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BIOLOGICAL EFFECT OF A NOVEL ALCOHOLIC AND AQUEOUS PLANT EXTRACTS OF MORINGA AS A BIOACTIVE IRRIGANT ON RADICULAR DENTIN ULTRA-STRUCTURAL SURFACE MORPHOLOGY

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ABSTRACT

Background/purpose: Commonly used irrigants for root canal treatment are bactericidal and important for pulp remnants removal but these irrigants may adversely affect the ultrastructural morphology and structural arrangement of the radicular dentin. These changes might dramatically affect the sealing of the root canal system. The aim of this study was to assess the biological effect of the alcoholic and aqueous extracts of moringa on the ultra-structural surface morphology of the radicular dentin.

Materials and methods: A total number of 32 extracted maxillary anterior teeth were decoronated, longitudinally sectioned, and polished. Root sections were randomly and equally divided into 4 groups: group 1 immersed in an alcoholic extract of moringa, group 2 in an aqueous extract of moringa, group 3 in sodium hypochlorite (NaOCl), and group 4 in distilled water (control). All groups were immersed for 10 minutes followed by examination of the dentinal surface using scanning electron microscope. Calculation of percentage of dentinal tubules and intertubular dentin was performed. Statistical analysis was performed, and significance was set at 5%.

Results: Both alcoholic and aqueous extracts of moringa widened the dentinal tubule orifices (more patent) and increased the percentage of the intertubular dentin compared to sodium hypochlorite with a statistically significant difference.

Conclusion: Both alcoholic and aqueous extracts of moringa could be used as a better bioactive irrigant in root canal treatment to enhance bonding to radicular dentin by widening (increasing patency) of the dentinal tubules and decreasing the intertubular dentin surface area compared to sodium hypochlorite.

KEYWORDS: Bonding, dentinal tubules, intertubular dentin, irrigation, moringa, sodium hypochlorite

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INTRODUCTION

Dentin is formed from inorganic hydroxyapatite crystals constituting approximately 70% by volume, while collagen fibrils and organic matter and water about 30% by volume. The structural arrangement of the dentin shows dentinal tubules surrounded by highly mineralized peritubular dentin and intertubular dentin matrix[1].

Dentinal tubules' arrangement follows a uniform pattern throughout all teeth. The dentinal tubules' number increases from near the dentino-enamel junction to near the pulp. The dentinal tubules' diameter increases from approximately 0.9μm at the enamel interface to about 2.5 μm at the pulp. The percentage of the dentin structure occupied by dentinal tubules increases as well from less than 1% near the DEJ to more than 22% near the pulp[2].

Proper chemo-mechanical preparation of the root canal system is deemed mandatory for the long-term success of root canal treatment[3]. Different root canal irrigants were used to effectively remove pulpal tissue remnants and kill intracanal microorganisms. Unfortunately, these chemicals adversely affect the morphology and structural arrangement of the underlying dentinal surface[4]. These changes might dramatically affect the sealing of the root canal system. Sodium hypochlorite (NaOCl) is the most common irrigant used in root canal treatment due to its unique tissue-dissolving activity[5].

Moringa extract has been advocated to be used as a root canal irrigant owing to its good antibacterial properties. Moringa oleifera originally grows in Southwest Asia, southwest Africa, northeast Africa, and Madagascar. Due to its innumerable benefits, Moringa is considered a medically supreme plant. The leaves of this plant have shown antioxidant, antitumor, anti-inflammatory, and antimicrobial properties. The leaves of Moringa trees contain flavonoids such as myricetin, quercetin, kaempferol, isorhamnetin, and rutin, as well as phenolic acids. In addition, fresh leaves are a good source of carotenoids such as lutein, β-carotene, and zeaxanthin[4,6–12].

The aim of this study is to evaluate the biological effect of alcoholic and aqueous moringa extract on radicular dentin ultra-structural morphology in comparison to sodium hypochlorite. The null hypothesis tested was that there is no difference in the effect on radicular dentin morphology between the tested root canal irrigants.

MATERIALS AND METHODS

Study design

The study protocol was approved by the ethical committee at the Faculty of Dentistry, Assiut University, Egypt. Ethical approval number 04/2023/300067 was obtained prior to starting the study.

The study design was experimental laboratory study including four groups.

Sample size calculation

Based on the previous study of Elgendy and Amen[13], Sample size was calculated with probability (power) 0.9,power analysis yielded a sample size of 32 samples (i.e. 8 samples per group). The Type I error probability associated with this test of this null hypothesis is 0.5.

Sample selection and preparation

A total of 32 extracted permanent human maxillary anterior teeth were obtained from uncontrolled diabetic patients or periodontal affection with severe bone loss beyond treatment visiting the clinic of oral surgery department according to ethical approval and infection control protocols. The patients' age was ranging between 28 and 40 years old. After thorough teeth examination, teeth with a fully formed apex were selected whereas roots with resorption defects, fractures, or open apices were excluded. Teeth were thoroughly washed, scrubbed,

and scaled to remove blood, mucous, shreds of periodontal ligament, and calculus. The teeth were examined using magnification lens of ×7 to exclude any tooth with cracks or structural defects. Teeth were decoronated (crowns and roots are separated at cervical line) using diamond discs followed by longitudinal sectioning in a buccolingual direction into two equal halves. Dentin surface was then polished using 400, 600, 800, 1200, and 1500-grit polishing papers under distilled water. Further polishing was then performed using diamond dust. Finally, all root sections were ultra-sonicated in purified distilled water for 20 minutes.

Samples classification (grouping)

Random.org was used to divide all roots into four equal groups according to the irrigant tested. All roots were immersed in the tested irrigant for 10 minutes. Group 1, alcoholic extract of moringa, Group 2, aqueous extract of moringa, Group 3, sodium hypochlorite 5.25%, Group 4, distilled water as a control group.

Moringa extract preparation

Ten grams of moringa leaves' powder were soaked in 100 ml of 99% ethanol (analytical grade) for 48 hours with shaking several times. Then the alcoholic extract was collected and filtered.

Ten grams of moringa leaves' powder were soaked in 100 ml of water for 48 hours with shaking several times. Then the aqueous extract was collected and filtered.

Scanning electron microscope and morphometric analysis

Instantly after rinsing the specimens, they were dehydrated in water/ethanol mixture with increasing content of ethanol (70%, 80%,96 % and 100% for 24 hours each), then dried for 24 hours in a desiccator under vacuum with glass filter pump. The specimens were mounted on a single stub, and sputter coated with gold. The third and fourth authors examined the dentin surface morphology and ultrastructure at magnification 1,000X, 2,500X,5,000X and 10000X. Using image analysis software, Image J, the images were further analyzed. The brightness and contrast of the images were automatically corrected, then converted into 8-bit grayscale type. A red color code was then automatically assigned to the area of dentinal tubules or intertubular dentin in gray scale images. The area fraction, representing the percentage of the surface area of dentinal tubules or the intertubular dentin to the total area of the image, was then automatically calculated. The mean values were statistically analyzed from the tabulated data.

Statistical analysis

Mean and standard deviation values of percentage of dentinal tubules and intertubular dentin were calculated for each group. Data were parametric and showed variance homogeneity using Shapiro-Wilk's and Levene's tests. Therefore, data were analyzed using one-way ANOVA test followed by Tukey's post hoc test. The significance level was set at $p<0.05$.

RESULTS

Mean ± standard deviations of the percentage of dentinal tubule and intertubular dentin for all groups are shown in table 1. Treatment using alcoholic and aqueous extracts of moringa showed significant increase in the mean area percentage of dentinal tubules compared to the control group. In NaOCl group, there issignificant decreased mean area percent of dentinal tubules compared to the control group. The mean area percent of the intertubular dentin significantly decreased in groups 1 and 2 while significantly increased in group 3 when compared to the control group.

TABLE (1) Means ± standard deviations of the area percent of dentinal tubules and intertubular dentin for the four groups.

	Dentinal tubule area %	Intertubular dentin area %
Group 1 (Alcoholic Ext)	7.41±1.44[a]	91.63±1.81[a]
Group 2 (Aqueous Ext)	6.41±1.10[a,b]	92.49±3.86[b]
Group 3 (NaOCl)	3.38±0.83[b]	95.32±1.02[b]
Group 4 (Distilled water)	5.65±0.86[b]	93.12±2.77[b]

Different letters in the same column indicates a significant difference.

Significance was set at p<0.05

Electron microscope micrographs show the different groups with different histologic pictures (Figures 1 and 2). The first group treated with alcoholic extract of Moringa showed patent dentinal tubules, clear peritubular dentin rims surrounding the tubules and uniform relatively thin intertubular dentin which is on one continuous level.

The aqueous extract of Moringa showed patent dentinal tubules as well but to a lesser degree than the alcoholic extract. A clear peritubular dentin rim surrounds most of the dentinal tubules. The intertubular dentin was seen at different levels in contrast to alcoholic extract group, smooth intertubular dentin.

The sodium hypochlorite group showed narrowed dentinal tubules openings with no evident peritubular dentin rim and irregular intertubular dentin. In addition to well-presented surface morphology alterations and surface roughness.

The control group presented regular appearance of dentin with patent dentinal tubule openings surrounded with peritubular dentin rim and separated with uniform intertubular dentin.

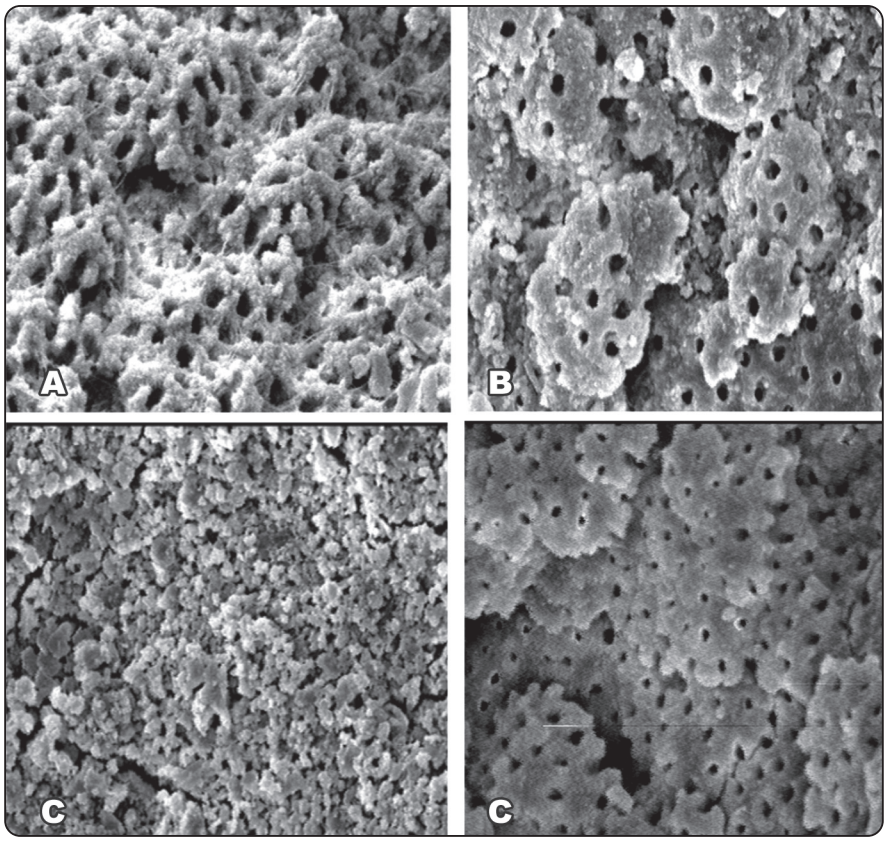

Fig. (1) Scanning electron microscope micrographs with magnification X2000, representing the dentin surface: A (alcoholic extract of Moringa), B (aqeous extract of Moringa), C (Sodium Hypochlorite solution) Moring. D: (distilled water as control)

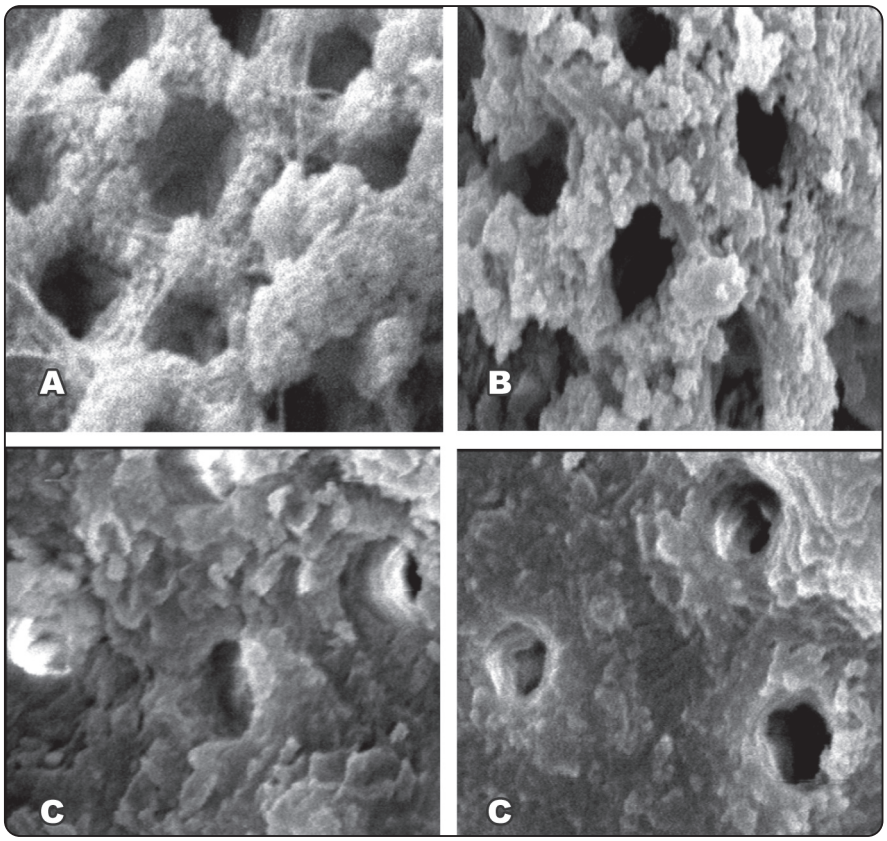

Fig. (2) Scanning electron microscope micrographs with magnification X 10,000, representing the dentin surface: A (alcoholic extract of Moring), B (aqeous extract of Moringa), C (Sodium Hypochlorite solution), D (distilled water as control)

DISCUSSION

Dentin tissue forming the main bulk of a tooth is really challenging and interesting tissue. It has specific molecular and histological characteristics. At the microstructural and molecular level, this heterogeneous mineralized tissue including hydroxyapatite in addition to some salts as amorphous calcium phosphates and carbonates is abundant with organic substances and extracellular matrix where the domination of Type I collagen fibers that is responsible of the stress response and distribution along the tooth. The meticulous arrangement of this heterogeneous tissue in the form of dentinal tubules filled with viscous fluid and containing the cytoplasmic process that is sensitive to biological irritation upon ingression of microorganism to 300 to 500 μm. The organic collagen fibers are subject to tissue-derived and microbial-derived enzymatic activities. Various root canal irrigants used during chemo mechanical preparation of the root canal system may affect the dentin structure and properties[14]. Maintenance of wide and patent dentinal tubules following root canal preparation dramatically affects bonding of different obturating materials to the radicular dentin[15].Thus, an important characteristic of the irrigants used is their diffusivity and their antimicrobial ability to this depth. Furthermore, the use of non-specific proteolytic chemicals such as sodium hypochlorite (NaOCl) to disinfect the infected dentin may elicit damaging effect on collagen.

Assessment of the ultra morphological changes of the dentin as a biological tissue is very crucial step. Scanning electron microscopy has been used to evaluate the ability of the various irrigants to remove the smear layer. Ultrastructural morphology of the radicular dentin differs from the coronal one third compared to the apical one third. Apically, the radicular dentin shows less number of dentinal tubules[16]. Therefore, root sections were selected

from the middle third in the current study to standardize the specimens.

As this preclinical study is very essential to accurately determine and properly interpret the mechanism of action of this novel strategy in endodontic irrigation before clinical evaluation, this study was designed so that all groups were immersed in an alcoholic extract of moringa, an aqueous extract of moringa, a sodium hypochlorite (NaOCl) solution, and the distilled water for 10 minutes, followed by examination of the dentinal surface using scanning electron microscope. Actually, this methodology doesn't mimic the clinical situation. However, the authors main concerns was to assess the effect of the used irrigants per se in dissolving debris and smear layers without the scraping action of endodontic files as well as their biological effect on the heterogenous nature of dentin tissue. Moreover, the immersion time selected in this study (10 minutes) aimed to reproduce the cumulative time of irrigation process through prolonged endodontic procedures clinical sessions.

Moringa extract is well known by its antimicrobial activity and good potential to be used as a root canal irrigant[8]. Khalaf et al's[10]study concluded that moringa increases the dentinal microhardness. The results of the current study presented biologically favorable results for moringa extract groups on the radicular dentin ultra-structural morphology compared to the sodium hypochlorite group that presented severe surface alterations, eventually, this may favor moringa extract uses as a bioactive irrigant solution as a replacement for sodium hypochlorite solution in endodontics to ensure a better sealing of the root canal system by obturating materials and decrease the risk of sodium hypochlorite accidents. The preservation and widening of the dentinal tubules in addition to the increase in the intertubular dentin could be attributed to the phenolic acid derivatives in the moringa extract. Rani et al[8]have shown the moringa leaves extract is rich in gallic acid as a major phenolic acid. These phenolic acids might lead to chelation of the inorganic components of the dentin structure and lead to opening of the dentinal tubules in the same mechanism of ethylenediaminetetraacetic acid 17% and citric acid.

Results of the current study could not be directly compared to previous studies as none could be found in the literature till now.

The decrease in the percentage of the dentinal tubules and peritubular dentin by NaOCl in the current study is a logic finding and comes in full agreement with the previous studies[13,17–19]. This could be simply explained by proteolytic nature of such an irrigant. Sodium hypochlorite is considered as a strong base that is capable of dissolving proteins. NaOCl, being a proteolytic agent, dissolves the organic ground substance, thus increasing the hydroxyapatite proportions on the treated dentin surface. This protein and tissue dissolving activity occurs through the reaction with the amino acids in a neutralization and chloramination reaction[5]. This will ultimately result in degradation of the collagen fibers which will lead to structural disorganization of the dentin structure. Evidence shows that these irrigants, adversely affect the physical and mechanical properties of the dentin with disproportionation of the inorganic and organic components.[17-19]

This change in the dentinal morphology by the sodium hypochlorite comes in full agreement with Inaba et al 1995 who demonstrated occlusion of the dentinal tubules following treatment with sodium hypochlorite. Oyarzunet al[17] and Hu et al[19] also showed disorganization of the dentinal collagen and increased surface roughness following the treatment with sodium hypochlorite.

Treatment of dentin samples using alcoholic and aqueous extracts of moringa showed less alterations of surface morphology compared to sodium hypochlorite group in addition to widened dentinal

tubules orifices and significantly increased their area percent. Collagen fibers were clearly demonstrated and exposed by 10000X examinations.

Therefore, within the limitation of this study, it could be concluded that both alcoholic and aqueous extracts of moringa could be used as a better bioactive irrigant in root canal treatment to enhance bonding to radicular dentin by widening (increasing patency) of the dentinal tubules and decreasing the intertubular dentin surface area compared to sodium hypochlorite.

LIMITATION OF THE STUDY

As any laboratory study there are several inevitable limitations that might affect the clinical consideration as the humidity, temperature, acidity, and the harbor of different microbial species. However, this preclinical study is very essential to accurately determine and properly interpret the mechanism of action of this novel strategy in endodontic irrigation before clinical evaluation.

CONCLUSION

Both alcoholic and aqueous extracts of moringa could be used as a better bioactive irrigant in root canal treatment to enhance bonding to radicular dentin by widening (increasing patency) of the dentinal tubules and decreasing the intertubular dentin surface area compared to sodium hypochlorite.

CLINICAL RELEVANCE

The present finding proved the efficiency of experimental naturally prepared Moringa alcoholic and aqueous extract as irrigant solution for root dentin while preserving the biological tissues. However, the findings of this study cannot be directly correlated with those of other materials because of the structural differences existing amongst the diverse types of irrigants.

RECOMMENDATION

Further studies are required to assess the effect of the natural Moringa extract as irrigation solution on sealer penetration and adaptation after longer periods of storage and under different experimental conditions.

Conflict of interest:

The authors have no conflicts of interest relevant to this article.

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