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ORIGINAL ARTICLE

Microhardness of the Radicular Dentine After Root Canal Irrigation With Salvadora Persica Extract: A Laboratory Study

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Abstract

Objectives: **To evaluate the effect of the conventionally used irrigant sodium hypochlorite (NaOCl) compared with prepared Salvadora persica (Miswak) root canal irrigant regarding the microhardness of root dentin.**

Materials and methods: **Forty-five extracted single-rooted mandibular premolars human teeth with closed apex were included in this study. Initially, the teeth were cleaned, prepared, and then disinfected, which were divided randomly into five equal groups according to the type of irrigant used [group 1: saline (negative control), group 2: 5.25 % NaOCl (positive control), group 3: 10 % Miswak, group 4: 15 % Miswak, and group 5: 20 % Miswak]. The specimens were embedded in acrylic resin, for examination, and positioned in a Vickers microhardness tester (Future-Tech Corp FM-700) to detect root canal dentin microhardness. The collected data were then statistically compared to detect differences between the tested root canal irrigants.**

Results: **Mean ± SD of dentin microhardness before irrigation showed an insignificant difference ($P = 0.942$), while the recorded Vickers hardness number values after irrigation showed a significant difference ($P = 0.046$) between the tested group. Only the NaOCl group showed a significant decrease in root canal dentin microhardness after the treatment and is preferred over Miswak irrigant.**

Conclusion: **Within the limitation of the present study, 20 % Miswak is comparable to 5.25 % NaOCl. Regarding its use as a root canal irrigating solution, 20 % Miswak does not affect the dentin microhardness negatively.**

Keywords: Microhardness, Sodium hypochlorite, Salvadora persica

Introduction

The occurrence of periapical and pulp pathoses is mostly influenced by a vast variety of bacterial species.[1] The root canal system's preferential anatomic localization aids bacterial fortification, and these bacteria are immune to the host defense system.[2] *Enterococcus faecalis* is the predominant bacterium linked to endodontic secondary infection, with prevalence in persistent endodontic infections ranging from 24 to 77 %.[3–7]

The primary microbiological objectives of the chemomechanical root canal preparation are to totally eradicate or significantly diminish the intracanal bacterial populations to promote periradicular tissue repair.[2] Therefore, to further diminish the microorganisms; it is essential to irrigate surfaces with solutions that have potent antibacterial properties. Sodium hypochlorite (NaOCl), which has great antibacterial properties against planktonic bacteria and biofilm formations, is the perfect root canal irrigant.[8–10] However, it has several drawbacks, including the possibility of allergies, tissue toxicity, and unpleasant taste and odor.[9] In endodontic dentistry, antimicrobial irrigants with minimal toxicity are essential.

In restorative dentistry, the microstructure and characteristics of the dentin are important. Four

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components make up the hydrated complex that is dentin: oriented tubules, a highly mineralized peritubular zone, and dentinal fluid surrounding the tubules. Type I collagen and apatite crystals make up the intertubular matrix. Understanding the mechanical characteristics of human root canal dentin will aid restorative procedures. Microhardness is measured based on the induced permanent surface deformation that persists after the load has been removed, and it is defined as the resistance to local deformation. Measurements of various mechanical characteristics, such as fracture resistance, elastic modulus, and yield strength, can be connected with measurements of hardness.[11]

The pharmacological effects of Salvadora persica (Miswak) are said to include anti-inflammatory, antibacterial, antifungal, antioxidant, chemosensitizing, and wound-healing abilities.[12] Both gram-negative and gram-positive bacteria were susceptible to the effects of *S. persica* aqueous formulations.[13] After chemomechanical preparation, it might be challenging to completely remove all of the *E. faecalis* that remain in the root canal system. To address this issue, numerous irrigation solutions and irrigation activation procedures have been developed. These approaches were utilized as an additional step in debriding resistant bacteria.[14] Microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues as it is sensitive to composition and surface changes of the tooth structure.

Therefore, the objective of this study was to evaluate the effect of the interaction of different irrigant solutions on the microhardness of root canal dentin.

2. Materials and methods

2.1. Materials

Materials and devices used in this study are expressed in Table 1.

Table 1. Materials, instruments, and devices used.

Materials, instruments, and devices	Manufacturer
Normal saline	Ghoneim Pharmaceutical Factory, Down Town, New Cairo, Egypt
NaOCl solution 5.25 %	Egyptian Household Cleaning Company, 10th of Ramadan, Egypt
Salvadora persica (Miswak)	Miswak sticks, Macca, KSA
Rotary evaporator machine	HAHNVAPOR, HS-2005S, HAHNSHIN S&T, Korea
Ultrasonic scaler	Guilin Woodpecker Medical Instrument Co. Ltd, Guangxi, China
Double-sided diamond disc	Kavo Kerr, Stockley Park, UK
Degotzen radiograph machine	Degotzen, Via Roma, 45–21057 Olgiate Olona, Italy
K-type file #15	Mani, Kiyohara Industrial Park, Utsunomiya, Japan
Race rotary nickel-titanium files	FKG Dentaire, SA, Switzerland
Electric endodontic motor (Denjoy)	Denjoy, Changsha City, Hunan Province, China
Side vented endodontic irrigation needle	Shanghai Mekon Medical Devices Co., Ltd, Shanghai, China
EDTA solution 17 %	I-dental Innovative Dental Products, Siauliai, Lithuania
Buehler (silicon carbide papers)	41 Waukegan Road, Lake Bluff, Illinois
Vicker microhardness tester	Future-Tech Corp FM-700, Tokyo, Japan
Phosphate buffered saline	PBS, Sigma–Aldrich, St Louis, USA
Hedstrom instrument #40	Mani, Kiyohara Industrial Park, Utsunomiya, Japan
Eppendorf microcentrifuge tube	Via Zante, 14, 20138 Milano MI, Italy
Polyethylene Glycol (PEG)	Oxford Lab Chem., Mumbai, India

NaOCl, sodium hypochlorite.

2.2. Methods

2.2.1. Miswak extract preparation with different concentrations

Miswak chewing sticks were purchased from Macca, KSA. Miswak sticks weighing 800 g were cut. The resulting Miswak pieces were processed via a food processor to a fine powder. One hundred milliliters of 70 % ethanol was added to 100 g of powder in a sterile flask with a tight-fitting cover.

After 3 days at room temperature, the mixture was allowed to sit before being filtered through number 1 filter paper. The extract was then heated to 40 °C in a rotary evaporator (HAHNVAPOR). The herbal irrigant material was formed with full concentration (100 %) (Fig. 1) and then the three different concentrations were made as follows: 10 % Miswak extract concentration was made by adding 10 mg of Miswak irrigant to 0.5 ml methanol and 0.5 ml saline, 15 % Miswak extract concentration was made by adding 15 mg of Miswak irrigant extract to 0.5 ml methanol and 0.5 ml saline, and 20 % Miswak extract concentration was made by adding 20 mg of Miswak irrigant extract to 0.5 ml methanol and 0.5 ml saline.

Sample size calculation was based on mean hardness between different novel plant extract irrigants retrieved from previous research.[15] Using G power program, version 3.1.9.4 to calculate sample size based on the effect size of 0.65, using the two-

Fig. 1. Miswak extract concentrations (10, 15, and 20 %).

tailed test, α error = 0.05, and power = 90.0 %, the number of studied groups are 5, the total calculated sample size will be 45 total.

2.2.2. Specimen collection and selection

Based on a study protocol that was approved by the Ethics Committee of the Faculty of Dentistry, Mansoura University (A 15020822), 45 single-rooted human teeth, closed-apex mandibular premolars that were extracted for orthodontic or periodontal reasons were included in the current study. Using an ultrasonic scaler (Guilin Woodpecker Medical Instrument), hard deposits and calculus were removed. The teeth were then washed, disinfected, and preserved in distilled water to keep them hydrated until usage.[16]

The present study included only teeth with developed apices, Vertucci type I root canal configuration, and visible canals in radiography.

The study did not include teeth with any of the following conditions: multiple-canalized roots, teeth with decay at the root, internally severely calcified root canals, internal/external root resorption is present, or roots with fractures or radicular cracks.

2.2.3. Specimens grouping and irrigations

After root canal preparation, and collection of baseline bacterial samples by determining the complete working length of the Hedstrom instrument #40 (Mani) was inserted into the root canal, and 10 filling strokes were made against the dentinal walls of the canal.[17,18] Then the specimens were randomly divided into five equal groups (n = 9); group 1: sterile saline solution was used to irrigate the root canals (negative control), group 2: 5.25 % NaOCl was used to irrigate root canals (positive control), group 3: 10 % Miswak was used to irrigate the root canals, group 4: 15 % Miswak was used to irrigate the root canals, and group 5: 20 % Miswak was used to irrigate the root canals. Each specimen's apical foramen was sealed with sterile sticky wax to prevent the leakage of the irrigant solutions (Fig. 2). The irrigant solution was allowed to soak in the canal for 4 min.

2.2.4. Specimens preparation for microhardness

The crowns of a 45 teeth were selected, and their lengths were decoronated to a standard (15 mm; Fig. 3) using a double-sided diamond disc (Kavo Kerr). With a conventional radiograph machine (Degotzen Via Roma), the radiographic periapical film was taken, and the K-type file #15 (Mani) was positioned 1 mm away from the radiographic apex to ensure the correct working length and determine samples sizes.

An electric endodontic motor (Denjoy) was used to prepare all canals using rotary Race nickel-titanium instruments in the following order: 15/0.06 file up to one-third of the working length, followed by (20/0.02), (25/0.02), (30/0.02), (35/0.02), and finally (40/0.02) file up to the working length. Between each file, 5 ml of 5.25 % NaOCl, was irrigated using a 30-G side-vented needle (Shanghai Mekon Medical Devices Co. Ltd).

All specimens were collected and put in an ultrasonic bath containing 5.25 % NaOCl and 17 % EDTA for 4 min to remove the smear layer after the root canal mechanical preparation. The specimens were then autoclaved for 20 min at 121 °C after being washed in sterile water for 1 min.[19–21]

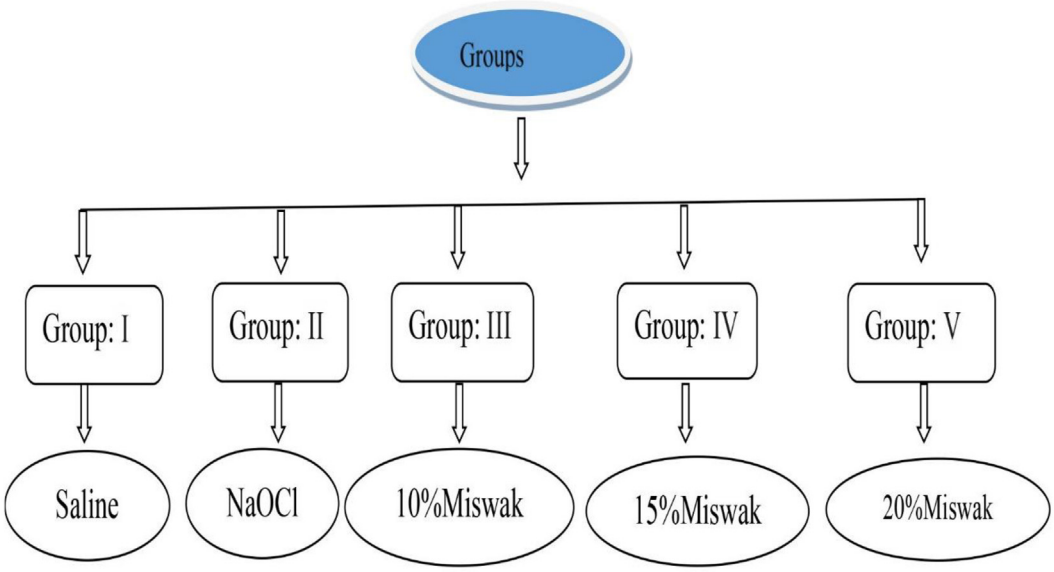

Fig. 2. The used irrigant solution.

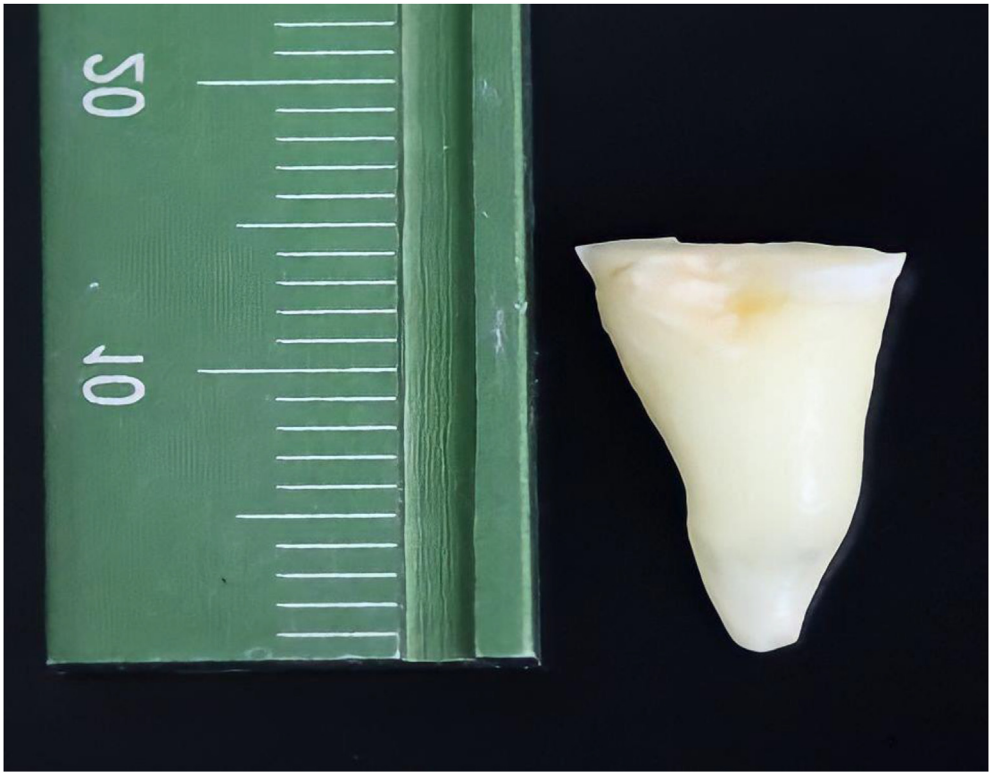

Fig. 3. Standardization of the working length.

2.2.5. Vickers microhardness measurement

With the exception of the first 1 mm of the cervical third, which was left extruded, the specimens were dried and embedded in acrylic resin. Before being polished with 400, 600, and 1200 grit silicon carbide papers (Buehler) to create a smooth surface free of gradients and being cleaned under running water.

Vickers hardness numbers (VHN) were measured using the prepared specimens in the Vickers microhardness tester (Future-Tech Corp) (Faculty of Dentistry, Mansoura University). The indenter was

pushed into the test specimen by a 50 g applied load that had no impact force. The dwell time of the indenter was 10 s.

A filar micrometer was used to read and concentrate the indentation after the load had been removed. And the two imprint diagonals (d1 and d2) were averaged (d) after being regularly measured to the nearest 0.1 mm.

The VHN was calculated using the following equation:

$$HV = 1854.4L/d^2$$

Where the load (L) was in gram force (gf) and the average diagonal (d) was in μm.

For each specimen, VHN was measured at three distances: the first indentation was produced about 0.5 mm from the outside surface of the root (distance 1), while the second indentation was carried out at ~0.5 mm from the root canal lumen (distance 3), and the third indentation was made at the middle of the distance between the previous indentations (distance 2).[19] All indentations were made on the buccal side.

2.2.6. Dentin microhardness measurement after irrigation

Each specimen's cervical 1 mm was cut into sections perpendicular to its long axis, polished once again with 400, 600, and 1200-grit silicon carbide sheets to provide a smooth surface free of gradients, and then rinsed under running water. The same procedure, as previously described, was used to measure the dentin microhardness following the application of the tested irrigants. As a measure of their microhardness effectiveness, the effects of five irrigation solutions on dentin microhardness were compared.

2.3. Statistical analysis

Data were subjected to statistical analysis by the SPSS for Windows statistical package, version 22.0. (Corp I. IBM SPSS statistics for Windows, version 22.0.; IBM Corp., Armonk, New York, USA) The one-way (analysis of variance) test was used for comparison between groups, followed by Tukey's post-hoc test for pairwise comparison between the differences among five groups, a *P* value less than or equal to 0.05 was considered significant. The difference between the values of dentin microhardness before and after irrigation with the tested solutions were measured. Twenty percent Miswak and NaOCl that's were detected by *t* tests.

3. Results

3.1. Results of microhardness test (Vickers hardness number)

The values of VHN before and after irrigation of the root canal using various irrigation solutions are shown in Table 2. The highest VHN value was recorded for group 1: saline (negative control) (62.364 ± 2.921), while the least value was recorded for group 2: NaOCl (positive control).

Analysis of variance test comparing the tested groups before irrigation, showed an insignificant difference ($P = 0.942$). While the recorded VHN values after irrigation showed a significant difference ($P = 0.046$) between the tested group. Tukey post-hoc test revealed a significant difference between groups 2 (NaOCl) and all other groups, while an insignificant difference was found between groups 1 (saline), 3 (10 % Miswak), 4 (15 % Miswak), and 5 (20 % Miswak).

4. Discussion

The effect of irrigants on the dentin microhardness was evaluated in the present study, since any reduction in the microhardness of root canal dentin could have a negative impact on the tooth's resistance to fracture and durability.[20] Furthermore, it inhibits the dentin's ability to close off and the adhesion of dental materials like resin cements and root canal sealers. In addition, it can provide a hint about the mineral content of tooth structure.[21,22] Because there are more widely opened dentinal tubules near the pulp, which provides the least resistance to the microhardness testing indenter, the microhardness of dentin decreases from the superficial to deep region.[20] Therefore, in the current study, seeked to make measuring to microhardness

Table 2. Mean ± SD of dentin microhardness before and after irrigation with the evaluated solutions of the tested groups.

Groups	Before (mean ± SD)	After (mean ± SD)	*P* value¥
Saline	62.736 ± 3.312	62.364 ± 2.921$^{(a)}$	0.125
Sodium hypochlorite	62.594 ± 2.953	54.937 ± 3.936$^{(b)}$	0.002*
10 % Miswak	62.016 ± 2.035	60.782 ± 1.943$^{(a)}$	0.327
15 % Miswak	62.082 ± 2.027	61.753 ± 1.946$^{(a)}$	0.427
20 % Miswak	62.304 ± 2.638	62.264 ± 2.737$^{(a)}$	0.570
P value€	0.942	0.046*	

Data with different superscripts at the same column are significantly different at *P* value less than or equal to 0.05 using Tukey post-hoc test.
*: Statistically significant.
E: one-way ANOVA test.
Y: paired t-test.

to the thickness level more than the length level of root dentin.

The Vickers microhardness tester was selected for the current study over the Knoop hardness tester to avoid measuring mistakes.[21] Additionally, it can accurately test small specimens and is less sensitive to surface circumstances. It also can detect surface changes in deep dental hard tissues. Furthermore, the Knoop hardness tester can only measure superficial dentin at 0.1 mm, not deep dentin.[23] Although a decrease in microhardness makes it easier to instrument a root canal, it can also weaken the root structure and cause a fracture of an endodontically treated tooth.[24]

The findings of the current study also demonstrated that 15 and 20 % of Miswak had no negative effects on root canal dentin microhardness while NaOCl dramatically decreased root canal dentin microhardness. The reason for the decrease in microhardness in the current study can be attributed to NaOCl organic dissolving properties on the collagen component of dentin. Another reason might be because NaOCl caused phosphate levels to drop.[25,26] NaOCl lowered the microhardness by breaking down lengthy peptide chains and chlorinating the protein terminal groups. The drop in microhardness seen in this investigation is consistent with the findings of Oliveira and colleagues.[27–29]

Ari et al.[30] agreed with the current study regarding microhardness test examined the impact of frequently employed endodontic irrigating solutions on root canal dentin microhardness. As irrigations, used 3 % H_2O_2, 5 % and 2.5 % NaOCl solutions, 17 % EDTA solution, and 0.2 % chlorhexidine gluconate. According to the results, all irrigants reduced the microhardness of root dentin, with the exception of 0.2 % chlorhexidine gluconate. Also, in agreement with the current study, Almas[31] examined the impact of intracanal irrigation on the effects of calcium hydroxide paste, 2 % chlorhexidine gel, and Miswak, found that Miswak extract had no negative effects on root canal dentine microhardness. The results of previous research that concluded that herbal irrigants had no negative effect on dentin microhardness were supported by the use of 15 and 20 % Miswak as irrigating solutions of herbal sources, which revealed no influence on dentin microhardness.[12,32,33]

In contrast to the present study, Saha et al.[34] reported that Miswak extract and 3 % NaOCl irrigation solution had no negative effects on dentin microhardness. These disagreements may be attributed to difference NaOCl concentration. This result did not agree with other research, revealing that the Miswak did not have an effect on the microhardness of root dentin. Nibal et al.[33] revealed that Miswak extract was discovered to raise the microhardness values of the surface of demineralized enamel. The researcher attributed their results to the reaction of fluoride ions with dentin surface that results in the formation of fluoride-containing compounds mainly calcium fluoride, thus making the root surface harder.

4.1. Conclusions

Within the parameters and conditions of this study, the following conclusions could be drawn: Miswak (10, 15, and 20 %) had no adverse effects on dentin microhardness, while the microhardness of root dentin is significantly reduced when using 5.25 % NaOCl as a root canal irrigation.

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Data availability

The collected and analyzed datasets are available from the corresponding authors upon reasonable request.

Conflicts of interest

There are no conflicts of interest.

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