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### RESEARCH ARTICLE

## COMPARATIVE EVALUATION OF HERBAL IRRIGANTS ON MICROHARDNESS OF DENTIN- AN IN VITRO STUDY

Somiya Gupta, Ajay Kumar Nagpal, Sunil Kumar, Shivangi Dokaniya and Jugal Kishore Das

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#### Abstract

**Introduction:** Success in endodontic therapy depends on the effective chemo-mechanical debridement of the root canal system using instruments and irrigants. Common chemicals, such as 17% EDTA and sodium hypochlorite, are used to remove the smear layer. t NaOCl primarily dissolves organic tissue and CHX is an antimicrobial, while EDTA is the chelating agent specifically for the smear layer's inorganic components. However, the rise of antibiotic-resistant strains and the side effects of synthetic drugs have led to increased interest in herbal alternatives. Herbal products, known for their antimicrobial, biocompatible, anti-inflammatory, and antioxidant properties, are gaining popularity in dental and medical practices.

**Aim:** To compare the effect of different herbal Triphala, Green tea extract, Neem and Tulsi on the microhardness of dentin.

**Material and Methods:** Thirty freshly extracted human mandibular premolars with fully formed roots were collected, cleaned with ultrasonic scalers and stored in saline. Teeth were decoronated at the CEJ with a diamond disc, and pulp tissue was removed with a barbed broach. Roots were sectioned vertically into buccal and lingual components cleaned with distilled water and set in acrylic blocks exposing the dentinal surface. Specimens were smoothed using silicon carbide abrasive papers (180, 320, 600, 800, 1000 grit) and polished with alumina. The specimens were randomly divided into six groups (n=10 each): Group I – 2% Chlorhexidine, Group II – Green Tea extract, Group III – Triphala extract, Group IV – Neem, Group V – Tulsi and Group VI – Distilled water. Specimens were immersed in their respective solutions for 15 minutes, rinsed with distilled water and tested for microhardness using the Vickers Microhardness test with a 200g load and 20-second dwell time. Three indentations were made on each specimen at the coronal, middle and apical thirds.

**Results:** Chlorhexidine showed the highest reduction in dentin microhardness, followed by Green Tea, Triphala, Neem, Tulsi and Distilled water. There was no statistical significance among the groups.

**Conclusion:** Within limitation of this study it can be concluded that herbal irrigants showed less effect on microhardness of dentin rather than chemical irrigants.

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#### Introduction:-

Complete eradication of bacteria and their byproducts is essential for successful endodontic therapy. This is achieved through mechanical instrumentation and primarily with antimicrobial irrigants.<sup>1</sup>



An ideal irrigant should have debridement, lubrication, antimicrobial action, and the ability to dissolve organic and inorganic materials.<sup>2</sup>

During biomechanical preparation, canals are irrigated to disinfect and remove debris. Commonly used irrigants include sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX) and EDTA. NaOCl is effective in dissolving organic tissue and disinfecting the root canal but may damage periapical tissues at high concentrations. CHX, a broad-spectrum, less toxic antimicrobial agent, is used both as an irrigant and intracanal medication.<sup>3</sup>

Chemical irrigants can alter dentin's composition, affecting microhardness and the adhesion of dental materials.<sup>4</sup> Herbal medicines are gaining popularity for their safety, availability and therapeutic properties.<sup>5</sup>

Triphala which has antibacterial and anti-inflammatory properties.<sup>1</sup> Green tea polyphenols show antibacterial, antioxidant, anti-inflammatory effects and natural fluoride.<sup>5</sup>

Neem possesses antimicrobial, antiviral, antifungal and anti-inflammatory properties, with active compounds like Azadirachtin and Nimbidin. It effectively reduces *E. faecalis* adhesion.<sup>6</sup>

Tulsi (*Ocimum sanctum*) rich in Eugenol and other phytochemicals, exhibits antimicrobial, antioxidant and immunomodulatory effects.<sup>6</sup>

Despite their efficacy, chemical irrigants may negatively affect dentin microhardness, increasing fracture risk.<sup>4</sup> Thus, safer alternatives like herbal irrigants—Triphala, Green Tea, Neem and Tulsi—are being explored for their low toxicity and high antimicrobial potential.

## Material and Methods:-

### Sample Preparation

Freshly extracted 30 permanent mandibular premolars with completely formed roots were collected. Teeth were cleaned with the help of ultrasonic scaler followed by storage in physiological saline solution until the start of the experiment. Teeth were decoronated at the CEJ with diamond disc. Pulp tissue was removed by barbed broach. Using a diamond disc, roots were sectioned vertically from the cervical to the apical extent splitting the roots into buccal and lingual components giving sample specimens. The root segments were cleaned with distilled water to clear all of the debris. Sectioned specimens were set in an acrylic block exposing the dentinal surface. The surface of the specimens were smoothened using a fine gritted silicon carbide abrasive papers 180,320,600,800,1000 grit followed by polishing of the specimens were done with alumina.

### Preparation Of The Test Solutions

#### Preparation of Testing Solutions

##### Triphala:

500 mg of Triphala powder (Himalaya) was dissolved in 10 ml of 10% DMSO resulting in a 0.5% w/v Triphala solution. Distilled water was added to make up a final volume of 100 ml. The solution was mixed thoroughly for uniformity.

##### Green Tea Extract:

5 grams of green tea extract (BRM) was added to 100 ml of boiling sterile water, steeped for 5 minutes, and then filtered using filter paper.

##### Neem and Tulsi:

100 grams each of dried neem and tulsi leaves were tied in a cloth pouch and soaked in 800 ml of distilled water. The pouch was boiled gently over a low flame. After cooling, the pouch was removed, and the solution was filtered to obtain a clear liquid. A 25% concentration was achieved by measuring the final volume post-filtration.

### Grouping of Samples:

Based on the irrigating solutions, specimens were randomly divided into 6 groups of 10 samples each, namely-

**Group I** – 2% Chlorhexidine [Positive control]

**Group II** – Green tea extract

**Group III** -Triphala extract

**Group IV** – Neem

**Group V** - Tulsi

**Group VI** – Distilled water [Negative control]

Each sample was immersed in respective irrigating solution for 15 minutes.

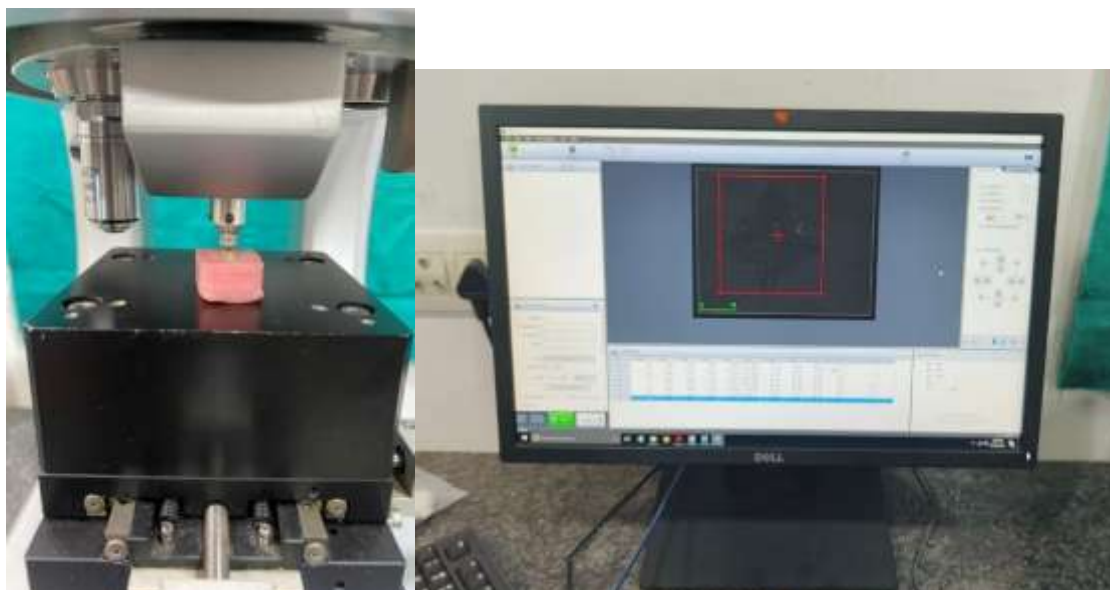




**Figure 1:-** Sample Immersed In Irrigating Solution.

### Microhardness Testing

Specimens were tested for microhardness using Vickers Microhardness test with a 200g load with a dwell time of 20 seconds . Specimen were horizontally mounted on Vickers testing machine and three indentations for each specimen were made on the coronal, middle and apical thirds of the root. Mean length of the diagonals of indentations were recorded and computed.



**Figure 2:-** Sample Mounted On Vickers Testing Machine And Screen Showing Mean Length Of Diagonals.



### Statistical Analysis

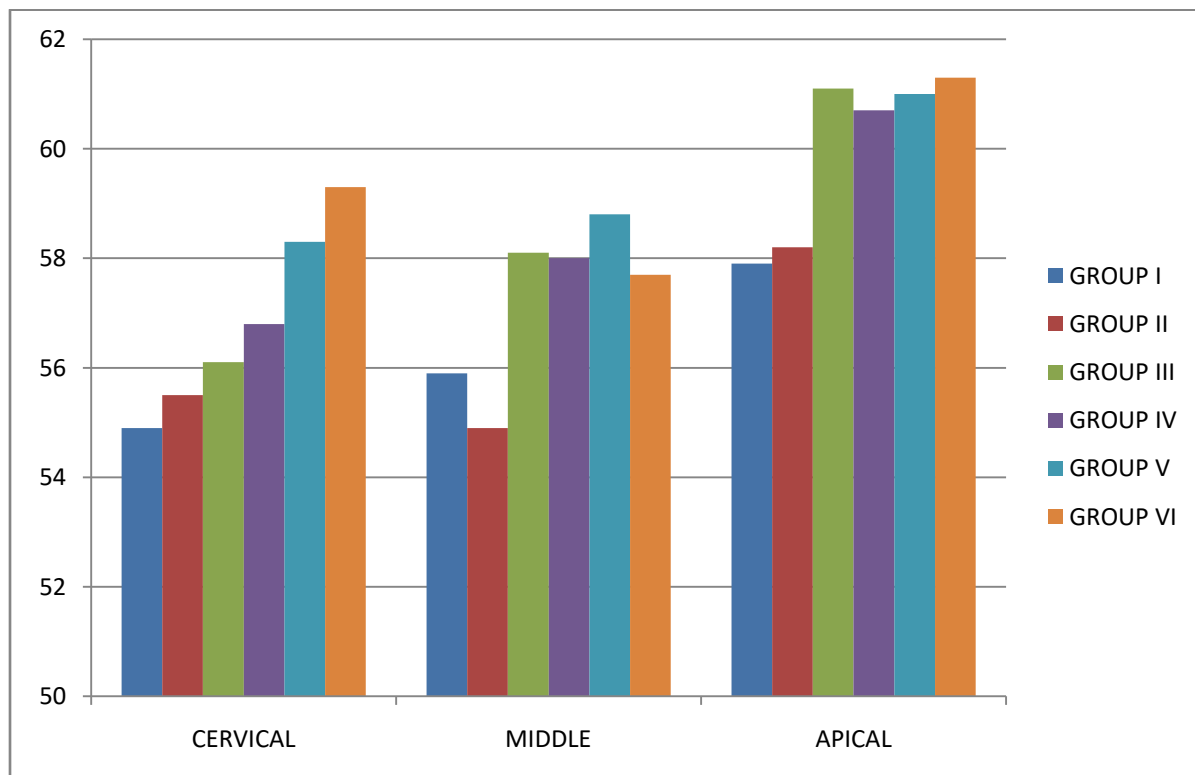
The data were analysed using statistical package for social sciences version (SPSS) 20.0. The level of statistical significance was set at 95% ( $P=0.05$ ).  $P\text{-value} > 0.05$  was non-significant. The data of the present study were subjected to statistical analysis to interpret the differences and significance among groups. One-way ANOVA test was applied to compare measurements of microhardness values among six study groups at each root level. Post hoc tukey test analyses multiple pair-wise individual group comparisons.

### Results:-

The microhardness values across all groups were highest at the apical region, followed by the middle, and lowest at the cervical region. Group I showed the highest value at the apical (57.90), with the lowest at the cervical (54.90). Group II also had the highest at the apical (58.20), but the lowest at the middle region (54.90). Groups III, IV, and V followed a similar trend, with peak values at the apical (61.10, 60.70, and 61.00 respectively) and the lowest at the cervical. Group VI showed the highest at the apical (61.30), followed by cervical, and lowest at the middle (57.30). Overall, the apical region recorded the highest mean microhardness (60.03), with the middle (57.23) and cervical (56.82) showing progressively lower values as seen in TABLE 1

**Table 1:-** Intergroup Comparison of Microhardness at Different Region.

GROUP	N	CERVICAL		MIDDLE		APICAL	
		MEAN	STD. DEVIATION	MEAN	STD. DEVIATION	MEAN	STD. DEVIATION
GROUP I	10	54.90	4.149	55.90	2.767	57.90	3.071
GROUP II	10	55.50	3.472	54.90	2.183	58.20	1.619
GROUP III	10	56.10	2.961	58.10	2.998	61.10	1.792
GROUP IV	10	56.80	3.084	58.00	3.399	60.70	2.406
GROUP V	10	58.30	3.199	58.80	3.293	61.00	2.309
GROUP VI	10	59.30	3.129	57.70	3.129	61.30	3.234
Total	60	56.82	3.568	57.23	3.175	60.03	2.768
SIGNIFICANCE		.039*		.041*		.004*	



**Graph 1:-** Microhardness at Different Region.



**Discussion:-**

Irrigating solutions can influence the microhardness of radicular portion of dentin, which could impact the clinical performance related to endodontically treated teeth. While several irrigants for root canal therapy offer benefits such as elimination of debris, disinfection, elimination of smear layer as well as lubrication of dentinal walls they may also cause negative changes in the physical structures of dentin, which is including its microhardness.<sup>7</sup>

The microhardness of the dentin is influenced by many factors such as mineral content, particularly the quantity of hydroxyapatite in the intertubular substance, tubular density, and the diameter of the tubules. Dentin, being a biological structure, is not homogeneous. The density of dentinal tubules increases as one moves from the dentino-enamel junction (DEJ) in the coronal part of the dentin toward the pulp chamber. In the radicular part of dentin (the root portion), this increase in density happens from the cervical area toward the apical area.<sup>8</sup>

Hardness correlates with resistance to fracture, modulus of elasticity, yield and bond strength. Although a change in composition of dentin makes the instrumentation process easier during the root canal treatment, it also compromises the root construction by decreasing the microhardness. As a result, teeth that have undergone root canal therapy are more prone to fracture.<sup>9</sup>

All samples were submerged in their designated irrigating solutions for 15 mins prior to undergoing microhardness testing. Goldberg et al. recommended an application duration of 10-15 mins to achieve optimal outcomes that aligns better with practical clinical settings.<sup>8</sup>

The choice of the "Vickers microhardness tester" over the "Knoop hardness tester" was based on the Vickers test's greater suitability and practicality for assessing surface alterations in deeper hard tissues of teeth. In contrast the "Knoop hardness tester" is typically utilized for evaluating superficial dentin at a depth of 0.1 mm rather than for deeper dentin.<sup>2</sup>

Distilled water was utilized as a control because it does not make any chemical alteration on dentin.<sup>11</sup>

This study was designed to evaluate the efficacy of different root canal irrigants viz. CHX, Triphala, Green tea, Neem, Tulsi and Distilled water to know the alterations of the microhardness of the dentin. The findings of this study observed all the irrigation solution reduced microhardness of the dentin structure and might be affected the mechanisms of dentin structure. All the irrigants used did not show significant impact on microhardness reduction of radicular dentin.

In this study, it was also obtained that CHX decreased the dentin microhardness. The result was in accordance with Oliveira et al., the microhardness of the dentin treated with CHX (2.0%) was reduced. CHX being a cationic chemical, which has the capability to bind to anionic molecules viz., the phosphates in the hydroxyl apatite structure, which may change the Ca/P ratio and help to explain the decreased microhardness of the dentin when exposed to CHX.<sup>12</sup>

Aslantas et al. also found the least effect of CHX on microhardness as it was not able of dissolving the necrotic tissues or eliminating the smear layer. The smear layer may act as a barrier, limiting irrigant interaction with dentin and thus allowing for only minimal alterations in microhardness, which also correlated with reduced Ca and P levels and root dentin microhardness. They suggested that this alteration in microhardness of the dentin might be relied on the application duration of CHX.<sup>18</sup> Amin et al. reported reduction of microhardness with the use of CHX.<sup>13</sup>

The current study also found that the green tea extract and neem did not significantly reduce the microhardness of dentine was similar to the study of Durgavandi et al. and they also reported that green tea and neem had no significant impact on microhardness of the dentin both the neem and tulsi have a neutral pH of 6.8 and 6.3, respectively. The dentin which contains 22% organic material have a neutral pH value less than 5.5. Although green tea and neem observed slight variation in pH value, but these were not statistically significant in the study.<sup>14</sup>

Nikhil et al. found green tea decrease the microhardness because of presence of catechins. Catechin a polyphenol with known anti-inflammatory, antioxidant and anticarcinogenic properties. These catechins with an acidic nature may contribute to demineralization of dentin.<sup>15</sup>

The findings of Mirkarimi et al. in contrast observed that green tea extract enhanced the microhardness of eroded dentine and there was an improvement of its texture. Moreover, green tea extract mostly permitted to form a surface deposition by organic components on the dentin, or it could be ascribed to the existence of afresh induced collagen



crosslinks. Proanthocyanidin is well-known combination of “monomers, oligomers and polymers of flavan-3-ols (catechines)”, which are extensively exist in extract of green tea and might be interacted with the organic part of the dentin.<sup>58</sup> Moreover, Kato et al. also emphasized that extract of green tea helps to reduce dentin layer under erosive situations as it inhibits the activity of MMPs which in turn allow the maintenance of an organic layer on the eroded dentin.<sup>16</sup>

In this study, DMSO was used as a solvent to create a solution of Triphala as reported by Hebling et al. reported that DMSO might have little or no cytotoxic impacts on odontoblast-like cells.<sup>7</sup>

Triphala has demonstrated a lesser decrease in the dentin microhardness when compared to CHX and green tea. The similar result was observed in a comparative study after the usage of NaOCl (5.0%) and EDTA (17.0%). The most probable reason for this might be because of the citric acid present in the Triphala fruit, which plays a role on weak chelation.<sup>17</sup>

Tulsi exhibited comparable anti-microbial efficacy due to its active constituents such as “Eugenol, Ursolic acid, Carvacrol and Oleanolic acid. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)” the primary potential component in *Ocimum sanctum* L. (Tulsi) were identified as a major contributor to its therapeutic properties.<sup>6</sup>

In all the groups the microhardness was found least at cervical, which was followed by middle and highest at apical regions, but the decrease in microhardness did not show statically significant change.

Maria Philip et al. found the dentin microhardness increased from the cervical to the apical regions.<sup>18</sup>

Durgavandi et al. on comparing with different herbal irrigants at cervical, middle and apical third.<sup>19</sup> The dentin microhardness decreased as per tubular density increased likely due to a reduction in the quantity of intertubular dentin along with an expansion of tubular diameters individually.<sup>20</sup>

## Conclusion:-

The limitation of this study is that the experimental group condition comprised in this research varied considerably as per the clinical conditions. Furthermore, other properties such as biocompatibility, staining and substantively are necessary towards the efficient intracanal irrigation. Many in-vivo and in vitro studies are required to evaluate the efficacy of plant extracts like green tea, triphala, neem and tulsi to be used as endodontically irrigation clinically.

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