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Impact of Tulsi extract on sealer penetration in dentinal tubules as compared to 17% EDTA as the final root canal irrigant (in vitro analysis)

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Abstract:

Introduction: Penetration of endodontic sealers into dentinal tubules is essential for ensuring successful root canal treatment by creating a hermetic seal that prevents reinfection. Traditionally, 17% EDTA has been used as the final irrigant for smear layer removal. However, concerns regarding its limited antimicrobial properties and potential for dentin erosion have prompted the exploration of alternative solutions. This in vitro study compared the effectiveness of Tulsi extract with that of 17% EDTA in promoting sealer penetration within the dentinal tubules.

Methodology: Thirty extracted single-rooted teeth were randomly divided into three groups: Group 1, 17% EDTA; Group 2, Tulsi extract; and Group 3, saline control. After preparation, the root canals were irrigated with their respective soluti ons, filled with gutta-percha, and sealed with a calcium hydroxide-based sealer. Sealer penetration was analyzed using scanning electron microscopy (SEM) at the 2 mm and 5 mm levels from the root apex.

Results: The results showed no significant difference in sealer penetration at 2 mm among all the groups. However, at 5 mm, both EDTA and Tulsi groups exhibited significantly greater sealer penetration than the control group ($p < 0.001$). No significant difference was observed between the EDTA and Tulsi groups at 5 mm ($p = 0.706$).

Conclusion: Tulsi extract demonstrated similar efficacy to 17% EDTA in facilitating sealer penetration, suggesting its potential as a biocompatible and antimicrobial alternative for root canal irrigation protocols. Further studies are warranted to explore its clinical applications and commercial viability.

Keywords: Hermetic Seal, Dentinal Tubule Penetration, Sealer, Tulsi Extract, EDTA, Final Irrigation.

Introduction:

The complex root canal system architecture, characterized by the presence of dentinal tubules, poses a challenge in achieving optimal sealing during endodontic treatment. The penetration of sealers into these tubules is critical for ensuring a hermetic seal, which is essential for preventing reinfection and promoting healing (1-3). Owing to its strong antibacterial properties, sodium hypochlorite (NaOCl) has historically been the recommended irrigant and its ability to dissolve organic tissue (4). However, the use of NaOCl alone may not adequately remove the smear layer, a residual layer of debris that can obstruct the penetration of sealers into dentinal tubules (5, 6). Ethylenediaminetetraacetic acid (EDTA) is frequently employed as a chelating agent to effectively remove the smear layer, thereby enhancing the penetration of sealers into dentin (6, 7). However, several disadvantages associated with EDTA warrant further consideration. One significant drawback of this method is its limited antimicrobial activity. Although EDTA is effective in chelating calcium ions and removing inorganic debris, it does not possess inherent antibacterial properties, which can leave viable bacteria in the root canal system (8, 9). This limitation is particularly concerning, given that the primary goal of endodontic treatment is to eliminate microbial infection and prevent reinfection (10). Another critical disadvantage of EDTA is its potential to cause dentinal erosion. Studies have shown that prolonged

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exposure to EDTA can lead to significant demineralization of dentin, compromising its structural integrity and increasing its risk of fracture (11, 12). Erosion can extend to depths of up to 300 µm, which may weaken the tooth structure and predispose it to future complications 46. Furthermore, when used in conjunction with sodium hypochlorite (NaOCl), EDTA can exacerbate dentin erosion due to the synergistic effects of these agents (12, 13). This erosion not only affects the mechanical properties of dentin but may also hinder the adhesion of endodontic sealers, thereby compromising the overall success of treatment (9). Additionally, the high surface tension of EDTA limits its penetration into dentinal tubules, which is essential for effective cleaning and sealing (8, 14). This limitation may result in inadequate removal of debris and smear layer from complex root canal systems, particularly in areas that are difficult to access (14, 15). The inability of EDTA to effectively penetrate and clean these areas can lead to persistent infection and treatment failure (10). Studies have shown that the combination of EDTA with other agents can significantly improve the depth of sealer penetration, which is crucial for the success of endodontic treatment (2, 16). In recent years, there has been growing interest in the use of natural extracts, such as Tulsi (Ocimum sanctum), as alternative irrigants in endodontics. Tulsi is renowned for its antimicrobial properties, which may complement the effects of traditional irrigants, such as EDTA (1, 16). Additionally, Tulsi extract may provide a biocompatible alternative to conventional chemical irrigants, potentially reducing the risk of adverse effects associated with synthetic agents (17). The dual action of Tulsi extract, both as an antimicrobial agent and a potential smear layer remover, is a valuable adjunct to endodontic irrigation protocols. Studies have suggested that herbal extracts can effectively enhance the cleaning efficacy of root canals, thereby improving the penetration of sealers into dentinal tubules (18, 19). Furthermore, the incorporation of Tulsi extract in irrigation protocols may not only facilitate better sealer penetration, but also contribute to the overall success of endodontic treatments by reducing microbial load and promoting healing (20, 21). The aim of this in vitro study was to evaluate and compare the impact of Tulsi (Ocimum sanctum) extract and 17% EDTA as final root canal irrigants on the penetration of endodontic sealer into the dentinal tubules. This study aimed to determine the effectiveness of Tulsi extract as a potential alternative to EDTA for enhancing sealer penetration, which may contribute to improved root canal obturation and long-term clinical outcomes. Methodology: The current in vitro investigation followed the checklist for reporting in vitro studies (CRIS) requirements. Seventeen months later, the Institutional Review Board (IRB) approved DSH/IRB/2024/0054 for the study. This study was evaluated and approved by the Liaquat College of Medicine and Dentistry IRB.Purposive sampling, which is non-probability, was used to collect the samples. Using PASS version 11 for one-way ANOVA, the sample size was determined using the averages and standard deviations of earlier research, with a 95% confidence interval and 90% power (22). For each group, the minimum sample size of eight samples was determined. At the LCMD, in the Department of Oral Maxillofacial Surgery, teeth were extracted. Each tooth was radiographed to confirm the presence of a single canal. The teeth were extracted, rinsed with tap water to remove any apparent debris, and then refrigerated in 0.1% thymol at 4 °C until needed. The following inclusion criteria were used to select samples: single-rooted permanent human teeth with developed apices, and patients aged between 18 and 40 years. Patients with Intracanal calcification were excluded. (Determined from the preoperative radiograph prior to extraction displacement of the roots, shattered or cracked teeth, root caries, root resorption, and previously treated or started root canal treatment). The old irrigants were not included in this study. Granular, flaccid, and turbid irrigants were discarded. Tulsi extract: Curcuma longa rhizomes were chopped into irregular pieces and oven-dried for twenty-four hours at 45°C and/or 5°C. This resulted in the production of the ethanolic extract. After confirming that it was moisture-free, it was ground into a powder. The powder was soaked in 200 mL of 95% ethanol and stored at room temperature. After maceration for one week, it was filtered. The ethanol was then evaporated by heating the filtrate to 40–50°C using water baths. The dense paste that was developed contained 100% extract. The extract was stored in a refrigerator at 4°C (23). Sample preparation: The current study included 30 teeth, with ten samples in each group. An access aperture was made in each sample using a # 4 diamond cutting round bur in a high-speed hand piece under an air–water spray. Each root canal was filled with a #10 K-type file (Maillefer/Dentsply) until it protruded past the apical foramen. The length was reduced by 1 mm to determine the working length. Initially, a single operator prepared root canals using K-type files (Maillefer/Dentsply); later, root canal preparation was completed up to the F2 file size using the ProTaper Universal System (Dentsply-Maillefer). Irrigation was carried out using 3% NaOCl (2.5 mL) at every file update. Three sets of specimens were randomly selected using a lottery method. In group 1 (n=10): Three millilitres of 17% EDTA was used as the last irrigation, and the sample was left in the canal for a minute. Group 2 (n=10): 3 ml of tulsi extract irrigant was used for the final irrigation, lasting one minute. Control group (n = 10): Using a normal irrigation process, the sample was finalized with a 1-minute irrigation with 0.9% saline. F2 sterile absorbent paper points were used to dry the specimens, and an F2 gutta-percha (GP) cone was chosen to serve as the master cone. After mixing the Sealapex (Sybron-Endo, Glendora, CA, USA) sealer according to the manufacturer's instructions, the canal was filled. After inserting the mastercone and extending the sealer to the working length, the tug's back was measured. Once the sealer was evenly applied to the

apical 2 mm of the master cone, GP was inserted up to the working length. To create a strong coronal seal, excess GP was burned off using a hot condenser and the coronal GP was vertically crushed. The GIC restorative was used to seal the access cavity. For one week, all samples were kept in an incubator (Biner GmbH, Germany) at 37 °C and 100% humidity. Selaer penetration: Penetration of Sealers Utilizing SEM, or scanning electron microscopy for every group, ten sectioned teeth had markings of two and five millimeters. The specimens were placed on metal stubs and titanium was sputtered in a JEC-3000FC Auto Fine Coater at 20 mA for 30 s. Images were acquired using an electron microscope (JEOL, Tokyo, Japan; Model No. JSM-IT 100). The samples were inspected by an impartial, blinded observer to check for dentinal tubule penetration of sealers at two different levels (2 mm and 5 mm) from the root apex. The distance between the locations and the sealer-dentin contact was used to assess the depth of penetration.

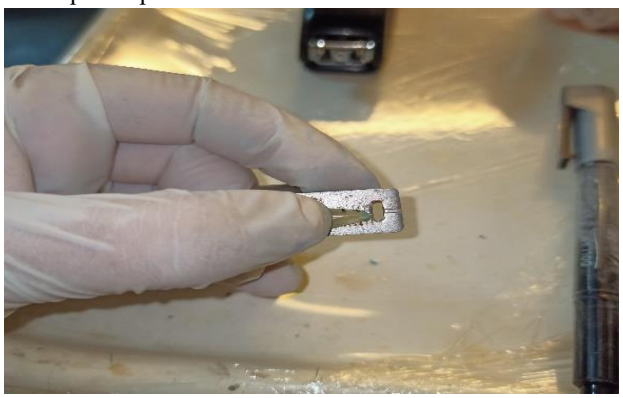

Figure 1: Markings on 2mm and 5mm in tooth apical region after obturation.

Figure 2: Scanning Electron microscope (JEOL, Tokyo, Japan, Model No. JSM-IT 100).

Figure 3: Metal stubs and titanium sputtered in the JEC-3000FC Auto Fine Coater.

Statistical analysis:

For the statistical version 23 (IBM Corp., Armonk, NY, USA's SPSS version 23 was used for the statistical analysis. The sealer penetration data, the Kruskal-Wallis test was used to evaluate sealer penetration data. Sealer penetration was analyzed using the Mann-Whitney test to compare the groups. Wilcoxon signed-rank test was used to compare sealer penetration at various tooth levels. Statistical significance was set less than 0.05.

Results:

The findings showed that at 2 mm, there was no significant difference ($p=0.67$) in sealer penetration into the dentinal tubule. There was a statistically significant ($p<0.05$) variation in sealer penetration into the dentine at 5 mm between the groups. A significant difference was seen in the 5 mm sealer penetration between the control group and EDTA ($p\leq0.001$) as well as between the control group and tulsi extract ($p\leq0.001$). However, there was no significant difference between tulsi extract and EDTA ($p=0.706$) at the 5 mm level.

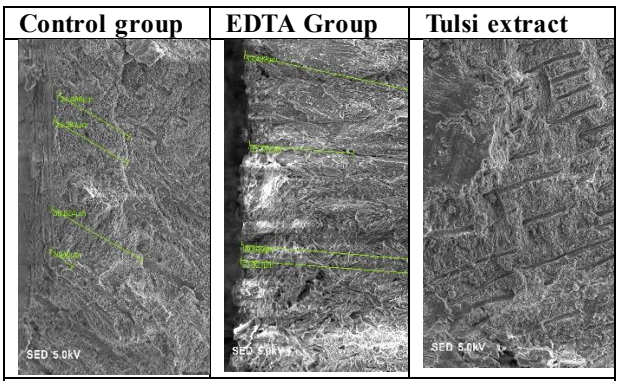

Figure 2: Sealer Penetration under SEM for control group, EDTA group and Tulsi extract

Table 1: Mean comparisons of sealer penetration among groups

Parameters	Control group	EDTA group	Tulsi Extract group	P value
Penetration at 2mm (µm)[b]	0.00 (0.00-4.5)	0.00 (0.00-9.5)	0.00 (0.00-10.1)	0.768
Penetration at 5mm (µm)[b]	7.4 (4.4-8.5)	13.7 (8.9-20.5)	13.2 (11.5-16.2)	< 0.001^

[b] Values are represented as median (Interquartile range). [β] One Way ANOVA; ^Kruskal Wallis

Table 2: Group-wise comparison of micro leakage & sealer penetration.

Parameters (unit)	Control vs. EDTA	Control vs. Tulsi	EDTA vs. Tulsi
Penetration at 5 mm (µm)[$]	< 0.001	< 0.001	0.706

[$]Values are represented as p-value computed using the Mann-Whitney test.

Table 3: Comparison of sealer penetration among different levels of the tooth among all investigated groups

Group	2 mm, penetration	5 mm, penetration	p-value
Control group	0.00 (0.00-5.5)	7.4 (4.4-8.5)	0.002∞
EDTA group	0.00 (0.00-12.6)	13.7 (8.9-20.5)	< 0.001∞
The Tulsi Extract (TE)	0.00 (0.00-13.4)	13.2 (11.5-16.2)	0.001∞

Values are reported as (Median ± Q1-Q2 µm). ∞2-mm vs. 5-mm level: Wilcoxon signed-rank test, $p < 0.05$.

Discussion:

After considering the findings of the current study, we failed to reject the null hypothesis, which states that there is no significant distinction between EDTA and Tulsi extract as final irrigants for sealer penetration. To avoid the dangerous impacts of EDTA, Tulsi Extract irrigant is a useful tool for removing smear layers. Additional research is needed to infer the results of the current study and to facilitate commercial availability. To prevent root canal organisms from communicating with periapical tissue, the sealer must seal the root canal apically. The penetration of the sealer into dentinal tubules, which stops bacterial activity within the tubules and inhibits bacterial recolonization, is the ideal result of root canal therapy. A sealapex sealer based on calcium hydroxide (Ca (OH)2) was used in this study. There are two main reasons for the use of calcium hydroxide-based sealers. First, due to its antibacterial qualities, which promote healing, it stimulates the periapical tissues. Suitable biological, physical, and chemical properties, such as sealability, are present in the seal apex. At the apical 2 mm level, sealer penetration across various final irrigation techniques showed no statistically significant difference between the groups under investigation. The results of the current study are consistent with those of previous studies. Ahmet et al, reported that that lower sealer penetration was observed in the apical third due to the presence of sclerotic dentin and a reduced number of dentinal tubules, which further complicates effective sealing in this critical area(24). Pawar et al, evaluated the apical sealing ability of various sealers and noted that the sealing efficacy was generally lower in the apical regions compared to more coronal areas(25). This study underscores the importance of considering anatomical variation when assessing sealer penetration. Evidence indicates that the following factors, which are directly related to sealer penetration, may cause the following outcomes: difficulties in adequately irrigating the apical area and removal of the smear layer. Furthermore, a homogeneous distribution of sclerotic dentin cannot be guaranteed, even with careful sample selection, which could have an effect on dentin penetration. The findings of this study are consistent with those of previous studies. There were notable disparities in sealer penetration at the 5 mm level between the groups. The maximum sealer penetration was observed when EDTA was used as the final irrigant, followed by Tulsi extract applied as an irrigant. At a 5 mm depth, the enhanced penetration of sealers due to the application of EDTA is particularly significant, as it allows for more effective sealing of the root canal system, thereby reducing the risk of reinfection and improving treatment outcomes. Although tulsi extract has been explored for its potential benefits in endodontic treatments, it does not demonstrate the same level of efficacy in enhancing sealer penetration as EDTA. Literature suggests that natural extracts such as tulsi may not sufficiently disrupt the smear layer or enhance dentinal permeability to the same extent as EDTA (26). This is crucial because the effectiveness of a sealer is highly dependent on its ability to penetrate the dentinal tubules, which is influenced by the properties of the irrigants used during the cleaning and shaping of the root canal (26). Moreover, confocal laser scanning microscopy (CLSM) in various studies has consistently shown that sealers achieve greater penetration depths when EDTA is employed, particularly at the 5 mm level, compared to other methods or natural extracts (27, 28). For example, one study indicated that the penetration depth of sealers was significantly greater in samples treated with EDTA than in those treated with alternative irrigants, including natural extracts (28).

Conclusion:

When used as the final irrigant in root canal disinfection, tulsi leaf extract produced results similar to those of 17% EDTA in terms of smear layer removal and sealant

penetration. Tulsi, therefore, has the potential to be combined with NaOCl as an adjunct final irrigant.

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Author's Contribution:

Dr. Syed Ashar Imtiaz: Idea and Concept of study
Dr. Urooj Musheer: Data analysis and interpretation
Dr. Hassan Khursheed: Data collection and final Proofing of study
Dr. Salman Ahmed: Writeup and alignment of final manuscript
Dr. Bilal Ansari: Proof reading, Referencing and final approval