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Effect of Nano Forms of Propolis and Antibiotic Pastes as canal Medicaments on Radicular Dentin Microhardness (In-Vitro Study)

Aya G. Abd El Nasser[1], Abeer A. El Gendy[2], Amr A. Bayoumi[3]

ABSTRACT

Background: In order to increase affected tooth' life span, a successful endodontic treatment is required. Although mechanical preparation and proper irrigation play an important role in root canal treatment's success rate, yet application of intracanal medicament is still a major key in obtaining an endodontically favourable outcome. ***Objectives:*** to asses the change in dentin micro-hardness after the use of Nano-Propolis, Double Antibiotic Paste (DAP) and Triple Antibiotic Paste (TAP) as an intracanal medicament by Vickers Test. ***Materials and Methods:*** Forty-two extracted human single rooted lower premolars were collected and longitudinally sectioned in a bucco-lingual direction into two halves using a low-speed saw with a diamond disc. One half received the canal medicament while the other half was used as a control for dentin microhardness measurements. Teeth were divided into 3 different experimental groups equally according to the type of testing material; Propolis, Double and Triple Antibiotic Paste. Each group was further divided into 2 equal subgroups (7 each) according to the used medicament particle size either nano or micro sized. After two weeks, the difference between Propolis, DAP and TAP in nano and micro forms was investigated by Vicker's test to evaluate the micro-hardness. ***Results:*** A statistically significant difference was found in the median % reduction in radicular dentin microhardness. ***Conclusion:*** DAP, Nano TAP, Propolis, Nano DAP and TAP when used as intracanal medicaments adversely affects radicular dentin microhardness percentage reduction respectively, while Nano Propolis is considered safe as intracanal medicament regarding microhardness percentage reduction of root canal dentin.

Keywords: Propolis, DAP, TAP, Nano-form, Microhardness.

INTRODUCTION

A successful endodontic treatment is considered as a challenge nowadays; as it is facing a tremendous fight against ongoing mutant microbial species. Therefore, probing

1. Postgraduate Student, Endodontic Department, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt.
2. Professor of Endodontics, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.
3. Associate Professor of Endodontics, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt.

for medical solutions to eliminate such bacteria is our major scope as endodontists. The purpose throughout the whole endodontic root canal treatment is to apply proper canal disinfection, either through eradication of bacteria, removal of diseased tissues, or prevention of future recontamination. This is carried out through the use of irrigation and intracanal medication during canal preparation.[1,2] The use of intracanal medicaments between visits in endodontic therapy is considered to be a beneficial asset as these medicaments relief/reduce the pain, reduce the inflammation of the pulp and periapical tissues, and contribute to healing; result in fewer flare ups. They also maximize the chemo-mechanical disinfection, destroys all types of microorganisms with the exception of Enterococcus faecalis. In addition, they preserve the inertness of the canal and debris neutralization, act as a barrier against temporary filling leakage, and finally dry the canals.[3,4]

Propolis is one of the most valuable natural products in the medical field and recently in dentistry, specifically in endodontics. Propolis is a resinous material collected by a special type of honey bee called Apis Mallifera which is then derived by plants, and could vary from yellow green to red and dark brown color. It consists of bioflavonoids which is considered to be the active ingredient of Propolis as it is the reason behind its anti-inflammatory, anti-bacterial and antioxidant action.[5]

Using a single type of antibiotic as an intracanal medicament in canal infection is never enough to efficiently disinfect the complex radicular system that harbours polymicrobial species, which makes the mechanical cleaning and shaping alone insufficient, hence the use of antibiotics in order to attain a bacterial-free environment. On top of that is using a combination of different antibiotic spectrums, as in triple Antibiotic Paste (TAP), which consists of metronidazole, minocycline, and ciprofloxacin, in addition to the Double Antibiotic Paste (DAP), which has the same components but with the exclusion of minocycline, Both have been proven to eradicate radicular bacterial infection.[6,7]

Nano-materials that have been introduced in endodontics are present in the form of disinfectants as irrigants and intracanal medicaments or form of fillers as in gutta- percha, sealers and cements. These nano-materials have overcome the major side effects of the micro-sized form of endodontic materials such as cytotoxicity, decreasing dentin hardness and affecting root dentin

chemical structure. Nano particles is of 1 to 1000 nanometer in dimension, which is considered to be a very small sized particle. Any biocompatible material that is in nano form will increase the product's surface area gaining the most beneficial effect of the therapeutic agent. Nano-form intracanal medicaments will have better penetration ability into the root dentin complex and control release of the active ingredients with a higher concentration in the canal.[8]

MATERIALS AND METHODS

a. Sample Selection:

Single rooted extracted mandibular premolars were selected from Misr International University's teeth bank, with approved written consent that these teeth will be used in dental experiments. Teeth were stored in distilled water until the time of use. Teeth were decoronated to a standard 16 mm, an apical patency was checked using K-file #10 then 1mm was subtracted in order to establish an accurate working length. Mechanical preparation was done using Mpro three files rotary system with 3ml of 2.6% sodium hypochlorite irrigation with manual dynamic agitation subsequent to each filing step. Canals were rinsed with saline as a final flush and dried with paper point. The prepared samples were subjected to longitudinal sectioning resulting in buccal and lingual halves by an isomet[1]. All buccal halves were considered the experimental half **(Figure 1)**, while lingual halves were considered the control half **(Figure 2)**. Each half was mounted in acrylic resin for easy material application and machine testing. Each lumen was encircled by pink wax to preserve medicament in place.

Figure (1): Showing experimental half.

Figure (2): Showing control half.

b. Sample Classification:

Total of forty-two extracted single rooted teeth were randomly classified into three different experimental groups equally (14 each) according to the type of testing materials: group A (Propolis-PRP), group B (Double Antibiotic Paste- DAP) and group C

[1] Isomet 1000; Buehler, Lake Bluff, IL, USA.

(Triple Antibiotic Paste-TAP). Each group were subdivided into two subgroups (7 each) according to the particle size used to prepare the medicament either micro or nano sized. Subgroup A1 (Micro PRP), subgroup A2 (Nano-PRP), subgroup B1 (Micro-DAP), subgroup B2 (Nano- DAP), subgroup C1 (Micro-TAP) and subgroup C2 (Nano-TAP).

c. **Sample Preparation:**

Micro-form of raw Propolis[2] material were obtained from the manufacturer[3] in the form of powder. Dispersing 1.5gm of Propolis in 5ml of absolute ethanol. 0.5gm of HPMC[4] was sprinkled gently and gradually over the solution under mild temperature 40^{o}C with vigorous stirring ≈ 1000 rpm by using hot plate and stirrer to get homogenous gel. (100g) of raw Propolis was placed in a flask with (500 mL) of 80 % ethanol, which was placed on hot plate and stirrer for 7 days, producing a filtered solution called ethanolic extract, which was added at 1:10 ratio to distilled water in order to isolate pure Propolis particles. The suspension (isolated pure Propolis particle) was placed in an ultrasonic bath for 20-30 minutes to obtain Propolis NPs. Nano Propolis was re-dispersed in DH2O. 0.5gm of HPMC was sprinkled gently and gradually over the solution under mild temperature 40^{o}C with vigorous stirring ≈ 1000 rpm by using hot plate and stirrer in order to get nano Propolis medicament. Double Antibiotic Paste (DAP) was prepared using ciprofloxacin and metronidazole antibiotics, which were obtained in the form of capsules. The antibiotic capsules were crushed and opened producing the antibiotic in powder form. The micro-DA was dispersed in DH2O to get 1mg/ml. 0.5gm of HPMC was sprinkled gently and gradually over the solution under mild temperature 40^{o}C with vigorous stirring ≈ 1000 rpm by using hot plate and stirrer to get homogenous gel.

Nano DAP was obtained by using (ciprofloxacin, metronidazole) powder which was then loaded into chitosan nano-particles.[9] The loaded DA nanoparticles was re-dispearsed in DH2O to get 1mg/ml. 0.5gm of HPMC was sprinkled gently and gradually over the solution under mild temperature 40^{o}C with vigorous stirring≈ 100 rpm, by using hot plate & stirrer to get homogenous nano medicament, re-dispersing the loaded DA nanoparticles in DH2O to get 1mg/ml. 0.5gm of HPMC was sprinkled gently and gradually over the solution under mild temperature 40^{o}C with vigorous stirring ≈

[2] Powder, IMTENAN, Cairo, Egypt.
[3] Nano-Gate, Cairo, Egypt.
[4] Tablet Binder, Loba Chime, Mumbai, INDIA.

1000 rpm, by using hot plate and stirrer to get homogenous nano medicament.

Triple Antibiotic Paste (TAP) was prepared using ciprofloxacin, metronidazole and minocycline antibiotics, which were obtained in the form of capsules. The antibiotic capsules were crushed and opened, producing the antibiotic in powder form. The micro-DA was dispersed in DH2O to get 1mg/ml.0.5gm of HPMC was sprinkled gently and gradually over the solution under mild temperature 40ºC with vigorous stirring ≈ 1000 rpm by using hot plate and stirrer to get homogenous gel. Nano TAP was obtained by using (ciprofloxacin, metronidazole and minocycline) powder were then loaded into chitosan nano-particles.[9] Re-dispersingthe loaded DA nanoparticles in DH2O to get 1mg/ml. 0.5gm of HPMC was sprinkled gently and gradually over the solution. Under mild temperature 40ºC with vigorous stirring ≈ 1000 rpm, by using hot plate and stirrer to get homogenous nano medicament. According to the different groups, each experimental dentin half received precisely a 1ml of the assigned medicament; PRP or DAP or TAP inside the lumen. Samples with medicaments were left untouched and stored in incubator for 14 days. The incubated dentin halves were placed in 37°C and 100% humidity during the whole observation period. After two weeks, the medication was washed away using ultrasonic activation of 2.5% NaOCL irrigation for 60 seconds, with power 9 using ultrasonic file #15, according to the manufacture. Afterwards, the lumens were flushed thoroughly with 5 ml distilled water. Samples were subjected to testing; in order to check the microhardness of both control and experimental groups.

a. Microhardness testing (Vickers):

All control and experimental samples were tested using Vickers **(Figure 3)**.

Figure (3): Vickers Microhardness Tester.

Three indentations have been done at coronal, middle and apical parts. The indenter placed at 1 μm form the lumen creating indentations at the edge towards the dentin outer border. (50 g) load was applied smoothly, without impact of forcing the indenter into the test specimen. The indenter is held in place for (10) seconds.

The physical quality of the indenter and the accuracy of the applied load must be controlled in order to get the correct results. The indenters were measured via an optical microscope connected with a digital camera and an image analysis software. After the load is removed, the indentation is focused with the magnifying eye piece and the two impression diagonals were measured, usually to the nearest 0.1-μm with a micrometer, and averaged. The percentage change in radicular dentin was calculated as follows: (Microhardness post-treatment - Microhardness pre-treatment) / Microhardness pre-treatment˝ 100.

b. Statistical analysis:

The collected data were tabulated and statistically analyzed using statistical analysis, which was performed with IBM® SPSS® Statistics for Windows, Version 23.0. Armonk, NY:IBM Corp. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate both parametric and non-parametric data. ANOVA test was used to describe the parametric data followed by Bonferroni's post-hoc test when ANOVA test was significant. While for non-parametric data; Kruskal-Wallis test was used followed by Dunn's post-hoc test when ANOVA test was significant. The significance level was set at $P \leq 0.05$.

RESULTS

Comparison of the percentage reduction in radicular dentin microhardness for the six experimental groups showed that least percentage reduction was seen within Nano Propolis group (-3.6%). The highest percentage reduction was recorded in DAP group (-11.1%), while Nano DAP, Propolis and Nano TAP groups showed intermediate values (-8%, -8.3%, -9.4%) respectively. Statistically, the percentage reduction in radicular dentin microhardness values for the six experimental groups were significantly different at $P \leq 0.05$. **(Table 1) (Figure 4)**

According to coronal root level, there was no statistical significance difference between the materials, while in middle root level there was a statistically significant difference showing that DAP and Propolis had the highest statistically significant median percentage decrease in microhardness. Nano DAP, TAP and Nano TAP all showed statistically significantly lower median percentage decrease in microhardness. Nano Propolis showed the statistically significantly lowest median percentage decrease in microhardness. Also, at apical root level Nano TAP showed the statistically significantly highest median percentage decrease in microhardness. DAP, Nano DAP, TAP and Propolis all showed

Table (1): Descriptive statistics and results of Kruskal-Wallis test for comparison between percentage changes in microhardness of different materials.

Material	Coronal	Middle	Apical	Overall
	Median	Median	Median	Median
Propolis	-6.6	-13.4 ^{A}	-7.2 ^{B}	-8.3 ^{B}
Nano Propolis	-5.4	-3.2 ^{C}	-1.3 ^{C}	-3.6 ^{D}
DAP	-12.7	-12.3 ^{A}	-6.6 ^{B}	-11.1^{A}
Nano DAP	-11.7	-8.1 ^{B}	-4.5 ^{B}	-8 ^{B}
TAP	-6.3	-7.1 ^{B}	-4.1 ^{B}	-5.7 ^{C}
Nano TAP	-9.7	-8 ^{B}	-10.2 ^{A}	-9.4 ^{B}
P-value	0.276	0.016*	0.006*	0.020*
Effect size *(Eta squared)*	0.073	0.496	0.628	0.464

**: Significant at P ≤ 0.05,*
Different superscripts in the same column indicate statistically significant difference between materials.

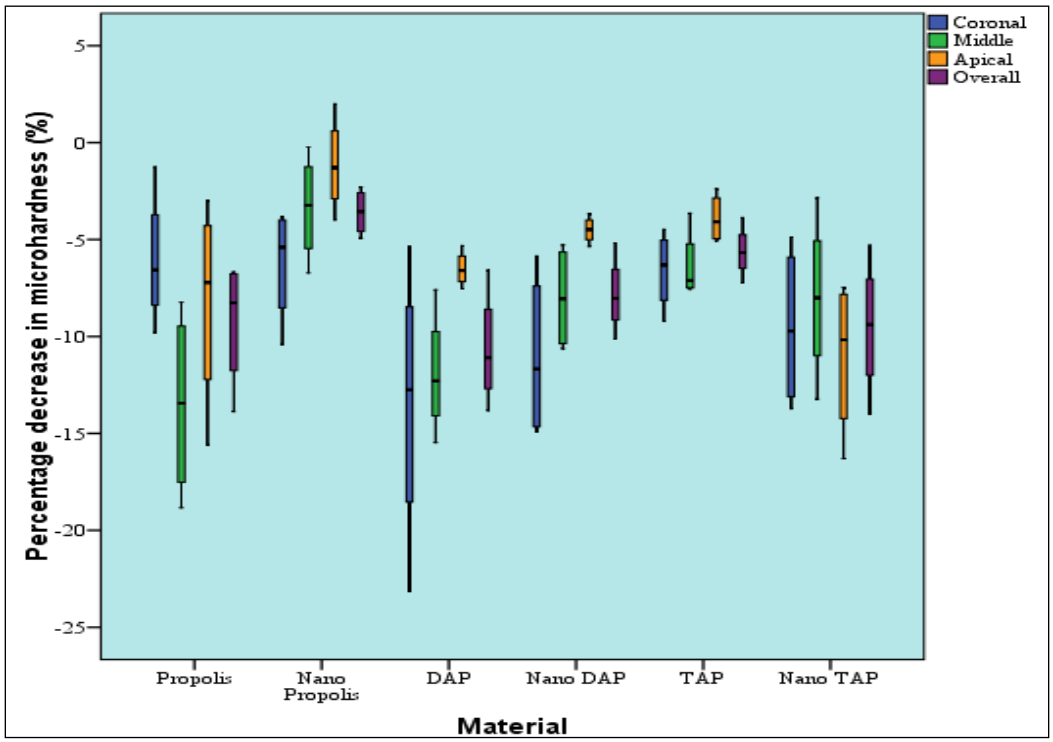

Figure (4): Box plot presenting median and range values for percentage decrease in microhardness of different materials.

statistically significantly lower median percentage decrease in microhardness, while Nano Propolis showed the statistically significantly lowest median percentage decrease in microhardness.

DISCUSSION

Endodontic treatment aims to eradicate infection, shape, and seal of the root canal system in three dimensions to prevent recurrent microbial invasion by performing a chemo-mechanical preparation to achieve an aseptic and bacterial-free environment. This is a combination of well-executed mechanical instrumentation with the help of an antibacterial chemical adjunct. Despite the evolution of instruments to shape the canals, 35% of the canal surfaces remain untouched, leaving the cleaning of the canals and elimination of bacteria strongly dependent on the adjunctive action of chemical irrigation and intracanal medication.[10]

Intracanal medicaments are considered an antiseptic agent that could be found in a chemical form like TAP and DAP, or in a natural and herbal form like Propolis; which will be introduced into the dental canals with a certain goal of eliminating microorganisms and their toxins. They are set to enhance canal disinfection through filling the canal space inter-appointment, act as a blockade against leakage through temporary restoration and prevent any source of nutrition to reach the domed bacteria leaving the canal inert, hence prohibiting any further bacterial mutation and reinfection. Also, medicaments have the ability to reduce pain by reducing inflammation[11] although it may have certain negative effect on root dentin such as crown discoloration, allergic reactions and dentin demineralization.

The purpose of this study is to investigate the effect of these material in micro and nano forms on radicular dentin microhardness before recommending its use as intracanal medicament.

In this study, Propolis has been used in both micro and nano sized particles in a form of intracanal medicament. It is a resinous sticky material, originally collected by bees from certain plants, in order to protect and cover the hive holes. Propolis is introduced into dentistry due to; it’s antimicrobial, antioxidant, anti- inflammatory, anti-proliferative and anti-cancerous activity. One of the main contents of propolis is flavonoids, resulting in its antibacterial effect. The reason behind the antioxidant advantage, is the radical scavenging ability. Propolis intracanal medicament has bactericidal activity similar to calcium hydroxide medicament. It is even capable of treating the

most resistant type of bacteria, E. faecalis bacteria.[12,13]

Hoshino and colleagues were the first to develop and investigate the use of Triple antibiotic paste and its effectiveness on enucleating micro-organisms. TAP has the ability to affect gram-negative, gram-positive and anerobic bacteria effectively. TAP is a combination of metronidazole, minocycline and ciprofloxacin. Metronidazole is considered an antimicrobial agent against anaerobic bacteria and protozoa. Minocycline has bacteriostatic effect against gram negative and gram-positive bacteria, while ciprofloxacin has a bactericidal activity against gram-negative bacteria. Double Antibiotic Paste consists of metronidazole and ciprofloxacin. The effectiveness of these drugs against bacteria is increased by using TAP and DAP in nanoparticle form, which allows for thorough diffusion into the radicular dentin tubules, resulting in a more powerful antimicrobial effect.[14,15] Also, utilizing the appropriate low concentration of antibiotic paste ranging from (0.1 – 1 mg/ml) and the duration of usage of these medicaments in certain procedures; led to decrease in radicular dentin hardness, which is attributed to the resulted acidic media (ph.=2.9). This acidity acts by reducing the phosphate/amide ratio thus causing radicular dentin demineralization. To avoid the harmful effects escalated from usage of DAP/TAP, it should be removed within 2-4 weeks following intracanal application, in order to gain its effect without risking the remaining tooth structure.[16-18]

Micro-hardness (the hardness of a material) referres to the amount of gain or loss of the minerals that is available in the dental hard tissues.[19] Usually, hardness measurements of dentin discs are performed using micro-indentation techniques such as Knoop, spherical or Vickers indenters. In this study, Vickers microhardness machine (Tukan 1102 Wilson) was used to evaluate the effect of intracanal medicament according to the classified groups. The Vickers machine was invented in early twenties by Smith and Sandland, who worked through application of a load on the testing material by indentations in a non- destructive manner. In this research, three indentations were done at disc's coronal, middle and apical thirds; in order to assess the amount of mineral change on radicular dentin, following the application of the proposed intracanal medicament. It has the ability to detect the amount of mineralization/demineralization of the hydroxyapatite crystals in intertubular substance within the radicular dentin. The indenter is connected to an optical

microscope, digital cameras, and photo analysis software, which determine the ultimate dentin hardness value in this study.[20,21]

Propolis had an effect on the percentage reduction in radicular dentin microhardness. It led to a decrease in mineral content percentage, which could be attributed to the presence of ethanolic extract in Propolis that has a high amount of phenolic acids and flavonoids. These weak acids could be adsorbed easily into hydroxyapatite molecules. Surface complexation is considered one of the chemical reactions that take place at the interface between two different products with active surface groups which could be the cause of Propolis medicament reduction on root dentin.[22,23] Propolis reduction was less than DAP, although nano Propolis had the least reduction among the tested materials.

Comparison between the micro and nano forms of different antibiotic paste was made. DAP led to less radicular mineral loss. This could be attributed to DAP acidity (ph=3.4), while Triple Antibiotic Paste (TAP) has a higher (ph= 2.9) and contains a minocycline which has the ability to chelate dentin calcium ions, resulting in dentin demineralization.[24,25]

These results came in accordance with previous different studies done with different investigators who used similar or different antibiotic pastes and micro-hardness tests.[26] Furthermore, Yassen et al.[27] concluded that both DAP and TAP showed decrease in radicular dentin micro-hardness.

Regarding the period of time, there was a statistically significant decrease in mean microhardness in dentin samples post-treatment, which may be related to the mixed acids present in case of antibiotic pastes, that are important to regulate their tonicity and to preserve the chemical steadiness of the antibiotic pastes.[14,28] This result was in agreement with Amonkar et al.[29] who stated that the exposure time is directly proportional to dentin demineralization, considering time an important factor when using intracanal medicament. Also, Bahandi et al stated that using intracanal medicament for a period of time less than 24 hours can be well tolerated with harmful effects on samples.[30]

This study showed that coronal root level had statistically significantly highest mean microhardness values among middle and apical root levels. This could be attributed to the histological pattern of dentin; which has a greater amount and type of dentinal tubules present in coronal root level, while there is

lesser or even devoid areas of these tubules in apical region.[31-33]

Conclusion

Based on the findings of this investigation, it could be concluded that:

1) All experimental groups had an effect on radicular dentin microhardness, especially Nano Propolis, which had the least negative effect on root dentin microhardness.

2) Utilizing Propolis, DAP and TAP intracanal medicaments in a nano-form did not improve the material's effect on the radicular dentin microhardness.

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Conflict of interest

The authors deny any conflict of interest related to this study.

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