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Research Article

Effect of Different Irrigant Solutions on Root Dentine Microhardness: An *In Vitro* Study

Tenzin Rapgay*, Preeti Gupta, Hitesh Gupta, Arvinder Singh and Anjula Jain

Department of Conservative Dentistry and Endodontics, HIDS, Himachal Pradesh, India

***Corresponding Author:** Tenzin Rapgay, Department of Conservative Dentistry and Endodontics, HIDS, Himachal Pradesh, India.

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Abstract

Aim: To evaluate and compare the effect of QMix, Tea tree oil, *Tamarindus indica*, Green tea extract and 17% EDTA on root dentine micro-hardness.

Methodology: Sixty freshly extracted human single rooted premolars were selected and divided into six groups and subjected to various treatments as follows: Group 1-Q mix, Group 2-Tea tree oil, Group 3 - 5% *Tamarindus indica,* Group 4 - 5% Green tea, Group 5 - 17% EDTA and Control group-normal saline. Each group was immersed in their solutions for 5 minutes and then subjected to Vickers micro-hardness testing.

Statistical analysis: Results were subjected to one way Anova and Tukey's test.

Results: Maximum reduction in micro-hardness was seen in the EDTA group, followed by Qmix and then Tamarindus indica groups. Tea tree oil group and Green tea groups did not show significant reduction in microhardness. Least reduction was seen in the control group saline.

Conclusion: EDTA induced maximum reduction in root dentin microhardness, followed by Qmix and Tamarindus indica. There was no significant reduction by Green tea, Tea tree oil and saline ($p > 0.05$) Tamarindus indica caused significantly less reduction than EDTA ($p < 0.001$).

Keywords: Qmix; Tea Tree Oil; Tamarindus indica; Green Tea; EDTA; Micro-Hardness

Introduction

Success in endodontic therapy depends on chemo-mechanical debridement of the root canal system through the use of instruments and effective irrigant solutions [1]. Mechanical instrumentation of the root canals produces a smear layer composed of organic and inorganic substances such as dentin particles, necrotic debris, and odontoblastic processes. Effective cleaning of the canal system requires the use of irrigation solutions throughout during instrumentation which serve variety of purposes including flushing of debris, antibacterial action, tissue dissolution, cleaning and chelating action to remove the smear layer [2].

Different chemicals have been used to remove smear layer. Most commonly used is 17% EDTA followed by sodium hypochlorite [3]. Qmix is a novel endodontic irrigant for smear layer removal with added antimicrobial agents. It contains EDTA, CHX, and a detergent Tween-80. EDTA is a chelating agent. Chlorhexidine is an antibacterial agent and has a high substantivity. Detergent i.e. Tween 80 is added to reduce the surface tension as it helps the irrigant to penetrate deeper and to lift up broken particles of smear layer by its frothing action [4].

The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives [5]. Green tea polyphenols is the traditional drink of Japan and China and is prepared from the young shoots

of Camellia sinensis. GTPs have demonstrated antioxidant, anti-inflammatory and antimicrobial properties in numerous human, animal in vitro studies. Green tea contains flavonoids that inhibit the growth of bacteria associated with tooth decay. Tea also contains natural fluoride, which may be helpful in preventing dental caries [6].

Tamarindus indica has been known in traditional herbal medicine. It was suggested that pulpatamarindorum contains various organic acids such as tartrate, lactate, malonate acid, 12 - 15% citric acid, to K-bitartrate, pectin, tannin, invert glucose [7].

Tea tree oil or *Melaleuca alternifolia* is a native Australian plant, the oil of which is used in dentistry. It is an antiseptic as well as an antifungal agent [8].

It has been reported that some of the chemicals used for endodontic irrigation are capable of causing alterations in the chemical composition of dentin [9,10]. Micro-hardness determination helps to provide an indirect evidence of losing or gaining any mineral substance in the dental hard tissues [11]. Changes in the mineral content of dentin may adversely affect the sealing ability and adhesion of dental materials such as resin based cements and root canal sealers to dentin [12].

Materials and Methods

Preparation of material

Qmix 2 in 1	It is manufactured by Dentsply Tulsa specialities. It contains 17% EDTA, 2% chlorhexidine and tween 80. No preparation required.
17% EDTA	17% EDTA was freshly prepared by using EDTA with the following composition: Disodium salt of EDTA (17g) Aquadest (100 ml) 5M sodium hydroxide (9.25 ml)
5% Green tea extract	It was supplied by Tetrahedron beverages Pvt. Ltd. Kakkalur, Tamil Nadu, India. 5g of green tea extract was mixed with 100 ml of sterile boiling water for 5 minutes and filter sterilized.
Tea tree oil (Melaleuca alternifolia)	It was taken from Falcon lab. Bengaluru, which was prepared by steam distillation of the foliage of Melaleuca alternifolia. It was then prepared to have miscibility in 85% (v/v) ethanol at 20^0C, to get a concentration of 2% by volume.
5**% Tamarindus indica:**	It was supplied by A.K.J.K. Gaum, Karnataka. 5g Pulpa tamarindo'rum was added to 100 cc of sterile aquabidest and mixed using magnetic stirring until the pulpa was homogeneously dissolved and mixed. It was then centrifuged at 250 rpm for 15 minutes. The Tamarindus indica supernatant was filtered using millipore 0.45 mm.

Specimen preparation: 60 straight single rooted premolars, freshly extracted from adult patients were collected for the study. Soft and hard tissues were removed. The teeth were radiographed to confirm the presence of a single canal. The samples were decontaminated by immersion in 5.25% sodium hypochlorite for 30 mins and thereafter stored in sterile saline solution at room temperature throughout the study. The crowns of the teeth were sectioned at the cemento-enamel junction. The root canals were enlarged upto master apical file 40k using step back technique and recapitulated using distilled water as an intracanal irrigant during instrumentation. Each root was sectioned into buccal and lingual segments. One half of each specimen was embedded into acrylic resin so that the dentin surface were exposed (Figure 1).

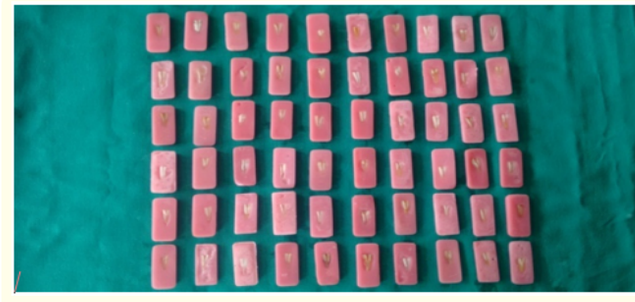

Figure 1: Samples mounted in acrylic resin.

The 60 specimens were randomly divided into six groups. Baseline values for microhardness for all the specimen were recorded before application of irrigants. The specimens were treated with irrigant solutions as follows:

- Group 1: Qmix for 5 minutes
- Group 2: Tea tree oil for 5 minutes
- Group 3: 5% *Tamarindus indica* for 5 minutes
- Group 4: 5% Green tea extract for 5 minutes
- Group 5: 17% EDTA for 5 minutes
- Control group: Saline for 5 minutes (Figure 2).

At the end of the treatment, the specimens were rinsed with 30 ml of saline and dried with sterile paper. The microhardness was measured after treatment in the same way as the baseline measurements.

Micro-hardness testing: The specimens were mounted on Vickers microhardness tester (Miyoto, Japan).

The indentations were made with Vicker's diamond indenter at three points for each sample; on the cervical, middle and apical thirds. The indentation was made on the dentin surface approximately 0.5 mm from the root canal space. Each measurement was

carried out using 200g load for 15s, oriented perpendicularly to the root surface.

Figure 2

Figure 3: Specimen mounted on Vickers.

Results

Pretreatment values ranged from 50.43 to 55.27. No significant difference in baseline microhardness values were seen among the groups. Post treatment changes in microhardness values are shown in table 1. Mean reduction was highest in the Group 5 (EDTA), followed by Group 1 (QMix) and then Group 3 (*Tamarindus indica*). Control group saline showed the least reduction in microhardness. Group 2 (Tea tree oil) and Group 4 (Green tea extract) showed no significant reduction in microhardness values ($p > 0.05$). Figure 4 is a schematic diagram showing changes in microhardness values for the test groups.

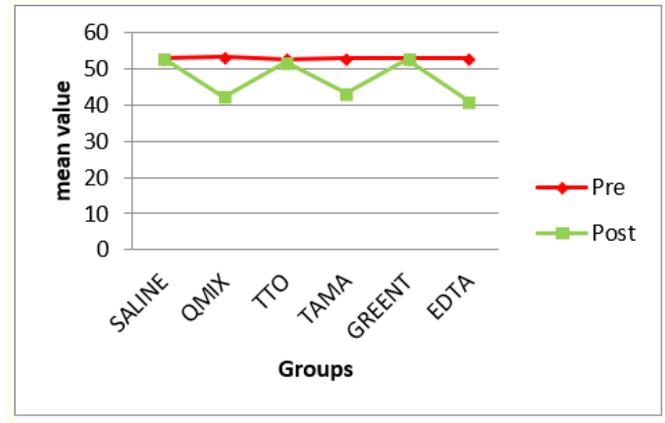

Figure 4: Schematic diagram showing changes in microhardness values for the test groups.

Group	N	Mean	SD	Minimum	Maximum
1 Qmix	10	11.091	0.661	10.01	12.07
2 TTO	10	0.703	0.409	0.04	1.36
3 TAMA	10	9.55	0.495	8.47	10.17
4 GreenT	10	0.319	0.325	0.00	0.94
5 EDTA	10	12.003	0.910	10.50	13.60
Control	10	0.119	0.215	0.00	0.67
Total	60	5.630	5.372	0.00	13.60

Table 1: Descriptive statistics showing mean reduction in microhardness post treatment with various irrigants.

Tukey's multiple comparison post hoc test showed that there was significant difference in the mean microhardness values of Group 5 (EDTA) and Group 3 (*Tamarindus indica*) i.e. $p < 0.001$. There was no significant difference between Group 1 (QMix) and Group 5 (EDTA) i.e. $p > 0.05$. No significant difference was seen between Control (saline) group and Group 2 (Tea tree oil) and Group 4 (Green tea) i.e. $p > 0.05$.

Discussion

It was found that the irrigant solutions can affect the microhardness of radicular dentin that consequently affects the clinical performance of endodontically treated teeth [13]. Apart from advantages of irrigating solutions such as flushing out debris, disinfection, smear layer removal, and lubricating dentinal walls, root canal irrigants may induce adverse changes in physical properties of dentin, including the microhardness [14]. Although a reduction in micro-hardness facilitates the instrumentation throughout the

root canal, it may also weaken the root structure. Microhardness determination can provide indirect evidence for losing or gaining any mineral substance in the dental hard tissues [12].

In the present study, all specimens were subjected to a 5 minute contact with the test solutions. Currently, there is a lack of consensus on the duration a decalcifying agent must be in contact with the root canal to adequately remove the smear layer [15]. As performed herein, De Deus., *et al.* (2011) limited the contact time of three chelator solutions (EDTA, EDTAC and citric acid) to 5 minutes, stating that this duration is more realistic in terms of clinical practice [16]. Other researchers have suggested extending the application time to 10 - 15 minutes to obtain optimal results. It has also been reported that EDTA can remove smear layer in 1 minute. In addition to contact time, the concentration of the irrigant solutions needs to be considered as another determinant in the post treatment microhardness values of dentin [17].

Microhardness of dentin may vary considerably between teeth, so in the present study the microhardness measurement was performed for each sample at baseline and after treatment with irrigation solutions to establish a reasonable evaluation for the effect of the irrigant solutions on the dentin surface. Post-treatment indentations were performed on each sample at same areas that were at symmetrical constant points of the baseline for both sides of the root canal to make evaluation of the tested irrigant [18].

Microhardness measurement was performed in three points at coronal, middle and apical third of the root canal dentin. Mean Vicker hardness number (VHN) was calculated for each specimen. The microhardness of dentin depends on the tubular density which varies from an area to another on the root dentin surface. Therefore, the current study design followed Pashley., *et al*. (1981), who stated that the tubular density affects microhardness, as the tubular density increases dentin microhardness decreases [19].

Distilled water was used initially as an irrigant solution for microhardness specimens because it has no effect on dentin surface, thus not considered as a variable which might affect the results. This followed by application of endodontic irrigation solutions on root canal dentin surface for 5 minutes in accordance with study by De Deus., *et al* [15].

Selection of Vickers microhardness tester over Knoop hardness tester was due to the suitability and practicality of Vickers test for evaluating surface changes of deeper dental hard tissues. Knoop hardness tester is used for superficial dentin at 0.1 mm rather than for deep dentin [20].

A possible limitation of the current study is that the experiments were performed at room temperature and not body temperature. Additionally, the volume of the irrigant in a root canal clinically is small compared with the immersing root dentin in irrigating solutions.

However, standardized circumstances for all study groups allowed for comparable results.

The present study revealed that QMix, 17% EDTA and 5% *Tamarindus indica* irrigation solutions decreased dentin microhardness. Green tea extract, tea tree oil and saline did not decrease the microhardness significantly.

This finding is in accordance with Saleh and Ettman (1999), who evaluated the effect of NaOCl and EDTA on the microhardness of root canal dentin and reported that both solutions decreased the microhardness of root dentin but EDTA irrigation induced more reduction [21].

Sayin., *et al.* (2007), also evaluated the effect of EDTA, EGTA, EDTAC and tetracycline HCL with and without subsequent NaOCl treatment on dentin microhardness and found that all tested solutions reduced dentin microhardness significantly. They concluded that significant alteration in dentin hardness after the irrigation treatment indicates potent direct effects of these chemical solutions on the components of dentin structure [3].

Flavia emi., *et al.* (2017) evaluated that QMiX and 17% EDTA reduced dentin microhardness at a greater depth when compared to 10% CA and 1% PA. Additionally, and differently from EDTA 17%, QMiX did not cause dentin erosion [30].

The present study confirms the finding of Eldeniz., *et al.* who stated that EDTA and citric acid (Tamarindus indica in the present study) solutions had the strongest effect on reducing dentin microhardness compared with NaOCl. The effect of EDTA was statistically similar to that of citric acid. In the present study the reduction in microhardness was statistically less for Tamarindus indica when compared with EDTA [22].

De Deus., *et al.* confirms the findings of the present study that citric acid was the less effective in reducing dentine microhardness than EDTA [15].

This is in contrast to studies by Scelza., *et al*. (2004) employing atomic absorption spectrometry; there was no difference between 10% citric acid and 17% EDTA regarding their ability to remove calcium ions in the present study [23]. EDTA induced the great-

est reduction in root dentine microhardness, followed by QMix and then *Tamarindus indica.*

Tea tree oil and Green tea did not show significant reduction in root dentine microhardness.

An insignificant difference in microhardness values were seen between the EDTA, QMix and between *Tamarindus indica* and Qmix groups. As QMix contains EDTA in its composition along with Chlorhexidine and a detergent, the effect of QMix on root dentine could have been almost similar to EDTA. There was a significant difference between *Tamarindus indica* and EDTA groups.

The present study confirms the findings of Kara TA., *et al.* (2015) that there was no significant difference between QMix and 17% EDTA +2% CHX but these groups presented significant dentine microhardness reduction [24].

Tamarindus indica has been known in traditional medicine. Tjitrosoepomo suggested that pulpatamarindorum contains organic acids such as tartrate, lactate, malonate acid, 12 - 15% citric acid, K-bitartrate, pectin, tannin, invert glucose. A material with an acid nature could contribute to demineralization [25]. Therefore in the present study, *Tamarindus indica* also reduced microhardness of dentine but it reduced it less than EDTA. This could be due to it being herbal in nature.

The present study revealed that tea tree oil and green tea extract did not reduce the microhardness of dentine significantly. Nor did it increase the microhardness of dentine. In contrast to this study, Mirkarimi M., *et al.* (2012) found that green tea increased the microhardness of eroded dentine and improved the eroded texture [26].

Demeule., *et al.* reported that different biologically active components from natural products such as catechins isolated from green tea, especially epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) inhibit matrix metalloproteinase (MMPs) activities [27].

Kato., *et al.* reported that green tea reduces dentin wear under erosive conditions [28].

Studies by Soukoulis S revealed that the tea tree oil decreased gingival inflammation. The components of TTO are known to decrease inflammation in *in vitro* and *in vivo* settings [29].

The present study did not reveal any increase in the microhardness of the tea tree oil group.

Conclusion

EDTA induced maximum reduction in root dentin micro-hardness, followed by Qmix and *Tamarindus indica*. There was no significant reduction by Green tea, Tea tree oil and saline ($p > 0.05$). *Tamarindus indica* caused significantly less reduction than EDTA ($p < 0.05$). Further studies need to be done on more herbal products.

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