

## A COMPARATIVE STUDY FOR DIFFERENT INTRACANAL MEDICAMENTS ON ROOT CANAL DENTINE MICROHARDNESS: AN IN VITRO STUDY

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### **ABSTRACT**

**Introduction:** This study was done to evaluate the effect of different intracanal medicaments on root canal dentine microhardness.

**Method:** Three intracanal medicaments were used in this study: Propolis, Calcium hydroxide and Chlorhexidine to evaluate their effects on microhardness of dentine in single rooted, human anterior teeth with a single canal. The medicaments were placed for three, seven and twenty one days. Samples were divided into three groups according to the intracanal medicaments used. Vickers microhardness tester was used to measure the surface hardness of the dentin of all specimens.

**Results:** The comparison of microhardness between the three intracanal medicaments on root canal dentin over the study period showed statistically significant difference ( $p < 0.001$ ) in all groups where chlorhexidine showed the highest mean values, followed by propolis and finally calcium hydroxide group. The largest decrease was demonstrated between day 7 and day 21 time period.

**Conclusions:** All the three internal medicaments decrease dentin microhardness in endodontically treated-teeth by time.

**KEYWORDS:** Propolis (PRP), Chlorhexidine (CHX), Calcium hydroxide ( $\text{Ca(OH)}_2$ ), microhardness, intracanal medicaments, root canal.

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## INTRODUCTION

Irrigation used during root canal preparation and intracanal medicaments could affect the physical and mechanical properties of radicular dentin negatively. Irrigation is presently the method of choice for the removal of tissue remnants and debris during root canal instrumentation<sup>(1)</sup>. Till today, no irrigant can completely eradicate all organic and inorganic materials in the canal and at the same time add a residual substantive antimicrobial effect to the canals. It should be also effective against the *Enterococcus Faecalis*, thus the combination of other solutions is mandatory to achieve the desired effect<sup>(2,3,4)</sup>.

Chemomechanical preparation of the root canal system should remove most of the irritants inside the root canal. However, total debridement is impaired because of the complex root canal anatomy due to the presence of accessory canals, fins and other communications within the main root canals<sup>(5)</sup>.

Sodium hypochlorite (NaOCl), which is an antimicrobial agent with tissue-dissolving action, is considered the gold standard irrigant to be used in root canal treatment. Despite of its germicidal actions, using it in high concentration might be cytotoxic to periapical tissues<sup>(2,3,4)</sup>; and will affect the dentin structure regarding its physical, chemical and adhesive properties<sup>(2,6,7)</sup>.

Many materials have been introduced as intracanal medicaments. Chlorhexidine gluconate (CHX) has been widely used as an intra-canal medicament in the treatment of infected root canal systems as it has a broad spectrum antimicrobial activity, lower toxicity compared to other medicaments, and water soluble<sup>(5,8,9,10)</sup>. It also has an important feature in which it provides antimicrobial substantivity and improves the adhesibility to dentine<sup>(2,3,4)</sup>. Using 0.2% chlorhexidine as an irrigation solution showed no effect on the microhardness of root canal dentin<sup>(11,12)</sup>. On the other hand, irrigation with 2% chlorhexidine solution reduced the dentin microhardness at both depths (500  $\mu$ m and 1000  $\mu$ m)<sup>(11)</sup>.

Additionally, calcium hydroxide is the most

commonly used as an intracanal medicament. It possesses a high pH which alters the biological properties of the lipopolysaccharides in the bacterial cell wall of gram-negative species, which inactivates the membrane transport mechanisms. However, dentine provides buffering action that neutralizes its action at deeper areas of dentinal tubules resulting in decreasing its effect on microorganisms<sup>(13,14,15)</sup>. Thus, the search for other alternative led to the discovery of newer intracanal medicaments<sup>(13)</sup>. The use of calcium hydroxide and others was found to reduce the dentin flexural strength, microhardness and root fracture resistance significantly<sup>(16)</sup>.

Recently, a new product which is found in nature, propolis (bee glue), a flavonoid-rich resinous substance obtained from the beehives, has antioxidant, anti-bacterial, anti-viral, antifungal, and anti-inflammatory activity, was introduced<sup>(5)</sup>.

Propolis when used as an intracanal medicament was found to adversely affect the fracture resistance of root canal dentin<sup>(5)</sup>. Yet, propolis is considered ten times less toxic to the cells than calcium hydroxide<sup>(13,17)</sup>. Recent studies<sup>(13,18,19)</sup> reported that propolis has more antibacterial effect against resistant microorganisms as well as its biocompatibility to the periapical tissues than existing medicaments used inside the canal. However, very few studies examined its effectiveness when used as an intracanal medicament, and literature lacks further investigations to prove its effectiveness<sup>(13)</sup>.

Therefore, the objective of this study was to evaluate the effect of propolis, chlorhexidine and calcium hydroxide, when used as intra-canal medicaments, on the micro-hardness of root canal dentin.

## MATERIALS AND METHODS

### Samples Selection

Thirty six extracted upper central incisors were used in this study. They were obtained from MIU teeth bank after having ethical approval number (IRB Number: MIU-IRB-2324-249). Teeth were

decapitated using a high-speed bur with water cooling and specimens were equally classified into three groups (twenty-one halves each) which were assigned for propolis, calcium hydroxide and chlorhexidine groups respectively.

### Materials

- **Propolis:** Raw powder microform was purchased from the manufacturer (IMTENAN, Cairo, Egypt). 1.5 grams of propolis were dispersed in 5 ml of absolute ethanol. 0.5gm of HPMC\*\* was sprinkled gradually and gently onto the solution under a temperature of 40°C with vigorous stirring at 1000 rpm by using a hot plate and stirrer until reaching a homogenous gel. 100 grams of raw propolis were placed in a 500 ml flask with of 80% ethanol, which was placed on a hot plate and stirrer for 7 days. This procedure produced a filtered solution called ethanolic extract.
- **Calcium hydroxide:** Metapex calcium hydroxide was used (Metapex, META BIOMED CO., LTD., Republic of Korea).
- **Chlorhexidine:** 2% chlorhexidine solution was used (CHX-Plus, Vista Dental Products, WI, USA).

### Sample size calculation :

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference between propolis and chlorhexidine when used as intracanal medicaments as compared to calcium hydroxide on dentin microhardness. By adopting an alpha level of (0.05) a beta of (0.2) i.e., power=80% and an effect size (f) of (0.586) calculated based on the results of a previous study with the same primary outcome which is the dentine microhardness. The predicted sample size (n) was found to be a total of (21) samples (i.e., 7 samples per group). Sample size calculation was performed using G\*Power version 3.1.9.7

### Préparation

A patency file of size 15 was used for all specimens before preparing the canals using three Hyflex edm files, reaching size 25 with taper of 6%. 5.25% NaOCl and saline were used as irrigants during canal preparation before being dried by absorbing paper points. The three intracanal medicaments were then placed in their assigned groups, according to their testing periods. Samples were rinsed using distilled water before being dried and tested.

### Grouping

After treatment of canals, specimens were classified into three groups depending on the intracanal medicament applied:

- **Group A:** propolis.
- **Group B:** Metapex calcium hydroxide.
- **Group C:** 2% chlorhexidine.

### Evaluation

Micro hardness of the various groups was tested after three, seven and twenty-one days using Vickers Hardness Tester (Matsuzawa® MHT2, High Quality Microhardness Tester, Matsuzawa SEIKI Co; Ltd, Tokyo, Japan). A Vickers diamond indenter was used to make indentations with a minimum of three widely similarly positioned points. They were made on the top surface of the coronal and middle thirds of each specimen using 300 g and a time of 20 seconds. The three values were averaged to produce mean hardness value for each specimen. The measurements were converted into Vickers numbers. (Equation)

### Statistical analysis:

The data were analyzed statistically using one-way analysis of variance (ANOVA), and the comparison of means was conducted using Tukey's multiple comparison test. Testing was performed at the 95% confidence level (p 0.05).

## RESULTS

The results of our study (Table 1) showed that there was a statistically significant difference between all groups throughout the different time intervals. Also, there was a statistically significant difference between each time interval within each of the three tested groups.

The results of our study showed that the difference between all groups throughout the different time intervals was statistically significant. At 3 days, microhardness (Vickers units) was the least in calcium hydroxide group ( $54.53 \pm 3.43$ ), followed by propolis group ( $61.91 \pm 3.43$ ) and the greatest values were in chlorhexidine group ( $65.21 \pm 3.02$ )

at ( $p < 0.001$ ). At 7 days, the values decreased with significant difference ( $53.91 \pm 3.56$ ), ( $61.88 \pm 3.18$ ), and ( $65.11 \pm 3.07$ ) for calcium hydroxide, propolis and chlorhexidine groups respectively at ( $p < 0.001$ ). Comparison showed that the chlorhexidine group had the highest microhardness values while the calcium hydroxide group had the least microhardness values. At 21 days, microhardness values continued to decrease to reach ( $41.07 \pm 3.2$ ), ( $58.11 \pm 3.59$ ) and ( $60.14 \pm 2.68$ ) for calcium hydroxide, propolis and chlorhexidine gluconate groups at ( $p < 0.001$ ). Comparison over time within the same group showed that all groups decreased over time with statistically significant difference ( $p < 0.001$ ). The largest drop was between day 7 and day 21.

TABLE (1) Comparison of microhardness between the study groups over the study period

	Group A	Group B	Group C	
	Propolis	Metapex Calcium hydroxide	2% Chlorhexidine	<i>P value</i>
<b>3 days</b> (Mean $\pm$ SD)	$61.91 \pm 3.43$	$54.53 \pm 3.43$	$65.21 \pm 3.02$	$< 0.001$
<b>7 days</b> (Mean $\pm$ SD)	$61.88 \pm 3.18$	$53.91 \pm 3.56$	$65.11 \pm 3.07$	$< 0.001$
<b>21 days</b> (Mean $\pm$ SD)	$58.11 \pm 3.59$	$41.07 \pm 3.2$	$60.14 \pm 2.68$	$< 0.001$
<i>P value</i>	$< 0.001$	$< 0.001$	$< 0.001$	

## DISCUSSION

The current study determined the effect of three intracanal medicaments; propolis, calcium hydroxide and chlorhexidine, on dentine microhardness in single canalled endodontically treated anterior teeth to simulate a similar clinical scenario.

The intracanal medicaments presented are considered to be widely used in endodontics. Root canal dentin microhardness testing was performed using Vickers indenter method<sup>(1, 20, 21, 22)</sup> that causes negligible damage to the dentin surface<sup>(23)</sup>. It can

provide an indirect proof of mineral changes in the teeth hard tissues<sup>(1,12)</sup> which plays a great role in evaluating surface changes when tissues are treated with chemical agents<sup>(12, 20, 21, 22, 24, 25)</sup>.

Previous researches<sup>(12, 23)</sup> demonstrated that there was an opposite relation between dentin microhardness and the tubular density. Moreover, the amount of hydroxyapatite and the degree of mineralization in the inter-tubular substance are important factors in determining the natural hardness profile of dentin structure.

Also, previous studies demonstrated,<sup>(2,3,26)</sup> that carious affected dentin is less hard one third to one fourth than non-carious sound dentin, showing that microhardness is very sensitive to tooth composition. Microhardness is also usually in relation with other mechanical properties such as, modulus of elasticity, fracture resistance and the respective bond strength<sup>(2)</sup>. Therefore, at that point we can predict the adhesive behavior at the dentin- restoration interfaces<sup>(2, 23, 27, 28)</sup>. Although a decreasing microhardness facilitates the mechanical preparation throughout the canal, it also weakens the root structure, as a consequence, root canal-treated teeth become more prone to fracture<sup>(2, 27, 28)</sup>.

There are multiple types of irrigants introduced & studied in literature where they showed that all irrigants produced a decrease in dentine microhardness and an increase in the brittleness of the dentin and consequently the root canal-treated teeth are more subject to fracture<sup>(29, 30)</sup>

It was found earlier<sup>(1,31,32)</sup> that chlorhexidine when used as an irrigant, it did not affect the root canal dentin microhardness, and it was emphasized that 0.2% chlorhexidine gluconate irrigation solution seems to be an appropriate irrigation solution as it has less harmful effect on dentine microhardness.

It was also claimed that CHX had lower effect on the dentinal structure compared to EDTA or NaOCL<sup>(2,33,34,35)</sup>, this was attributed to the fact that CHX has neither chelating properties, nor tissue dissolving ability<sup>(2,3,4)</sup>.

However, the results of our study contradicted those findings, where there was a statistically significant decrease in microhardness of the group treated by 2% chlorhexidine gluconate ( $p < 0.001$ ) at the different time intervals. This contradiction might be due to difference in exposure time and concentration of chlorhexidine gluconate.

Our findings came along with those of Oliveira et al.<sup>(11,29)</sup> who concluded that 2% chlorhexidine gluconate significantly caused a reduction in root canal dentine microhardness at 500 and 1000 $\mu$ m

from the dentine surface facing the pulp<sup>(29)</sup>. Microhardness studies showed no difference in the hardness of root dentin when canals were neither filled with calcium hydroxide nor left open to the oral environment. It was reported that the inner dentin walls, or open pulps, had no changes in the microhardness when the cavity was filled with calcium hydroxide<sup>(36,37)</sup>. Yet, the results of this study showed a decrease in the values of microhardness of root canals which used calcium hydroxide as an internal irrigant material gradually from 3 days and up to 21 days which agrees with the results of White et al.<sup>(38)</sup> who concluded that root dentin micro-hardness decreased after 5 weeks of exposure to calcium hydroxide and agrees with the conclusions of Rosenberg and White. Sahebi et al. and Andreasen who confirmed that Ca(OH)<sub>2</sub> had a weakening effect on dentin<sup>(2)</sup>. It is worth mentioning that the dentine strength depends on the collagenous fibrils link and hydroxyapatite which can be affected by the strong alkaline pH of Ca(OH)<sub>2</sub> which might reach 11.8. It leads to the collapse of dentine structure as carboxylate and phosphate groups get denatured<sup>(39)</sup>. The cause of disruption could also be due to neutralization, dissolution of proteoglycans and acid proteins which play an important role in binding the collagen network and the hydroxyapatite crystals in dentin<sup>(39,40)</sup>. Andreasen evaluated strength of dentine for a certain period of time in which the results showed a decrease in the strength of the root after application of Ca(OH)<sub>2</sub> for 10 days, which was around 15% decrease in the root hardness and reached 50% within a year<sup>(39,41)</sup>.

Moreover, Parashar et al. and Yassen et al<sup>(42,43)</sup> reported reduction in root dentin microhardness when they used calcium hydroxide as an intracanal medicament. They also explained another cause of this reduction which could be due to ability of the medicament to reach into the intrafibrillar structure of mineralized collagen fibrils as they have a minute molecular size, which led to changes in the three-dimensional configuration of tropocollagen, leading to a decreased dentine microhardness.<sup>(42, 44)</sup>

Nowadays, the recent trend is using natural products as a cure for different diseases. Propolis is a natural substance. The healing properties of propolis was first discovered by the Egyptian and Greek civilizations. Hippocrates, the founder of Modern Medicine, also used it for healing sores and ulcers. Propolis is a resinous material where honeybees collect them from plants. This collected material is mixed with wax and other substances. Honeybees used it to seal the unwanted open spaces in their hive. Propolis is constituted of pollen, resin and balsams, and other components which are minerals, amino acids, vitamins A, B complex and phenols. The active biochemical substance called bioflavonoid, and aromatic compounds. It is commonly brown in color. Flavenoids are famous plant compounds, which have antibacterial, antiviral, antifungal, antioxidant besides having anti-inflammatory characteristics. They are one of the most common groups of polyphenolic compounds used in human diet and found in plants. In the dental field, current research involving propolis highlights its antimicrobial and anti-inflammatory properties particularly in caries progression, oral surgery, oral pathology, periodontics, and endodontics.

As for the effect of use of propolis solution as an irrigant, previous studies proved that 4% propolis reduced the microhardness of root canal dentin significantly comparing it to 0.2% chitosan<sup>(29)</sup> which agrees with our findings in which it was found that when propolis was used as an intracanal medicament the root-canal dentine microhardness decreased throughout the study period in which the largest decrease was observed between day 7 and day 21.

It is claimed that the propolis ethanolic extract (EEP) is one of the wealthiest sources of phenolic acids and flavonoids<sup>(5,45)</sup>. Phenolic acids are weak acids that might be adsorbed by hydroxyapatite molecules<sup>(5,46)</sup>. After adsorption, the mechanism of the reaction might be surface complexation with hydroxyapatite<sup>(5,46)</sup>. Surface complexation is a method of chemical reactions (equilibrium

reactions) that might take place at the interface between the solution and a mineral surface<sup>(5,47)</sup>. This might be the cause for the noteworthy reduction in microhardness after the use of propolis as an intracanal irrigation<sup>(5)</sup>.

However, these findings contradict those who found that moringa-containing tooth-pastes and propolis did not significantly affect the surface microhardness when brushed on dentin with and without acidic challenge. This might be related to the chemical formula of the used toothpaste. Also, it was found that, propolis has the ability to obliterate dentinal tubules and to resist acid attack<sup>(48)</sup>.

The contradicting results regarding the effect of propolis on microhardness could be related to the different botanical sources, preparations, extraction and methods of delivery of propolis.

Under the limitations of this investigation, we can conclude that, all intracanal medicaments used reduced dentine microhardness. The least destructive effect was obtained by chlorhexidine gluconate followed by propolis and finally the calcium hydroxide.

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