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COMPARATIVE EVALUATION OF THE EFFECT OF ACACIA NILOTICA, CYMBOPOGON CITRATUS AND SODIUM HYPOCHLORITE ON THE ROOT CANAL DENTIN MICROHARDNESS OF SINGLE ROOTED MANDIBULAR PREMOLARS WHEN USED AS ENDODONTIC IRRIGANTS: AN IN VITRO STUDY

Dental Science

Dr. Jayendra Desai Post Graduate Student, Ranjeet Deshmukh Dental College and Research Center, Nagpur, India.

Dr. Rajesh Kubde Professor, Ranjeet Deshmukh Dental College and Research Center, Nagpur, India.

Dr. Pratima Shenoi Professor and Head of Department, Ranjeet Deshmukh Dental College and Research Center, Nagpur, India.

Dr. Gautam Badole Associate Professor, Ranjeet Deshmukh Dental College and Research Center, Nagpur, India.

Dr. Ankita Ramteke Senior Lecturer, Ranjeet Deshmukh Dental College and Research Center, Nagpur, India.

ABSTRACT

Aim: The aim of this study was to assess and compare the effect of Acacia nilotica, Cymbopogon citratus, and sodium hypochlorite as endodontic irrigants on the microhardness of root canal dentin in mandibular premolars. **Methodology:** Thirty-two recently extracted mandibular premolars were divided longitudinally into two halves, after being decoronated at the cementoenamel junction (CEJ). Before exposing the samples to irrigants, the Vickers microhardness test was used to determine the baseline dentin microhardness. The samples were randomly divided into four groups (n = 8): Group 1- normal saline (control); Group 2- 5.25% sodium hypochlorite; Group 3- Cymbopogon citratus (lemongrass); and Group 4- Acacia nilotica (babool). Samples were immersed in the respective irrigants for 15 minutes, after which microhardness was re- evaluated. Data were analyzed using one-way ANOVA followed by post hoc Tukey test ($p < 0.05$).**Results:** All irrigants caused a reduction in dentin microhardness compared to baseline values. However, Cymbopogon citratus and Acacia nilotica showed a comparatively lesser reduction than 5.25% sodium hypochlorite. **Conclusion:** Herbal irrigants demonstrated less detrimental effects on dentin microhardness, suggesting their potential use as alternative endodontic irrigants.

KEYWORDS

Acacia nilotica, Cymbopogon citratus, Dentin microhardness, Sodium hypochlorite.

INTRODUCTION

The most crucial element in the management and prevention of endodontic diseases is root canal disinfection using irrigation and instrumentation.[1] The purpose of irrigation and instrumentation is to get a clean, debris-free canal prepared for obturation.[2] The canals are cleaned or irrigated with a solution that can dissolve organic matter and disinfect them during each instrumentation procedure.[3]

The most popular irrigating solution for root canal treatment is sodium hypochlorite, which has a concentration range of 1% to 5.25%. It is regarded as an efficient antibacterial agent and a superior organic solvent for fixed, necrotic, and vital tissues. [4] The use of sodium hypochlorite (NaOCl) is associated with several drawbacks. It can corrode endodontic instruments, cause burning of the surrounding tissues, and has an unpleasant taste. Furthermore, NaOCl is highly toxic and ineffective in removing the smear layer. Additionally, it has been observed to lower dentin's elastic modulus and flexural strength.[5]

Also, these solutions can change dentin chemical structure, especially the amount of calcium in its hydroxyapatite crystals, which can affect important tooth properties like microhardness. Dentin microhardness evaluation allows us to infer changes in dentin's chemical and physical characteristics, including its mineral concentration and modulus of elasticity. The dentin's modulus of elasticity decreases as the microhardness decreases.[6] Thus, it would be preferable to have a potent, safe, and effective substitute irrigant. Over the years herbal medicine is becoming a more popular alternative therapy because of its greater effectiveness, biocompatibility, affordability, and ease of access. Additionally, a number of herbal medicines are gaining popularity in the dental field due to their strong antibacterial and anti-inflammatory properties.[7] Two examples of these natural extracts include lemon grass (*Cymbopogon citratus*)and babool (*Acacia nilotica*). Both *Cymbopogon citratus* and *Acacia nilotica* are used as an endodontic irrigants in the previous literatures.[8,9]

Herbs like lemon grass are members of the Poaceae family. Apart from its culinary use, lemon grass has several therapeutic advantages like its antipyretic, anti-inflammatory, antiseptic, and antibacterial characteristics. It includes ingredients with antioxidant and pain-relieving properties.[9]

Acacia nilotica (Leguminosae) commonly known as babool is an evergreen tree found in different parts of India in abundance. This plant has traditionally been used to cure a variety of systemic illnesses, including dental issues, and it plays a vital part in the native medical system.[10]

There is limited literature comparing the effect of *Acacia nilotica* and *Cymbopogon citratus* with sodium hypochlorite on the root dentin microhardness Of mandibular premolars when used as endodontic irrigants. Thus, this in vitro study's objective is to assess and contrast the effects of *Cymbopogon citratus*, *Acacia nilotica* and NaOCl on the microhardness of mandibular premolar root dentin when used as endodontic irrigants.

METHODOLOGY

Herbal extract preparation (*Cymbopogon citratus*)

A decoction of lemon grass leaves was prepared by placing the leaves in boiling distilled water for 5 minutes using a solid:liquid ratio of 1:1. The vessel was covered, taken off the heat source, and left to cool for about five minutes after boiling. The decoction was then obtained by filtering the combination of liquid and herbal material through a cheese cloth. The prepared extract was subsequently transferred into 100 ml reagent bottles and stored at 4 °C until further use. [9]

Herbal extract preparation (*Acacia nilotica*)

The extract was prepared using the maceration method. In this process, the powdered material was kept in suspension in 50%-90% ethanol for 7 days. After the maceration period, the mixture was filtered, and the ethanol was evaporated. The remaining extract was then oven- dried at 60°C until it became completely dry. Finally, the dried extract was re-dissolved in ethanol to obtain a 10% concentration for further use.[11]

Specimen preparation

Thirty-two recently extracted human single-rooted teeth free of restorations, cavities, and fractures were collected and kept in a 0.1% thymol solution. To standardize the canal length, a diamond-impregnated disc was used to decoronate the samples at the cementoenamel junction. The samples underwent no endodontic therapy.

After making grooves on the buccal and lingual exterior surfaces of the roots to segment them longitudinally, each root was split in half using a double-faced diamond disc while being cooled by water. After that, the dentin samples were ground polished using 600-grit and 1200-grit

silicon carbide abrasive sheets. Plastic rings of uniform diameter were filled with newly mixed autopolymerized resin. All specimens were embedded in 2 × 2 cm acrylic resin blocks with the polished surface facing outward (Figure 1). Repolishing was done to get rid of any extra material on the tooth surface after the specimens were mounted in the resin molds.

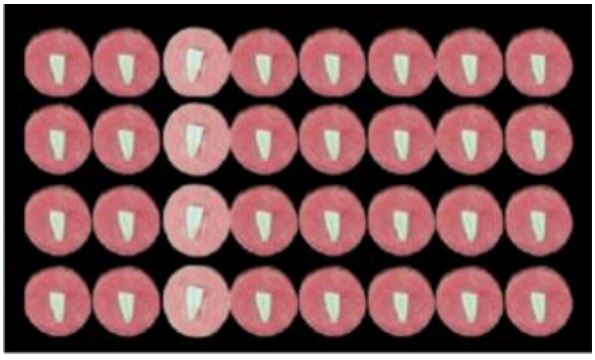

Figure 1. Mounting of samples.

Baseline microhardness evaluation (T0)

A Vickers microhardness testing machine with a 200 g load was used to measure the microhardness of each sample (Figure 2). The Vickers hardness number was noted after the diamond indenter was given 20 seconds to penetrate the apical third of root canal dentin surface. For each specimen, three indentations were made at an average distance of 100 µm from each other. The average of these three readings was used to determine each specimen's representative hardness value. The sample's baseline (T0) microhardness was determined by this value. Subsequently, the samples were randomly allocated into four groups. All specimens were subsequently immersed in their respective irrigating solutions for a mean period of 15 minutes according to the groups listed below in table 1.

Table 1. Grouping of samples.

Group 1	Irrigation with Normal saline.
Group 2	Irrigation with Sodium hypochlorite.
Group 3	Irrigation with Lemon grass (Cymbopogon citratus)
Group 4	Irrigation with Babool (*Acacia nilotica*).

Figure 2. Vickers hardness machine.Post treatment microhardness evaluation (T_1)

To reduce prolonged contact with the irrigants, washing of the samples was done with distilled water after being immersed in the irrigating solutions. Subsequently, the specimens were thoroughly dried, and the microhardness was measured again. This measurement was considered the post-treatment (T1) microhardness value of the samples.

Statistical analysis

SPSS version 21, the Statistical Package for the Social Sciences, was utilized to examine the data. Descriptive statistics were shown by the lowest, maximum, mean, and standard deviation (SD) values. The overall changes between baseline and post-treatment microhardness values were assessed using analysis of variance (ANOVA). A post hoc Tukey test was used to compare groups. In order to determine if the baseline and post-treatment measurements differed significantly, a paired t-test was also employed. For every analysis, the level of statistical significance was established at $P < 0.05$.

RESULTS

Table 2 summarizes the mean microhardness values at baseline and after treatment, as well as the mean and standard deviation (SD) for each group.

Table 2. Baseline and post-treatment mean microhardness values, mean and SD of all the groups

Group	Pre		Post		Difference	p-value
	Mean	SD	Mean	SD		
Saline	55.49	2.78	53.84	2.59	1.65	<0.001*
NaOCl	54.26	3.53	44.18	3.10	10.08	<0.001*
Cymbopogoncitratus	55.61	4.05	47.96	3.76	7.65	<0.001*
Acacia Nilotica	53.64	3.48	45.03	3.24	8.61	<0.001*

A paired t-test was used for statistical analysis.* denotes a statistically significant difference at $p \leq 0.05$. There was no significant difference in the baseline microhardness values across the groups, suggesting that the samples were evenly distributed. After immersion in the irrigating solutions, all the groups demonstrated a reduction in microhardness, except Group 1 (control group), which did not show any noticeable change.

Table 3 compares the % change in microhardness values between four groups. Maximum reduction was seen in the NaOCl group followed by *Acacia nilotica* and *Cymbopogon citratus* groups. The reduction in microhardness (in %) in the *Cymbopogon citratus* and *Acacia nilotica* groups did not show a significant difference.

Table 3. Comparison of % change in microhardness values between four groups.

Group	Mean	SD	p-value
Saline	2.96^{a}	0.98	<0.001*
NaOCl	18.59^{b}	2.13	
Cymbopogoncitratus	13.76^{c}	1.64	
Acacia Nilotica	16.06^{c}	2.36	

A one-way ANOVA test was used to examine the data, and a post hoc Tukey test was used to make multiple comparisons between the groups. A statistically significant difference at $p<0.05$ is denoted by the symbol *. Furthermore, a substantial difference between the corresponding pairings of groups is indicated by various superscript letters inside the column.

DISCUSSION

Biomechanical preparation of root canals involves the combined action of endodontic instruments and irrigating solutions, with the primary aim of eliminating pre-existing organic and inorganic debris produced during operative procedures, as well as reducing the microbial load and its by-products. However, the irrigating solutions used during endodontic treatment may alter the chemical structure of dentin, which can subsequently influence its mechanical properties. Irrigants employed for smear layer removal may act not only on the smear layer but also on the underlying root dentin. This action can result in the exposure of the collagen matrix and may ultimately lead to a reduction in dentin microhardness.[12]

A reduction in dentin microhardness may therefore contribute to a higher frequency of cracks or fractures. The Vickers hardness test is extensively used and regarded as a dependable, appropriate, and useful technique for assessing alterations in both the surface and deeper hardtissue structures.[1] Prior to microhardness testing, the specimens in this study were immersed for fifteen minutes in the appropriate irrigating solutions.

Goldberg et al.[13] suggested that, ten to fifteen minutes application time is sufficient to achieve best possible outcomes, which appears to be

more practical and clinically relevant in routine dental practice. Previous studies have reported that the use of 5.25% NaOCl as an irrigating solution leads to a reduction in the microhardness of dentin[14].

The reduced hardness of the root dentin may be caused by a reduction in the stiffness of the intertubular dentin matrix. This alteration is probably related to the unevenly dispersed mineral phase in the collagen matrix, which could compromise the structural integrity of the dentin[15]. Herbal irrigants can be utilized as a safe irrigating solution with less detrimental impact on the hardness of root dentin, according to a study by Elika et al. (2021).[1] In the present study Lemon grass showed less impact on the root canal dentin's microhardness after treatment. This result was in accordance to the study performed by Syed Abrar et al (2018) which demonstrated that Lemon grass do not cause erosion of the dentinal wall.[9] Also, the study showed a less reduction in microhardness with the use of *Acacia nilotica*. The present study aimed to evaluate the microhardness of root canal dentin following the use of both synthetic and herbal irrigants. The findings of the present study indicate that the percentage reduction in microhardness was comparatively lower when herbal irrigants were used. This suggests that herbal irrigants may have a less detrimental effect on dentin microhardness. However, before they may be widely advised for clinical usage, more clinical research is required to evaluate their safety and biocompatibility.

Standardized specimen preparation, baseline and post-treatment Vickers microhardness assessment, and random assignment into control and experimental groups, which improves internal validity, are among this in vitro study's strong points. Clinical relevance is provided by using 5.25% sodium hypochlorite as a gold standard comparator, and results are morereliable when suitable statistical analysis (ANOVA with post hoc Tukey test and paired t-test) is performed. Herbal irrigants like *Acacia nilotica* and *Cymbopogon citratus* are added for novelty and translational value. The study's in vitro approach and small sample size, however, may make it difficult to recreate clinical settings including dynamic irrigation and dentinal fluid flow. Furthermore, thorough clinical extrapolation was limited since only microhardness was assessed, not other mechanical characteristics, antibacterial activity, or long-term impacts.

CONCLUSION

Considering the constraints of this in vitro study, all of the irrigants utilized had an impact on the microhardness of human radicular dentin. However, lemon grass and babool demonstrated comparatively less harmful consequences on the microhardness than Sodium hypochlorite. Furthermore, the reduction in microhardness observed in the lemon grass and babool groups was not statistically significant.

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