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The Antibacterial Effect of Calcium Hydroxide Modified with Dragon Blood Tree Extract Compared to other Intercanal Medicaments and Their Effect on Crown Discoloration and Dentin Microhardness: An In Vitro Study

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ABSTRACT

Objective: The aim of this study compare the antimicrobial efficacy of dracaena cinnabari extract with the calcium hydroxide and triple antibiotic paste in primary root canals contaminated with E. faecalis then evaluate their effect on crown discoloration and dentin microhardness.

Material and methods: Eighty extracted (n=80), primary anterior teeth were collected from the outpatient clinic of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University and then rinsed with sterile saline , all teeth will be sterilized via an autoclave machine in 121°C and 15 PSI pressure for 15 minutes.

Results: Statistical analysis of the results of current study revealed that all the experimental groups showed reduction in the total bacterial counts (CFU/ml) compared with the untreated positive control group that had the highest (Mean±SD) value followed by Ca(OH)2 group then modified CH group while the lowest value was found with samples treated with (TAP).

Conclusions: Calcium Hydroxide Modified with Dragon Blood Tree

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Extract, TAP and Conventional Calcium Hydroxide as intracanal medicaments have antibacterial effect against E. faecalis. Calcium Hydroxide Modified with Dragon Blood Tree Extract can be used as a safe natural alternative to TAP and Conventional Calcium Hydroxide.

INTRODUCTION

The goal of endodontic treatment is to control pulpal and periradicular infections and to promote healing. The success of endodontic treatment is directly influenced by the elimination of microorganisms in infected root canals.(1)

Chemomechanical instrumentation removes the majority of bacteria, together with pulp debris. However, it should be supplemented by intracanal medicaments to eliminate the remaining bacteria, to prevent its regrowth, thereby making the environment conducive for periapical tissue repair.(2)

Enterococcus faecalis is the predominant microorganism and occasionally the only species detected in root canals of teeth associated with persistent periradicular lesions. (3)

Because of the complexity of the root canal infection, it is unlikely that any single antimicrobial agent could result in effective sterilization of the canal. More likely, a combination would be needed to address the diverse flora encountered. (4)

In last few decades, the use of alternative therapeutic agents has considerably increased and these agents which are derived from plants, insects, microorganisms, etc. are a part of a growing trend to seek natural remedies in dental treatment. (5)

Dracaena Cinnabari is a species plant in Agavaceae family. It is a tree endemic to the Island of Socotra, Yemen. It was used as a dye and medicine in Socotra and the Mediterranean basin.(6)

MATERIAL AND METHODS:

Study Population

Eighty extracted (n=80), primary anterior teeth were collected from the outpatient clinic of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University and then rinsed with sterile saline , all teeth will be sterilized via an autoclave machine in 121°C and 15 PSI pressure for 15 minutes.

Preparation of Dracaena Cinnabari extract:

Branches were air-dried then bark was decorticated. Bark and branches were ground into powder using the ceramic mortar. 250 g of powdered bark and 400 g of powdered branches were macerated in 95% ethanol for 3 days at room temperature (1.25 L and 2 L, respectively). Extraction was assisted by sonication for 3 intervals 10 min each to avoid excessive heating.(7)

The resultant extract was filtered through a Whatman n° 4 filter paper.The extract was evaporated under reduced pressure at 40 °C using a rotary type evaporator and the residual water was removed by lyophilization.(7)

Preparation of Triple Antibiotics Paste:

Triple antibiotic paste was prepared by crushing a tablet of metronidazole 500 mg and a tablet of ciprofloxacin 500 mg in sterile mortar, Then the content of a doxycycline capsule 100 mg evacuated in the same sterile mortar and will be mixed to form homogenous powder using a pestle. Few Saline drops added to the powder and mixed using the pestle till a creamy mix was achieved.

Preparation of Conventional Calcium Hydroxide Paste:

Ca (OH)2 Powder (JK Dental Vision , India) where mixed with saline in a ratio of 1.5:1 (weight/volume) to obtain a paste-like consistency.(8) The paste then were loaded into sterile plastic syringe , Tips associated with metapste where mounted on the disposable plastic syringe for easy application of intercanal medicament.

Preparation of Modified Calcium Hydroxide Paste with Dracaena Cinnabari extract

Ca (OH)2 Powder (JK Dental Vision , India) was mixed with dragon blood extract solution in a ratio of 1.5:1 (weight/volume) to obtain a paste-like consistency.(8)

The paste then were loaded into sterile plastic syringe , Tips associated with metapste where mounted on the disposable plastic syringe for easy application of intercanal medicament.

Determination of Antibacterial Effect

A- Preparation of Specimens

-file #15 (mani, Japan) was placed in the root canal until its tip appeared at the apical foramen to ensure patency of the canal. (9)

Root canals were then mechanically prepared using hand files (k-type), employing the step back preparation technique reaching master apical file size #40 to standardize the diameter of the root canal apices, . Irrigation of the root apices with saline was performed after the use of each file to prevent blockage of the canal.(10)

B- Biofilm Development:

The microbiological culturing was carried out in the (Department of microbiology Faculty of Medicine, Ain Shams University), in which a clinical isolate of E. faecalis from the Microbiology laboratory (Central laboratories, Ministry of Health, Egypt) was used for biofilm formation. The bacterial strains were inoculated in Brain Heart Infusion broth (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37oC for 24 hours. The experimental suspensions were prepared by cultivating the biological marker on the surface of bile esculin agar following the same incubation conditions. The bacterial cells were suspended in saline to reach a final concentration of about 3 x 108 cells/mL, adjusted to No. 1 MacFarland turbidity standard which was used to infect the samples.(11)Qmix. Methods: Dentinal tubules from the root canal side in semicylindrical dentin specimens were infected with Enterococcus faecalis by centrifugation of the bacterial suspension into the tubules. Scanning electron microscopy (SEM

C- Intracanal Medication Dressing :

After 21 days of incubation the infected samples were irrigated with 5 ml of sterile saline to remove the incubation broth then root canals filled with intercanal medicaments using sterile plastic syringe according to each group as follows:(12)

GROUP 1 : dressed with calcium hydroxide. GROUP 2 : dressed with modified calcium hydroxide. GROUP 3 : dressed with TAP. GROUP 4 (positive control) : were contaminated and irrigated with sterile saline GROUP 5 (negative control) : were not contaminated nor medicated (sterile). (13)

All samples will be sealed with wax and maintained in vials in a humid environment at 37o C for one week in the incubator.(12)

D-Removal of Dressing:

After one week (Treatment Period), all the samples irrigated with 20 ml sterile saline solution to remove the root canal contents.

E-Bacterial sampling and bacterial count:

Two sterile absorbent paper points were used for each root to absorb the irrigation fluid and transferred to a test tube containing 1.0 ml of saline, (Fig. 36). All samples were vortexed for twenty seconds and 10-fold dilutions were prepared in saline. Aliquots of 0.1 ml were

spread plated onto BHI agar plates (Fig. 37), incubated at 37°C for 48 hours, and colony-forming units (CFU) per 1 ml.(14)

RESULTS :

Descriptive statistics for bacterial count:

Table (1): Mean ± standard deviation (SD) of bacterial count (CFU/ml ^ 3) for different groups

Bacterial count (mean±SD)			p-value
Calcium Hydroxide	Modified Calcium Hydroxide	TAP	
23.402.70±^{A}	12.201.92±^{B}	3.201.30±^{C}	**<0.001***

Different superscript letters indicate a statistically significant difference within the same horizontal row*; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

Percentage reduction from the control group:

Table (4): Mean ± standard deviation of percentage of reduction of bacterial count from the control group

Percentage of reduction from the control group (mean±SD)			p-value
Calcium Hydroxide	Modified Calcium Hydroxide	TAP	
73.203.30±^{C}	87.392.03±^{B}	96.801.30±^{A}	**<0.001***

Different superscript letters indicate a statistically significant difference within the same horizontal row*; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

DISCUTIONS

The results of antibacterial effect of the used intracanal medicaments of current study revealed that all the experimental groups showed reduction in the total bacterial counts (CFU/ml) compared with the untreated positive control group that had the highest (Mean±SD) value followed by Ca(OH)2 (23.40±2.70) group then modified CH (12.20±1.92) group while the lowest value was found with samples treated with (TAP) (3.20±1.30). This finding could be attributed to the combined spectrum of antimicrobial activity and synergetic or additive actions of antibiotics ciprofloxacin, metronidazole, and minocycline found in TAP.(172) Metronidazole is effective against obligate anaerobes, which are common in the deep dentin of infected root canals and acts by disrupting bacterial DNA. Minocycline is a broad-spectrum tetracycline antibiotic and acts by inhibiting protein synthesis and inhibiting matrix metalloproteinase enzyme. Combination of these three antibiotics overcomes bacterial resistance and achieves higher antimicrobial action. The insufficient antibacterial efficacy of CH has been attributed to the buffering action of dentin.

This finding confirms with the study by

Madhubala et al. (2011) in which propolis and TAP showed higher antibacterial effects than CH on E. faecalis.

The current findings are in accordance with results obtained by Adl et al (2013) who evaluated the efficacy of the experimental medicaments in removing E. faecalis. the TAP was more effective than Ca (OH)2 in a seven-day period, and it eliminated EF from the root canal system.

Kim and Kim reported that TAP showed a larger inhibition zones against E. faecalis than calcium hydroxide.

The results of microhardness of root canal dentin after intracanal medication in current study revealed that control samples (74.69±5.18) and those treated with conventional calcium hydroxide (67.76±6.31) to have a significantly higher values in comparison to other groups (p<0.001). This could be explained by the strong acidic nature of TAP (pH = 2.9) as well as the chelating effect of minocycline present in TAP.

These results agreed with the vitro study by Yassen et al. (2014) who concluded that the use of Ca(OH)2 medicaments during endodontic may cause significantly less microhardness reduction and superficial demineralization of dentin compared to the use of TAP which caused significantly higher dentin demineralization and reduction in

dentin microhardness.

The results of current study regarding to the crown discoloration revealed that there was a significant difference in the values of crown discoloration (ΔE) for different groups ($p<0.001$). The highest value of discoloration was found with modified calcium hydroxide samples (3.46 ± 0.20) followed by samples treated with (TAP) (2.97 ± 0.17) then conventional calcium hydroxide treated samples (2.36 ± 0.14) while the lowest value was found with control samples (0.47 ± 0.08).

These results were comparable with the results of study done by **Lenherr et al. (2012)** reported that Significant differences were detected amongst the experimental groups after 12 months ($P < 0.0001$). The lowest color change values were observed in the empty group (3.8 ± 1.4) then calcium hydroxide (4.7 ± 1.5), and the most discoloration was measured in TAP group (66.2 ± 9.9).

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