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A Comparative Study of the Antibacterial Efficacy of two Natural Irrigating Solutions with Two Different Root Canal Sealers on *E-feacalis*

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ABSTRACT

This study aimed to: evaluate the antibacterial efficacy of two natural irrigating solutions (Green Tea, and Propolis) with two different root canal sealers (AH Plus sealer, and Total Fill BC Sealer) against *E. faecalis*. **Methods:** One hundred single rooted teeth were selected. All root canals were prepared manually to #50 file. Finally the teeth were sterilized by gamma rays. Each root canal was inoculated with 0.5 ml of *E. faecalis*. Specimens were divided randomly into two main groups (50 specimens each) according to the irrigating solution; A1 (Green Tea), and A2 (Propolis). Each main group was divided into two subgroups with (25 specimens each) according to the type of sealer used B1 (AH Plus sealer), and B2 (TotalFill BC Sealer). The bacterial sampling counting process was done three times throughout the study. Five specimens from each subgroup were examined by SEM for presence or absence of *E. faecalis*. **Results:** The highest mean value of bacterial count was found in (Green tea) (6.50x10x10 ± 1.29x10x10) while the least mean value of bacterial count was found in (Propolis) (with less antibacterial effect 2x10x10 ± 1.33x10x10) with higher antibacterial effect. The highest mean value of bacterial count was found in (AH Plus Sealer) (5.40x10x10 ± 2.44x10x10) with less antibacterial effect while the least mean value of bacterial count was found in (TotalFill BC Sealer) (3.10x10x10 ± 1.54 x10x10) with higher antibacterial effect. **Conclusions:** Propolis is an effective intracanal irrigant in eradicating *E. faecalis*. On other hand TotalFill BC Sealer is more effective in reducing *E. faecalis*.

- Paper extracted from Master Thesis entitled "A Comparative Study of the Antibacterial Efficacy of two Natural Irrigating Solutions with Two Different Root Canal Sealers on E-feacalis"

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INTRODUCTION

Bacteria cause pulpal and periradicular diseases. Hence, root canal treatment aims to eliminate bacteria from the infected canal system and to prevent reinfection. Clinical studies have demonstrated that chemo-mechanical preparation and use of antimicrobial medicaments are effective in reducing the bacterial load in root canal systems [1].

The presence of *E faecalis* in cases of persistent apical periodontitis is of particular interest because it is rarely found in infected but untreated root canals. *E faecalis* is the most consistently reported organism from such former cases, with a prevalence ranging from 22% to 77% of cases analyzed [2].

Recurrent root canal infections can occur even after endodontic treatment and are most commonly associated with Enterococcus faecalis. Sodium hypochlorite has been used as the irrigant of choice for endodontic procedures, but it has many deleterious effects if pushed beyond the apex. Herbal products have been used in dental and medical practice for thousands of years and now become more popular due to their antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties [3] .

Tea possesses anticariogenic and antibacterial properties. Green tea has antimicrobial activity on E fecalis and found to be a good chelating agent [4,5]. On other hand Propolis exhibits a wide range of biologic activities, including antimicrobial, anti-inflammatory, antioxidant, anesthetic and cytotoxic properties [6].

Moreover, Root canal sealers should adhere to dentin and the core material and fill the irregularities between the core material and the root canal dentin[7,8]. It is stated that, the root canal sealers show antibacterial activity that may contribute to the destruction of intracanal microorganisms and all sealers exhibit highest toxicity and antibacterial activity when freshly mixed that decreases during setting [9,10].

Therefore, the experimental design of the present study aimed to evaluate the antibacterial efficacy of two natural irrigating solutions (Green Tea, and Propolis) with two different root canal sealers (AH Plus sealer, and Total Fill BC Sealer) against *E. faecalis*.

MATERIALS AND METHODS

One hundred extracted single rooted teeth with mature apex were used in this study, the teeth were extracted for orthodontic, pulpal or periodontal reasons. After extraction, the teeth were cleaned and root planned to remove any soft or hard debris on its surface using ultrasonic scaler[a]. The selected teeth were radiographically examined from facial and mesial surfaces to exclude any tooth with root cracks, internal resorption, root caries, pulp space calcifications, root fracture or previously treated endodontically. The teeth were stored in normal saline[b] solution 0.9% at room temperature till time of use.

The roots of all teeth were sectioned at CEJ with high diamond tapered bur and high speed hand piece [c] with water spray. Flattening of the coronal portion of the roots was done with wheel stone. K-File No.10 [d] was introduced into the canal till the tip of the file is seen from the apex to ensured patency. Working length was determined by subtracting 1 mm from a K file size 15 just visible at the apical foramen. All root canals were then prepared by Step- back technique the canals were enlarged to a size #50 file, which served as master apical file.

a. Wood pecker china.

b. El Fath for drugs, and cosmetic industry (FIPCO).

c. W&H Burmoos, Austria.

d. Dentsply, Maillefer, Tulsa,OK.

One milliliter NaOCl of 2.6% concentration was the irrigant solution used after each file. The specimens received a final rinse with 2ml Smear Clear solution which agitated into the canal for 2 minutes by means of the master apical file. All the canals were dried with sterile paper points after irrigation. Apical foramina of all samples were sealed with light cured resin composite "FiltekTM Z250 , 3M-ESPE" and samples were coated with two layers of nail varnish to prevent bacterial leakage. Samples were inserted into sterilization bags and sterilized using gamma radiation[a].

Preparation of microorganism

The *E. faecalis* used in this study (obtained from the patient randomly selected for research purposes at Microbiology department, Faculty of Medicine for Girls, Al Azhar University, Cairo) was prepared by growing Pure *E. faecalis* on a bile-esculin plate for 24 hours at 37°c, the purity of the culture was confirmed by culture characteristics, microscopic examination and biochemical reactions.

Bacterial inoculation

From these prepared microorganisms, colonies of *E.Faecalis* were picked up by sterile bacteriological loop and emulsified in tube containing 5ml of Brain Heart Infusion Broth "BHI", using automated micro pipette and shacked while the bacteriological loop in the glass tube to evacuate its bacterial content. The turbidity of solution within the tube was then compared with a standardized solution within a vial with a turbidity level of 0.5 Mc Ferland [(11)].The turbidity level was adjusted by adding an amount of saline till the turbidity level match that of 0.5 Mc Ferland which is equivalent to 1.5 x10 ^{8} CFU/ml using a sterile micropipette.

Following sterilization of the samples, the root canal of each sample was flooded with a suspension of the isolated E.faecalis using sterile 1ml insulin syringe. For maximum penetration of the bacterial suspension into the entire root canal system, sterile size #15 K files was used in an up and down movement. Infused samples were placed individually inside eppendorfs with 2 ml BHI broth, closed then inserted inside a rack and placed in the incubator at 37°c for 48 hours, for allowing bacteria to multiply and proliferate.

Grouping of the Samples

The prepared specimens were divided randomly into two main groups with fifty specimens each according to the irrigating solution A1 (**Green Tea**), and A2 (**Propolis**). Each main group was divided into two subgroups with 25 specimens each according to the type of sealer used B1 (**AH Plus sealer**), and B2 (**Total Fill BC Sealer**).

Bacterial counting was done three times for each root throughout the study at;

1. After Incubation of root canals.
2. After irrigation of root canal with the tested irrigation A1, and A2.
3. After obturation with B1, and B2 sealers.

The bacterial sample was taken from each root canal by insertion of three sterile absorbent paper point size #20 successively inside each root canal to be saturated for 1 min for each point. The paper point specimen was removed from the canal using sterile tweezer and placed in a sterile plastic tube containing 1ml saline. The falcon tube was then sealed tightly with its screw and agitated by hand for 30 sec.

Cultivation, Incubation and Counting Procedures

After agitation, the three paper point was removed from the plastic tube and the cultivation process was done using serial dilution as follows

a. The Gammacell 220 ® (MDS Nordion, Ottawa, Canada).

1. The tube and four more sterile plastic tubes (each tube contain 0.9 ml of sterile saline) in a test-tube rack. The tube were then labeled 1:10, $1:10^2$, $1:10^3$ and $1:10^4$ representing different levels of dilution.
2. Two hundred μl of solutions from the original tube were placed in the first tube (1:10) of saline using A sterile automated pipettes and suitable different sterile tips used in between the different dilutions. To avoid carry-over of bacteria.
3. The solution was thoroughly mixed in the tube by sucking up and then expelling the content three times gently.
4. This process was repeated three more times using solution from 1:10 to dilute $1:10^2$ and from $1:10^2$ to dilute $1:10^3$ and so on.
5. Then culture every dilution on a separate bile oscaline plate
6. Then the plates with cultivated bacteria were incubated for 24 hours at 37°C and 100% humidity.

At the end of the incubation period, the number of colonies was counted, all of the plates containing less than 300 colonies were selected, because the greater than 300 colonies on the bile esculin plate leads to a high degree of error. A high count can be confounded by error in counting too many small colonies, or difficulty in counting overlapping colonies. Then the number of bacterial colonies forming units (CFUs) per milliliter was counted and calculated according to this equation:

Number of colonies (CFUs) = bacteria X ml dilution X amount plated.

Group A1 (Green Tea):

Green tea extract is obtained from five gram of the selected dry green teas leaf, steeped for 1.5- 2 minutes in 100 ml of distilled water. The coolest brewing temperature was below 70°C.The mixture was purified to obtain the 5% concentration solution of green tea [3].

The specimens were irrigated with 5 mL of sterile saline to remove the incubation broth, and then each specimen was irrigated by 2ml of 5% concentration solution of green tea followed by bacterial counting test.

Group A2 (Propolis):

The hand-collected propolis sample was kept desiccated in the dark until it was processed. Subsequently, 30 g crude propolis was dissolved in 70% ethanol by shaking 3 times in a day for 3 days. The aqueous ethanol extract was filtered through a filter paper, and evaporated at 50°C. The resin obtained was dissolved in 70% ethanol to a final concentration of 9.6 mg/ml. Ethanol extract of propolis was employed for the antimicrobial assays [12].

The specimens were irrigated with 5 mL of sterile saline to remove the incubation broth, and then each specimen was irrigated by 2ml of Propolis followed by bacterial counting test.

Specimen's obturation

Specimen's obturation was done by the tested sealer according the manufactures' instructions using lateral obturation technique. In this technique the spreader used for lateral condensation was placed within 2 mm from the working length when apical stop has been created. Master cone was selected to fit properly in the whole working length with tug-back action. Then each sealer was mixed according to the manufacturing instructions on sterile glass slap. The sealer was inserted inside the canal using the master cone which was then placed in the canal to the working length. The premeasured spreader was placed with a rotary vertical motion along the side of the master cone. The spreader was removed from the canal by rotated counter close wise motion to free the master cone, and then auxiliary cones size 25 gutta percha were inserted inside the root canal. This process was repeated until no more auxiliary cones could be introduced inside the root canal.

Excess of gutta percha was removed with a hot sterile condenser. Each sample of both groups was placed in a sterile eppendorf tube which was filled with sterile saline to keep samples under humid condition. The eppendorfs of both groups were put inside a rack and placed in the incubator at 37°c for 24h to allow sealer setting.

Preparation the specimens for evaluation:

At the end of the incubation periods, eppendorfs containing the samples were opened and samples were removed. Each sample was transversely cut into sections of one millimeter thickness each. Obturation material was removed from the sections using sterile H file. The sections and the removed obturation material of each sample were inserted inside sterile tube that contains one mL of BHI broth. Each tube was subjected to Vortex for one minute to allow the bacteria adherent to the root canal to fall down in the broth. The number of colony forming units (CFUs) was counted and calculated.

Scanning electron microscope:

Scanning electron microscope was used to observe presence or absence of bacteria was examined at the time of evaluation. Five specimens from each subgroup were sectioned in a bucco-lingual direction using a low speed diamond saw under copious irrigation with distilled water. The half which did not retain the obturation material was chosen for examination by the scanning electron microscope "Quanta 250 FEG, FEI Company, Netherlands". Specimens were gold sputter-coated before scanning electron microscopic examination by Sputter coater "EMITECH, K550X sputter coater, England".

Statistical analysis:

The mean and standard deviation values were calculated for each group. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed parametric (normal) distribution. Independent-sample t test was used to compare the difference between two groups in non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

Effect of irrigation solution on bacterial count regardless of sealer type (Table 1) (figure 1):

There was a statistically significant difference between (Green tea) and Propolis) groups where (p=0.001). The highest mean value of bacterial count was found in (Green tea) (6.50x10x10 ± 1.29x10x10) with less antibacterial effect while the least mean value of bacterial count was found in (Propolis) (2x10x10 ± 1.33x10x10) with higher antibacterial effect.

Table (1) *Mean and standard deviation values of bacterial count of each irrigation solution regardless of sealer type.*

Variables	Bacterial count	
	Mean	**SD**
Green tea (A1)	6.50x10x10 [a]	1.29x10x10
Propolis (A2)	2x10x10 [b]	1.33x10x10
P-value	**0.001***	

Different letters in the same row indicate statistically significant difference

**; significant (p<0.05) ns; non-significant (p>0.05).*

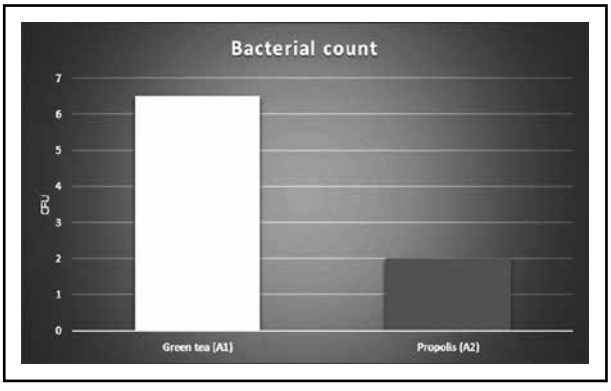

Fig. (1) Column chart of mean values of bacterial count of each irrigation solution regardless of sealer type.

Effect of sealer type on bacterial count regardless the type of irrigation solution (Table 2) (Figure 2):

There was a statistically significant difference between (AH Plus sealer) and (TotalFill BC Sealer) groups where (p=0.014). The highest mean value of bacterial count was found in (AH Plus Sealer) (5.40x10x10 ± 2.44x10x10) with less antibacterial effect while the least mean value of bacterial count was found in (TotalFill BC Sealer) (3.10x10x10 ± 1.54 x10x10) with higher antibacterial effect.

Table (2) *Mean and standard deviation values of bacterial count of each sealer type in each irrigation solution.*

Variables	Bacterial count	
	Mean	SD
AH Plus Sealer (B1)	5.40x10x10 [a]	2.44x10x10
TotalFill BC (B2)	3.10x10x10 [b]	1.54 x10x10
P-value	**0.014***	

Different letters in the same row indicate statistically significant difference

**; significant (p<0.05) ns; non-significant (p>0.05)*

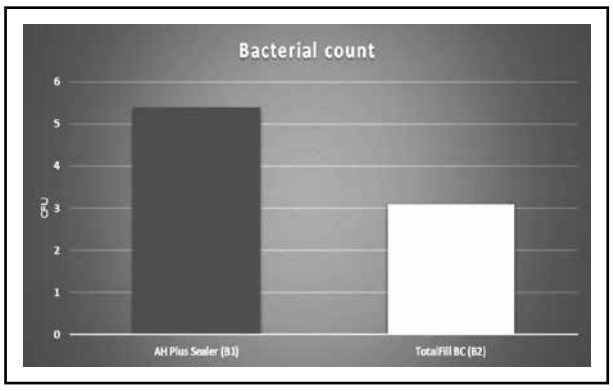

Fig. (2) Column chart of mean values of bacterial count of each sealer type regardless of irrigation solution.

DISCUSSION

The present study aimed to evaluate the antibacterial efficacy of two natural irrigating solutions (**Green Tea,** and **Propolis**) with two different root canal sealers (**AH Plus sealer,** and **Total Fill BC Sealer**) against *E. faecalis*. To simulate clinical conditions, natural teeth were used in this study and sectioned 15 mm from the apex to the amelo-cemental junction, at a level corresponding to the clinical gingival margin to standardize the root length [13].

All root canals used in this study were prepared by Step- back technique to a size #50 file. Finally the teeth were underwent for sterilization; to completely eradicate all forms of bacteria that may be founded in the root canal system; by gamma rays which is effective and introduce no detectable changes in dentin while other methods (ethylene oxide, dry heat, and autoclaving.) of sterilization alter the structure of the dentin [14], then bacterial sampling for checking of sterilization were done before inoculation of bacteria and this were serve as negative control [15].

5 specimens from each subgroup were sectioned in a bucco-lingual direction using a low –speed diamond saw under copious irrigation with distilled water. The half which did not retain the obturation material was chosen for examination by the **scanning electron microscope** for presence or absence of *E. faecalis***.**

E. faecalis, a Gram-positive facultative anaerobe, was selected in this study because it is commonly found in the root canals of failing endodontically treated cases [16].

In this study each root canal was inoculated with 0.5 ml of *E. faecalis*, with density adjusted to the turbidity of 0.5 McFarland standard (1.5 x 10^8 bacteria/ml) according to ***Blanscet et al***, [17], this was followed by incubation period 48 hrs.to allow deeper penetration of *E. Faecalis* into dentinal tubules according to ***Tewari et al*** [18].

The bacterial sampling counting process was done three times throughout the study; after incubation of root canals for 48 hrs. to count the number of bacteria that already inoculated into the canal to compare it with that found after irrigation of root canal with the tested irrigation A1 (**Green Tea**), and A2 (**Propolis**), and after obturation with B1 (**AH**

Plus sealer), and B2 (**Total Fill BC Sealer**), [19] on the number of inoculated bacteria [20].

The result of the tested irrigation A1 (**Green Tea**), and A2 (**Propolis**) in present study regardless of sealer type showed that there was a statistically significant difference between groups where ($p \leq 0.001$). The highest mean value of bacterial count was found in (Green tea) (6.50x10x10) with less antibacterial effect while the least mean value of bacterial count was found in (Propolis) (2x10x10) with higher antibacterial effect.

The reasons for these result may be due to Propolis contains predominantly phenolic compounds, including several flavonoids, aromatic acids, and their esters, and the major component was chrysin (flavonoid). Flavonoids are the most important pharmacologically active, and they are thought to account for much of the antimicrobial activity in propolis [21].

Other studies explain the effect of Propolis on *E. Faecalis* to the fact of Propolis extract contains compounds like aldehyde, aliphatic acid ester, carboxylic acids, cinnamic acid and its esters, ketone, terpene, alcohol, ether, hydrocarbon, and phenolic resin each having their own antibacterial effect. In addition, synergy between these compounds along with unique properties of each constituent is effective in occurrence of the antibacterial effects of Propolis. Also, it has been found that each of the constituents of Propolis alone is effective against microorganisms but Propolis itself has greater antibacterial activity against pathogenic strains when compared to its constituents [22-25].

Our findings agree with observation of ***Oncag et al*** that propolis had good in vitro antibacterial activity against *E. faecalis* in the root canals of extracted teeth, suggesting that it could be used as an alternative intracanal medicament [26]. Propolis's antimicrobial properties have also been evaluated in other studies the results of which, overall, are in accord with those of ours [21, 27].

Also the result of our study on the effect of sealer type B1 (**AH Plus sealer**), and B2 (**Total Fill BC Sealer**) on bacterial count regardless of irrigation solution found that There was a statistically significant difference between (AH Plus sealer) and (TotalFill BC Sealer) groups where ($p=0.014$). The highest mean value of bacterial count was found in (AH Plus Sealer) (5.40x10x10) with less antibacterial effect while the least mean value of bacterial count was found in (TotalFill BC Sealer) (3.10x10x10) with higher antibacterial effect.

The key antimicrobial properties of root canal sealers lie in their alkalinity and release of calcium ions [28], which stimulates repair via the deposition of mineralized tissue [29]. Bioceramic root canal sealer has been shown to have high pH (>11) as well as high tendency to release calcium ions [30].

Zhang et al. tested the antibacterial activity of bioceramic root canal sealer *in vitro* against *Enterococcus faecalis* through a modified direct contact test, finding that iRoot SP sealer had a high pH value (11.5) even after setting but that its antibacterial effect was greatly diminished after seven days. The investigators suggested two additional mechanisms associated with the antibacterial efficacy of bioceramic root canal sealer: hydrophilicity and active calcium hydroxide diffusion. Hydrophilicity reduces the contact angle of the sealer and facilitates penetration of the sealer into the fine areas of the root canal system to enhance the antibacterial effectiveness of bioceramic root canal sealer [31].

Pizzo et al reported that only fresh AH plus possessed antibacterial activity, whereas 24-hour and 7-day-old samples did not show antibacterial effect against E. faecalis [32]. Similar results were reported by Kayaoglu et al [33]. The antimicrobial effect of epoxy resin–based sealers might be related to the release of formaldehyde during the polymerization process [34].

Scanning electron microscope result of the present study indicated only presence *E. faecalis* in the specimens studded in each sub groups.The results

obtained by Clegg *et al*. showing that they tested only the antimicrobial activity of the agents, but not their capacity to disrupt the biofilm structure; and the SEM analysis was conducted only to confirm the presence of the biofilm on dentin surface and its removal by the glass beads [(35)].

CONCLUSIONS

Propolis is an effective intracanal irrigant in eradicating *E. faecalis*. On other hand TotalFill BC Sealer is more effective in reducing *E. faecalis*.

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