p = 0.008$ and $p = 0.013$ respectively. In group 5, no statistically significant difference

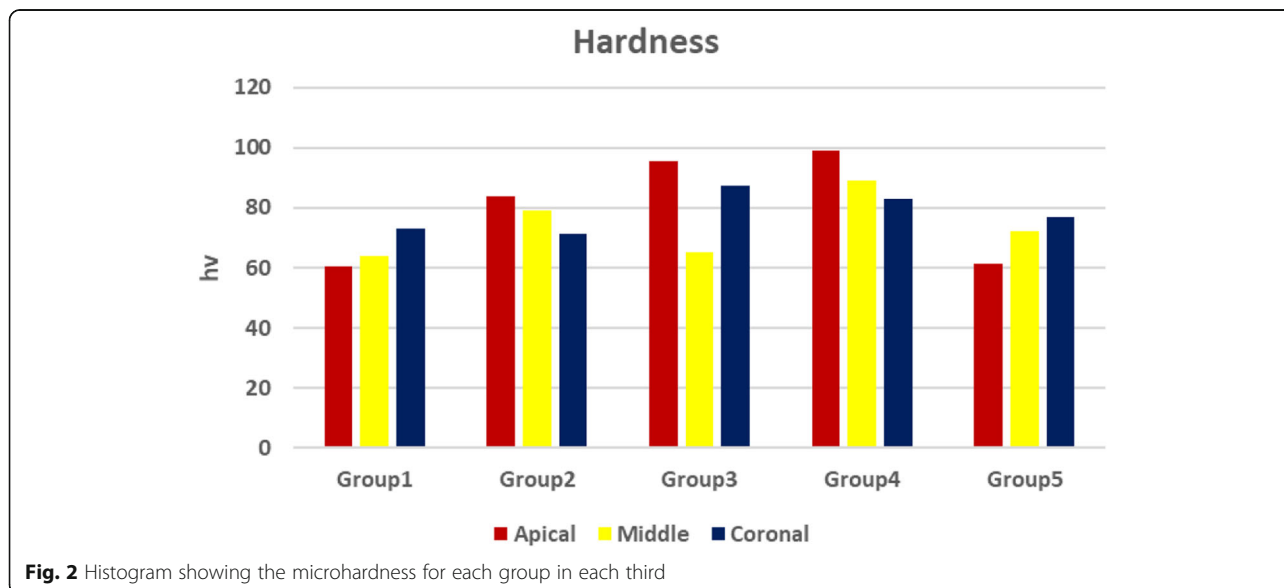


Table 2 The mean and standard deviation (SD) of debris percentage in each third in different groups

Variables	Debris percentage						p value
	Apical		Middle		Coronal		
	Mean	SD	Mean	SD	Mean	SD	
Group 1	32.85	20.95	24.33	13.68	14.80	11.67	0.020*
Group 2	10.30	6.80	5.83	5.72	1.30	2.01	0.041*
Group 3	35.75	23.32	11.79	15.92	5.29	8.19	0.032*
Group 4	29.23	11.24	9.17	5.39	6.05	2.15	0.005*
Group 5	35.08	22.32	25.70	22.55	13.77	5.17	0.115 ns
p value	0.192 ns		0.128 ns		0.024*		

ns non-significant ($p > 0.05$)
 *Significant ($p < 0.05$)

was found between the apical, middle, and coronal thirds where $p = 0.115$.

SEM evaluation

SEM examination showed that none of the used irrigants has the ability to completely remove smear layer. However, the *M. oleifera* group showed the least amount of smear layer on the canal wall (Figs. 6, 7, 8, 9, and 10).

Discussion

A number of chemicals have been previously investigated as irrigants to remove smear layer. The sodium hypochlorite solution (NaOCl) has been the mostly used irrigant for over four decades due to its proven effective antibacterial properties and its excellent action as an inorganic solvent material. However, high concentrations of NaOCl showed toxic effect to the periapical tissues (Ferraz et al. 2001; Kuruvilla and Kamath 1998).

That is why researches are now shifted towards the creation of more compatible irrigant solutions based on natural herbs. In many studies, chlorhexidine gluconate was proved to be an effective endodontic irrigant. Chlorhexidine could clear the microorganisms persisting in the root canal, even after biomechanical preparation, due to its adsorption capacity and slowly liberated active cations by the dental tissues, which lead to successful root canal therapy (Ayhan et al. 1999; Leonardo et al. 1999). Chemicals used for root canal irrigation may lead to changes in chemical and physical properties of root dentin (Cruz-Filho et al. 2011). Moreover, decrease in the dentin microhardness can affect the adhesion and sealing ability of the sealers to the root dentin walls

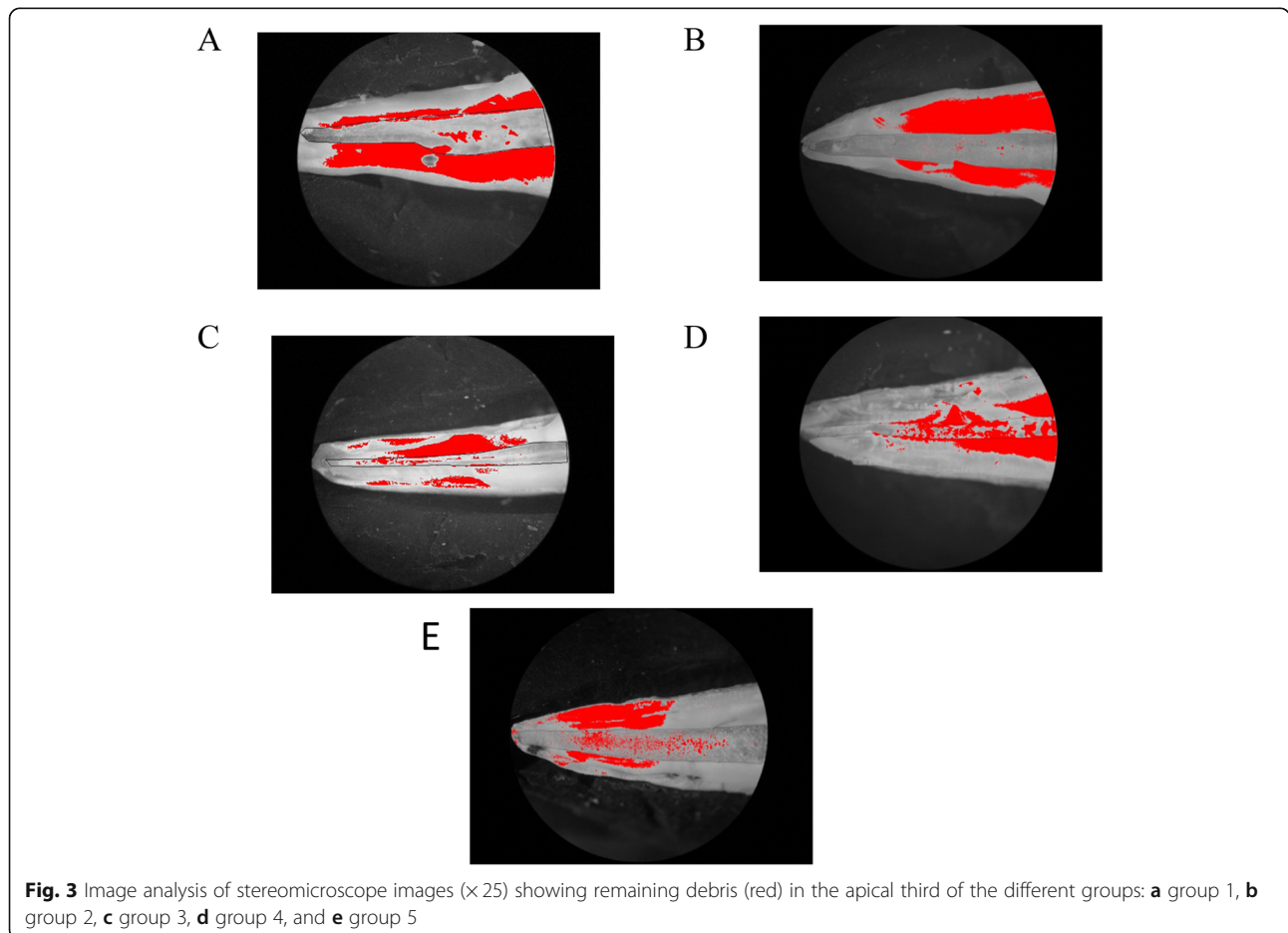
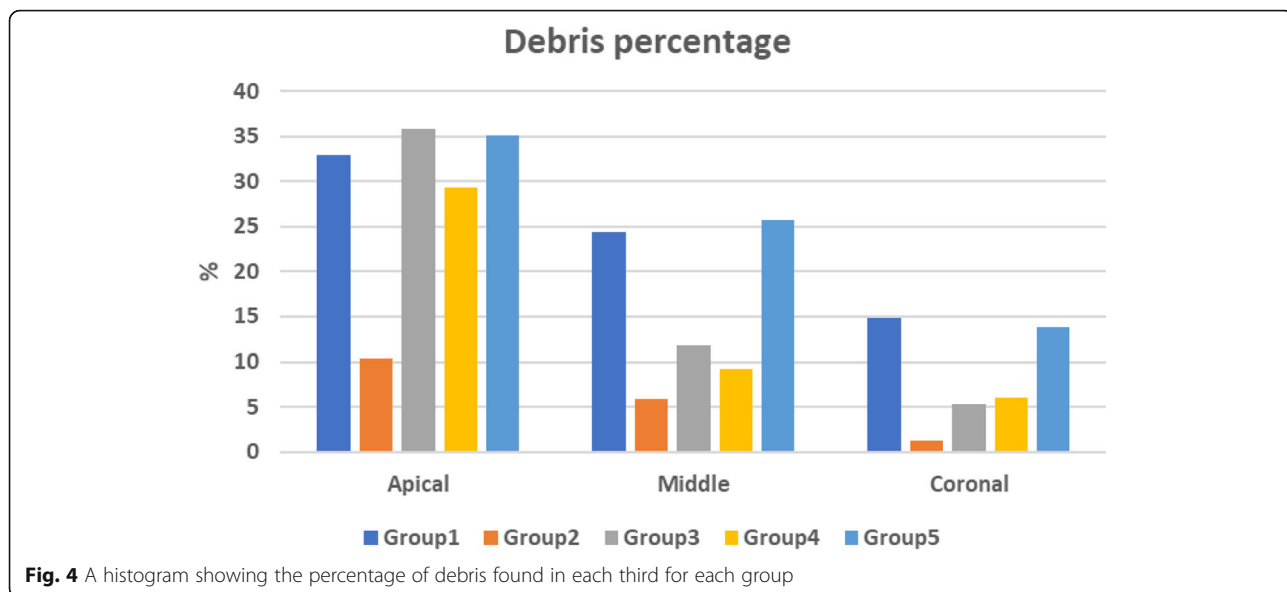
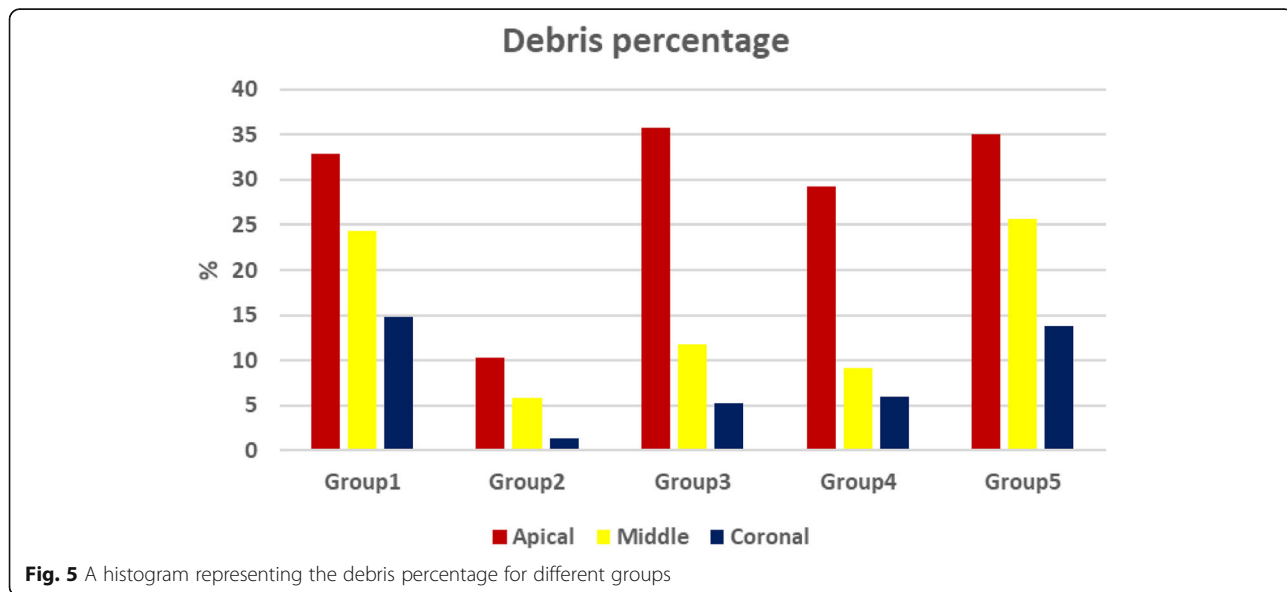


Fig. 3 Image analysis of stereomicroscope images (x25) showing remaining debris (red) in the apical third of the different groups: **a** group 1, **b** group 2, **c** group 3, **d** group 4, and **e** group 5



(Zehnder 2006). Irrigation with a tissue-dissolving antimicrobial solution is essential for effective removal of the smear layer and remnant pulp debris from the root canals. No irrigant can completely achieve total debridement of the root canal system; thus, the combination of more than one solution is necessary to achieve this goal (Zehnder 2006; Agrawal Vineet et al. 2014). In this study, a comparison of the effect on dentin microhardness and smear layer removal of the new irrigant solution *M. oleifera* (extracts were obtained from the Egyptian Scientific Society of the moringa trees, National Research Centre, Dokki, Giza, Egypt), *M. oleifera* +

CHX, CHX solution, NaOCl, and saline solution was done. The microhardness measurement was performed at the coronal, middle, and apical third of the root canal dentin. Mean Vickers hardness number (VHN) was calculated for each specimen. The current study design was done following Pashley et al. (Pashley et al. 2004), who stated that microhardness was affected by tubular density; as the tubular density increases, the dentin microhardness decreases. Microhardness was evaluated using a Vickers hardness tester. The selection of Vickers microhardness tester rather than the Knoop hardness tester was due to the former's ability of evaluating surface



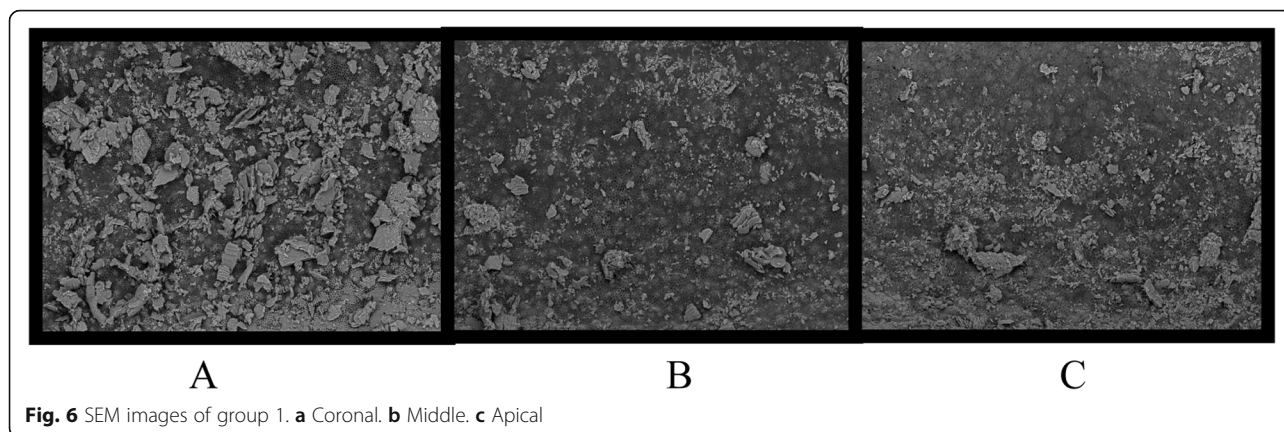


Fig. 6 SEM images of group 1. **a** Coronal. **b** Middle. **c** Apical

changes occurring in deep dental hard tissues. The Knoop hardness tester is limited to superficial dentin at 0.1 mm rather than deep dentin (Kandil et al. 2014; AL-Ashou WO. 2011). Although a reduction in microhardness facilitates root canal instrumentation, it may also weaken the root structure leading to fracture of the endodontically treated tooth (Baghdadi and Hassanein 2004; Ossareh and Kishen 2015). The measurement of microhardness indicates indirectly the evidence of mineral loss or gain in dental hard tissues (Panighi and G'Sell 1992; Ten Bosch 1992).

Therefore, this study was aimed to compare the effect of different root canal irrigant solutions on microhardness of the root canal dentin and smear layer removal. Sodium hypochlorite decreased the microhardness in most of the canal thirds; the organic dissolving property of NaOCl on the collagen component of dentin is an explanation of the cause of microhardness reduction (Cohen et al. 1970). Another possible hypothesis is that when in contact with the dentin, NaOCl promoted a decrease in the amount of phosphate (Tsuda et al. 1996). The microhardness reduction observed in the present

study is in accordance with the results obtained by Oliveira et al. (Oliveira et al. 2007). The concentration and time of action of NaOCl on dentin interfered with its effect on microhardness (Slutzky-Goldberg et al. 2004), although that effect was not clear in the apical third.

CHX recorded the highest mean hardness values in the apical, middle, and coronal parts. Furthermore, CHX with *M. oleifera* recorded a statistically significant higher mean hardness values than NaOCl in the apical part. This is found to be in agreement with the finding of Ari et al. (Ari et al. 2004) who concluded that 0.2% chlorhexidine gluconate seemed to be an appropriate endodontic irrigation solution because of its harmless effect on the microhardness of root canal dentin. Different formulations of *M. oleifera* are considered to increase the dentin microhardness in the three thirds of the canal; this could be attributed to the remineralizing effect of *M. oleifera* stated by Nagib et al. (Nagib et al. 2016).

The important goal of irrigation is to remove the smear layer and debris (Srirekha et al. 2013). The main two purposes of irrigation are to remove debris (the organic component) and to remove smear layer (mostly

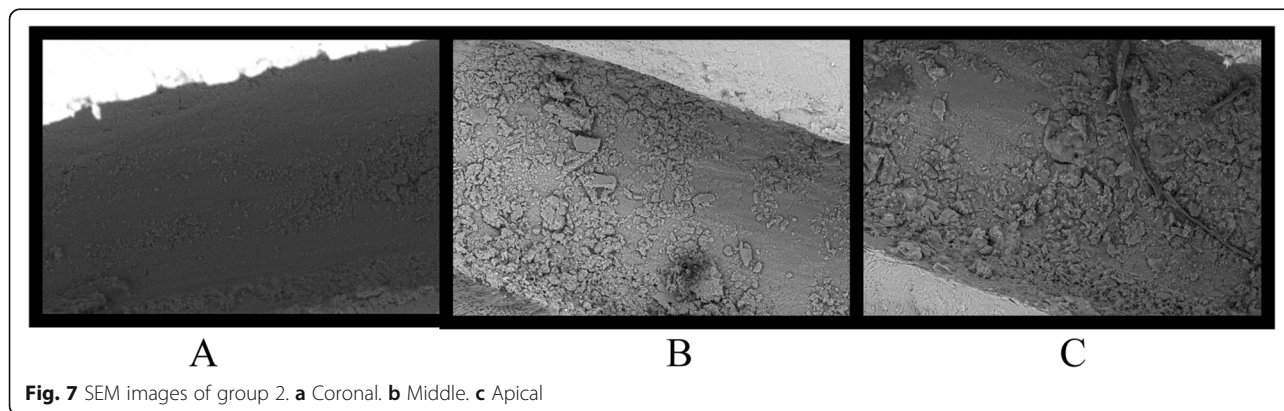


Fig. 7 SEM images of group 2. **a** Coronal. **b** Middle. **c** Apical

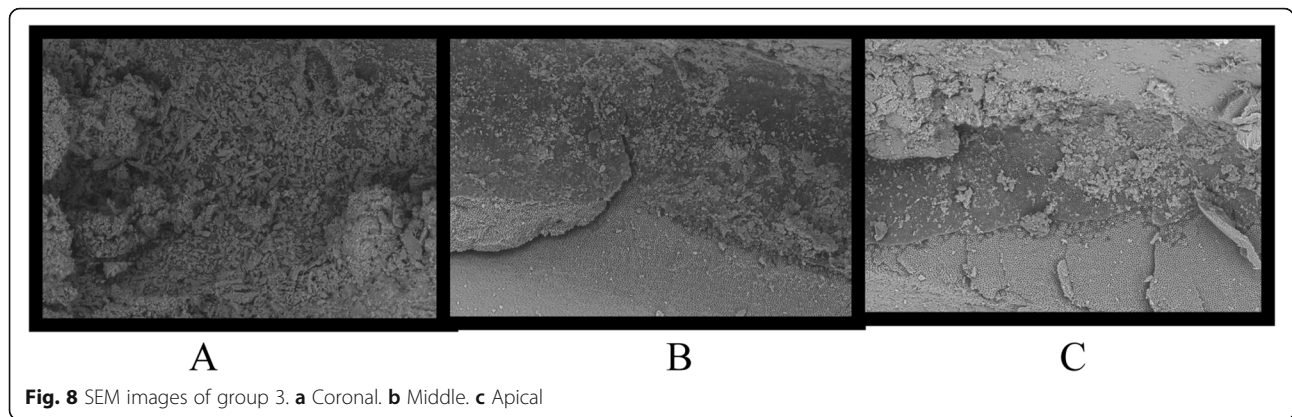


Fig. 8 SEM images of group 3. **a** Coronal. **b** Middle. **c** Apical

the inorganic component) (Sen et al. 1995). An in vitro study conducted by Orstavik and Haapasalo (Orstavik and Haapasalo 1990) showed that the smear layer removal decreased the time needed for dentin disinfection with intracanal medicaments. Other studies have showed that the removal of smear layer led to better adhesion of obturation materials to canal walls (Tidmarsh 1978; White et al. 1984). Scanning electron microscopy has been used to evaluate the ability of the various irrigants to remove the smear layer. SEM allows an examination of the prepared root canal surface morphology (Torabinejad et al. 2003). All irrigants used in this study were able to decrease debris in the coronal third with varying degrees; this is attributed to the increased amount of irrigant reaching the coronal part of the root (Karade et al. 2017). In this study, it was verified that *M. oleifera* as an irrigant alone or combined with CHX was capable to remove the remaining debris similar to the NaOCl and CHX. No statistically significant difference was found between the apical, middle, and coronal thirds in these groups. In the middle and apical thirds, the *M. oleifera* irrigant alone showed the lowest debris on the

canal wall, and this was also confirmed using the SEM images. This property may be attributed to its high effect as a chelating agent as proven by Zaroul et al. (Zaroul et al. 2014). These results are important for further research on the efficiency of natural herbs used as intracanal irrigant solutions having a similar effect as the routinely used ones.

Conclusions

- *Moringa oleifera* increased dentin hardness.
- *Moringa oleifera* is a promising final irrigant solution alone or combined with chlorhexidine throughout the instrumentation.

Recommendation

1. With the positive results obtained from this study, further studies should be carried out to investigate the *Moringa oleifera* antibacterial effect on root canal pathogens.

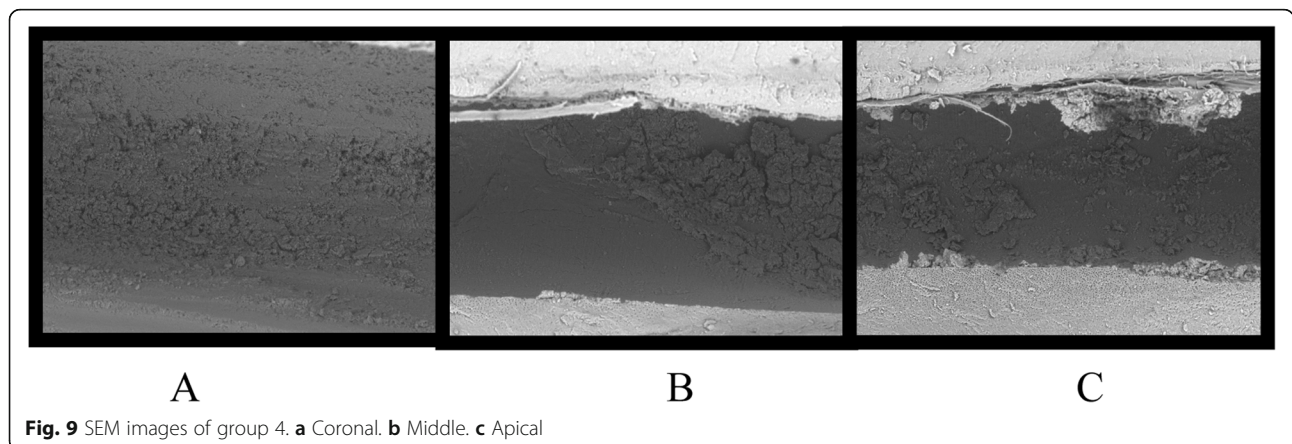
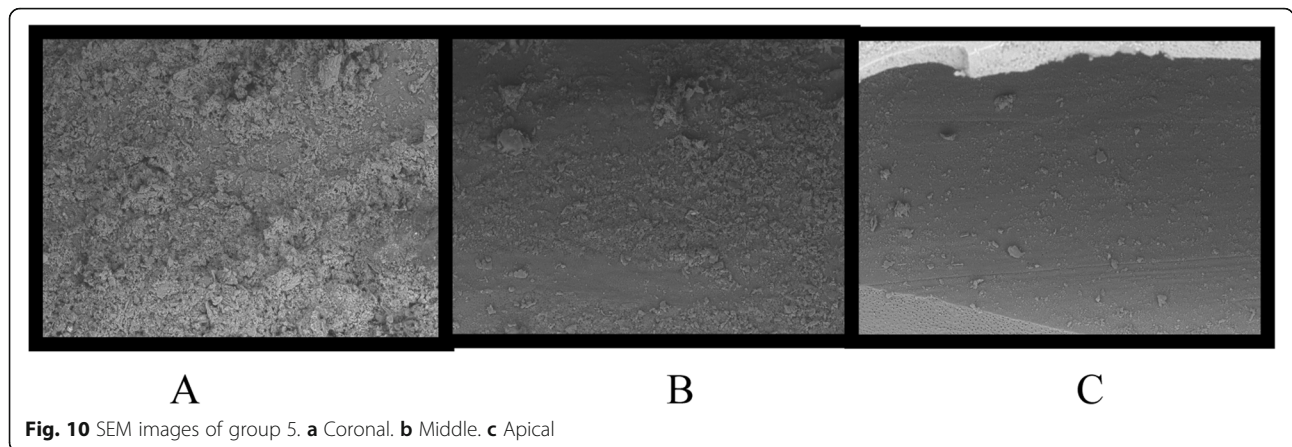


Fig. 9 SEM images of group 4. **a** Coronal. **b** Middle. **c** Apical



2. Further studies should be carried out to investigate the *Moringa oleifera* ability to be used as a chelating agent compared to EDTA.

Abbreviations

SEM: Scanning electron microscope; NaOCl: Sodium hypochlorite; *M. oleifera*: *Moringa oleifera*; CHX: Chlorhexidine; VHN: Vickers hardness number; EDTA: Ethylenediaminetetraacetic acid

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Not applicable in this section.

Authors' contributions

Dr. Nada Omar and Dr. Yousra Aly performed the biomechanical preparation of the collected specimen using the different types of irrigants. Dr. Maram Khallaf and Dr. Engy Kataia analyzed the data regarding the hardness test, scanning electron microscope, and stereomicroscope. Dr. Yousra Aly and Dr. M. Abdullah Mohamed were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

National Research Centre project; Ethics Committee approval number 16/344

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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