ORIGINAL ARTICLE

Comparative Evaluation of Microhardness of Radicular Dentin by Using Different Herbal Extracts (*Azadirachta indica, Morinda citrifolia, Green Tea*) as Root Canal Irrigant: An *In Vitro* Study

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Abstract

Aim and objective: To evaluate the effect of different herbal extracts such as *Azadirachta indica*, *Morinda citrifolia*, and green tea on the microhardness of radicular dentin and compare the effects of these herbal extracts with 2.5% sodium hypochlorite and saline.

Materials and methods: In all selected 60 single-rooted, human permanent teeth, standardized access opening and working length determination were done. Later, instrumented to the apical size of 30 (F3) with ProTaper universal using saline. Each tooth was sectioned longitudinally using a low-speed diamond disc into two halves. Then, one-half without any defects was selected and embedded horizontally in auto-polymerizing acrylic resin, by exposing their dentin. The dentine surfaces on either side of the root canal lumen of these mounted specimens were flattened and smoothened with series of ascending grades of silicon carbide abrasive paper and finally polished with a composite polishing kit and 0.1-mm alumina suspension on a rotary felt disk. Then, all teeth were divided into five groups ($n = 12$) group I: saline, group II: 2.5% sodium hypochlorite, group III: *A. indica*, group IV: *M. citrifolia*, and group V: green tea, based on final irrigant. Initial and posttreatment microhardness was recorded using Vickers microhardness tester at coronal, middle, and apical third.

Results: Group II and group IV showed statistical significance ($p < 0.05$) between initial and posttreatment values, while group I, group III, and group V, had no significance ($p > 0.05$). In comparison between group II and group IV, no significant difference was seen.

Conclusion: Among herbal extracts, *M. citrifolia* leads to structural changes in radicular dentin due to demineralizing effect on root canal dentin.

Keywords: Herbal extracts, Microhardness of radicular dentin, *Azadirachta indica*, *Morinda citrifolia*, *Green tea*, Root canal irrigant.

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Introduction

Irrigation becomes a foremost factor along with root canal instrumentation to sterilize the infected root canal during cleaning and shaping of complex root canal instrumentation.

A variety of chemical substances such as acids (citric and phosphoric), chelating agent (ethylenediaminetetraacetic acid, EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea, and potassium hydroxide), oxidative agents (hydrogen peroxide and Gly-Oxide), local anesthetic solutions, and normal saline have been proposed for irrigation due to their desirable properties. Among them, the most widely used irrigants are sodium hypochlorite, EDTA, chlorhexidine, and normal saline. But, they have their own limitations. Hence, the search for an ideal root canal irrigant still continues with the development of newer materials and methods.[1]

Nowadays, the "Herbal Renaissance" is happening all over the globe in all fields due to potential side effects of conventional allopathic formulations and advantages of natural products such as high antimicrobial activity, biocompatibility, anti-inflammatory, and anti-oxidant properties. In endodontics also, researchers focused on these products and conducted several studies on the feasibility to be an alternative to the conventional root canal irrigants and medicaments.[2–4]

Previous studies conducted on herbal extracts such as *Morinda citrifolia*, *Azadirachta indica*, and green tea proved that they have the potential to be a root canal irrigant by having good antimicrobial property, biocompatibility, and effectiveness in smear layer removal.[2–4] Along with these desirable properties, ideally an irrigant should not alter the mechanical, chemical, and physical properties of dentine when irrigant exposed to radicular and coronal dentin during irrigation.[1,5]

Unfortunately, some conventional root canal irrigants are capable of altering the chemical composition of human dentin which in turn affects the calcium/phosphorus (Ca/P) ratio of the dentin surface. The alterations in the Ca/P ratio may change the permeability and solubility characteristics of dentin.[6] These changes

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in dentin affect its interaction with materials used for obturation and coronal restoration, permitting coronal leakage leads to bacterial ingress.[7] Microhardness determination can provide indirect evidence for losing or gaining any mineral substance in the dental hard tissues.[5]

Previous studies conducted by several researchers proved that irrigating solutions such as sodium hypochlorite, EDTA, and hydrogen peroxide can reduce the microhardness of radicular dentin significantly.[8–12]

However, to the best of our knowledge, there have been no studies reported on how herbal extracts such as *M. citrifolia, Green tea*, and *A. indica* affects the microhardness of radicular dentin. Therefore, the present study is designed to evaluate the effect of different herbal extracts such as *A. indica* (Neem), *M. citrifolia,* and green tea on the microhardness of radicular dentin and compare the effects of these herbal extracts with sodium hypochlorite as a positive control group and saline as a negative control group.[9]

Materials and Methods

Preparation of the Test Solutions

Herbal solutions were prepared by following the method of preparation designed by Rosaline et al.[13] Fresh plants of *M. citrifolia, A. indica,* and green tea (Yucca Enterprise, Mumbai) were powdered and air-dried. Then weighed quantity of 500 g of each of these herbal extracts was macerated with 500 mL of 99% ethanol (BRG Biomedicals, Panipat, Haryana) and filtered using a double filter paper. Then, centrifuged at 10,000 rpm for 20 minutes and stored at 4°C until required.

Sample Preparation

Sixty ($n = 60$) intact single-rooted, human permanent teeth, having a single canal with fully developed apices, extracted recently for the orthodontic and periodontic purpose were collected and cleaned of any debris, soft tissues, calculus, and stored in saline solution. In all teeth, standardized access cavity preparation and working length determination were done. All root canals were prepared to an apical size of 30 (F3) with ProTaper universal rotary instruments (Denstply Maillefer, Ballaigues, Switzerland) following the manufacturer's instructions. During instrumentation, irrigation was performed using saline solution. Then, each tooth was sectioned longitudinally with a low-speed diamond disc (Horico, Berlin, Germany) underwater cooling into two halves. Among these two halves, one-half without any defects was selected. Later, the selected root segments were horizontally embedded in auto-polymerizing acrylic resin, leaving their dentine exposed to facilitate manipulation. The dentine surfaces on either side of the root canal lumen of these mounted specimens were flattened and smoothened with a series of ascending grades of silicon carbide abrasive papers (3M, India) (500, 800, 1,000, and 1,200 grit) under distilled water to remove any surface scratches and finally polished with a composite polishing kit (Microdont, Brazil) and 0.1-mm alumina suspension (Ultra-Sol R; Eminess Tec Inc., Monroe, NC, USA) on a rotary felt disk.

Microhardness Testing Procedure

Vickers microhardness test was preferred in the present study because of its suitability and practicality to evaluate significant surface changes of dental hard tissues after the application of irrigating solutions.[7,11,12]

Initial Microhardness Test

Each specimen was numbered. The initial microhardness values were measured to establish an initial value for comparison of the effect of final irrigant solutions on the dentin surface using a Vickers microhardness tester (Matsuzawa MMT7, Matsuzawa SEIKI Co., Ltd., Tokyo, Japan). In each sample, three separate indentations were made with a Vickers diamond indenter at 40× magnification at cervical (at cemento-enamel junction level), midroot (exactly halfway horizontally between the central lumen and root cementum), and apical region on one side at 0.5 mm level to the root canal wall using 50-g indentation load for 10 seconds at a depth of 100 µm from the pulp–dentin interface because some root canal irrigants penetrate up to 130 µm from this interface (Berutti et al.). Then, the obtained diamond-shaped indentations were carefully observed in an optical microscope with a digital camera and image analysis software, allowing the accurate digital measurement of their diagonals. The Vickers hardness number was calculated by using a formula-VHN = 1.854 (F/D^2) Where: F= Load in kgf, D = Arithmetic mean of the two diagonals, d1 and d2 in mm.

The initial microhardness value of each specimen on one side of the canal lumen was obtained as the average of three indentations results.

Final Irrigation Treatment Procedure

After measuring initial microhardness values, all specimens were randomly divided into five groups (n = 12), based on the final irrigant used,

Group I (n =12): Normal saline (negative control group) (Otuska pharmaceuticals, India).

Group II (n = 12): 2.5% Sodium hypochlorite (positive control group) (VIP, Vensons, India).

Group III (n = 12): *A. indica* (AI).

Group IV (n = 12): *M. citrifolia* (MC).

Group V (n = 12): Green tea (GT).

Each specimen of each group was irrigated with 5 mL of irrigating solution using a disposable 2 mL syringe with a 23-gauge, side-vented needle for 5 minutes.

Posttreatment Microhardness Test

After final irrigation treatment, three separate indentations were made on each specimen at depth and area that were at symmetrical points of the initial ones on the other side of canal lumen to measure the microhardness values. Then, the Vickers hardness number was calculated the same as the initial values.

The posttreatment microhardness value of each specimen on the treatment side of the canal lumen was obtained as the average of three indentations results.

Statistical Analysis

The initial and posttreatment mean values of each specimen obtained by the average of the cervical, middle, and apical indentations among all experimental groups were tabulated. The data were not normally distributed; therefore, a non-parametric test was used for statistical analysis. Data were statistically analyzed by using the Wilcoxon signed-ranks test for comparison of initial and posttreatment microhardness values of all experimental groups and Mann–Whitney test was used for inter-group comparison of posttreatment microhardness of all groups.

Table 1: Comparison of initial and posttreatment (mean ± SD) microhardness values of all groups using Wilcoxon signed-ranks test

Groups	*Initial microhardness (mean ± SD)*	*Posttreatment microhardness (mean ± SD)*	*Change in microhardness values (mean ± SD)*	*p value*
Group I: Saline	59.49 ± 5.12	58.76 ± 4.64	0.73 ± 4.15	0.126**
Group II: 3% sodium hypochlorite	61.61 ± 5.29	56.43 ± 5.52	5.18 ± 5.06	0.008*
Group III: *Azadirachta indica* (AI)	60.21 ± 4.63	60.18 ± 4.63	0.03 ± 4.63	0.109**
Group IV: *Morinda citrifolia* (MCJ)	57.28 ± 2.77	52.08 ± 3.07	5.20 ± 2.77	0.002*
Group V: Green tea (GT)	59.63 ± 4.52	59.28 ± 4.51	0.35 ± 4.52	0.058**

Wilcoxon signed-ranks test: *$p < 0.05$ (significant), **$p > 0.05$ (not significant)

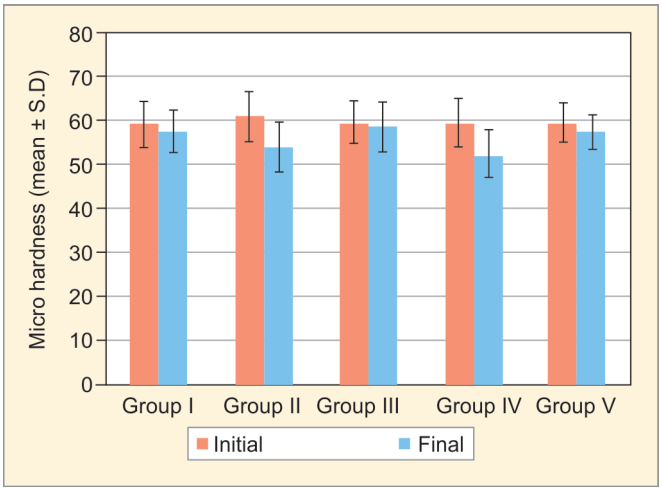

Fig. 1: Comparison of initial and posttreatment microhardness values of all groups

Table 2: Inter-group comparison of posttreatment microhardness of all groups using Mann–Whitney test

Groups	*Mean ranks*	*p value*
Group II vs group IV	13.42 vs 11.58	$p = 0.525$**
Group II vs group V	8.25 vs 14.75	$p = 0.019$*
Group II vs group I	8.33 vs 14.67	$p = 0.033$*
Group II vs group III	8.17 vs 14.83	$p = 0.006$*
Group IV vs group V	9.58 vs 15.42	$p = 0.045$*
Group IV vs group I	9.46 vs 15.54	$p = 0.035$*
Group IV vs group III	9.08 vs 15.92	$p = 0.018$*
Group V vs group I	12.46 vs 12.54	$p = 0.977$**
Group V vs group III	11.92 vs 13.08	$p = 0.686$**
Group I vs group III	11.83 vs 13.17	$p = 0.908$**

Mann–Whitney test: *$p < 0.05$ (significant), **$p > 0.05$ (not significant)

Results

A statistically significant difference was detected among group II (2.5% sodium hypochlorite) and group IV (*M. citrifolia*) irrigating solutions ($p < 0.05$) which indicates a significant change in initial and posttreatment microhardness values of radicular dentin in these groups. However, there is no significant change in these values in group I (saline), group III (*A. indica*), and group V (green tea) ($p > 0.05$) (Table 1 and Fig. 1).

In an inter-group comparison of posttreatment microhardness values, group II (2.5% sodium hypochlorite) and group IV (*M. citrifolia*) showed significant differences when compared with the remaining other groups. While on comparison between group II (2.5% sodium hypochlorite) and group IV (*M. citrifolia*), no significant difference is seen. Similarly, in between group I (saline), group III (*A. indica*), and group V (green tea) comparison, there is no much significance was seen (Table 2).

Discussion

The microhardness of dentin depends upon the degree of mineral content, amount of hydroxyapatite in intertubular substance, and tubular density and tubule diameter. A biological structure such as dentin is far less homogenous, with dentin tubule density increases while progressing to the pulp chamber than near the dentino-enamel junction in coronal dentin. While in radicular dentin, it increases from the cervical to apical region. Pashley et al. stated that the tubular density affects microhardness, as the tubular density increases dentin microhardness decreases presumably due to a decrease in the amount of intertubular dentin and an increase in individual tubular diameter.[14] Thus in this study, to minimize the structural variation and to establish a reasonable baseline for evaluation of the entire radicular dentin of each specimen, the mean values obtained by the average of the cervical, middle, and apical indentations were taken as a total microhardness value in both initial and posttreatment measurements.

The results of the present study showed that the initial microhardness of radicular dentin for all experimental groups ranges from 51 to 68 (VHN). These values correspond to hardness values of unaffected dentine which are present between 40 and 75 (VHN).[15,16]

Posttreatment microhardness values of all experimental groups revealed that 2.5% sodium hypochlorite and *M. citrifolia* showed a significant reduction in root dentine microhardness ($p < 0.05$). However, there is no significant reduction in microhardness in the green tea, *A. indica*, and saline groups ($p > 0.05$).

Sodium hypochlorite, which is chosen as a positive control group showed a significant reduction (mean change value of microhardness in this study is 5.18 ± 5.06 with *p* value 0.008) in root dentin microhardness while comparing initial and posttreatment microhardness values. This significant reduction may be attributed to its pH value and organic tissue dissolving property. pH value of 2.5% NaOCl is 12.65 which can cause 70% depletion of protein from hydroxyapatite surfaces.[17] Dentin contains 22% organic material mainly collagen type I which plays a major role in the microhardness of dentin. Depletion of the organic phase after irrigation using

NaOCl may cause a change in microhardness.[18] These results were correlated with other studies conducted by Ulusoy et al.,[10] Patil and Uppin,[11] Oliveira et al.,[12] and Ari et al.,[7] which reported that sodium hypochlorite decreased the dentin microhardness.

Saline, chosen as a negative control group showed that it does not have any significant changes (mean change value of microhardness in this study is 0.73 ± 4.15 with *p* value 0.126) in root dentin microhardness while comparing initial and posttreatment microhardness values. Saline has a pH value ranging from 5.5 to 6.0, which is nearer to neutral pH value 7.[19] Due to its near-neutral pH value; saline might not have shown any changes in the ratio of the organic and inorganic portion of radicular dentin, thus not altering the microhardness of root dentin. This was similar to other previous studies conducted by Ulusoy et al.[10] and Oliveira et al.[12]

On comparing herbal extracts with the negative and positive control groups, i.e., saline and sodium hypochlorite, only the *M. citrifolia* group showed significant changes (mean change value of microhardness in this study is 5.20 ± 2.77 with *p* value 0.002) in root dentine microhardness whereas green tea and *A. indica* groups did not show any significant changes (mean change value of microhardness of green tea and *A. indica* groups in this study are 0.35 ± 4.52 with *p* value 0.058 and 0.03 ± 4.63 with *p* value 0.109, respectively) in root dentin microhardness while comparing initial and posttreatment microhardness values.

The significant change in root dentin microhardness of the *M. citrifolia* group can be attributed to its pH value of 3.5.[20] This acidic pH value might be responsible for the changes in the ratio of the organic and inorganic portion of radicular dentin. This in turn may affect the microhardness of root dentin.

Azadirachta indica and green tea groups did not show any significant changes in root dentin microhardness in this study. This may also be due to its pH values 6.8 and 6.3, respectively, which are nearer to neutral pH value 7.[20,21] Therefore, green tea and *A. indica* groups might not have shown any significant changes in the ratio of the organic and inorganic portion of radicular dentin which in turn may not affect the microhardness of root dentin.

In a pair-wise group comparison of initial microhardness values, no significant difference was seen among all experimental groups. But, comparing posttreatment microhardness values, 2.5% sodium hypochlorite and *M. citrifolia* showed significant differences when compared with saline, *A. indica*, and green tea groups. This may be due to the alkaline pH value (12.65) of sodium hypochlorite and acidic pH value (3.5) of *M. citrifolia* juice may affect the ratio of the organic and inorganic portion of radicular dentin which may result in microhardness changes.

However, when 2.5% sodium hypochlorite and *M. citrifolia* groups were compared, there was no significant difference. This may be due to their surface tension property (surface tension value of 2.5% sodium hypochlorite and *M. citrifolia* juice are 41 and 41.64 dynes/cm).[22,23] Surface tension is defined as the force between molecules that produces a tendency for the surface area of the liquid to decrease. This force tends to inhibit the spread of a liquid over a surface or limit its ability to penetrate a capillary tube. The low surface tension property of endodontic solutions improves their dentin-wetting ability and improves their flow into narrow root canals. This same surface tension value for sodium hypochlorite and *M. citrifolia* might be the probable reason for not having a significant difference while comparing initial and posttreatment microhardness values in our study.

When compared to saline, *A. indica,* and green tea groups, did not show any significant difference. This may be due to their near-neutral pH value (pH value of saline, *A. indica*, and green tea are 5.5–6, 6.8, and 6.3, respectively). Dentin dissolves when the pH value is <5.5. Although saline, *A. indica,* and green tea showed slight variation in pH values, they may not be statistically significant.

The present observations suggested that among herbal extracts, canal irrigation with *M. citrifolia* juice leads to structural changes in radicular dentin due to demineralizing effect on root canal dentin. This softening effect on the dentinal walls could be beneficial in the clinic, as it permits rapid preparation and negotiation of tight root canals. However, the degree of softening and demineralization action might influence the physical and chemical properties of the heterogenic structure of dentin which in turn affect the adhesion of sealers and cements to the dentin.[21]

The limitations of the current study are that the experimental conditions (room temperature) employed in this study differed substantially from the clinical situations (body temperature).

Further *in vitro* and *in vivo* studies are needed to evaluate the efficacy of herbal extracts, *M. citrifolia*, *A. indica*, and green tea to be used as endodontic irrigants clinically.

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