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EVALUATION OF THE EFFICACY OF ACTIVATED CHARCOAL AND ACTIVATED CHARCOAL CALCIUM HYDROXIDE COMBINATION VERSUS CALCIUM HYDROXIDE INTRACANAL MEDICATION AGAINST *ENTEROCOCCUS FAECALIS* IN SINGLE ROOTED PREMOLAR TEETH: AN IN VITRO STUDY

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ABSTRACT

Objective: This study aimed to evaluate efficacy of activated charcoal and activated charcoal/ calcium hydroxide combination versus calcium hydroxide as intracanal medication against Enterococcus faecalis

Methods: 42 extracted single rooted premolar teeth were prepared, sterilized and inoculated with *E. faecalis* for 21 days. After incubation, initial bacterial counts were detected. Then, samples were divided randomly into three groups (n = 14) according to the used medication as follows: Group I: Ca $(OH)_2$ mixed with distilled water, Group II: activated charcoal /Ca $(OH)_2$ mixed with distilled water, Group III: activated charcoal mixed with distilled water, then samples were incubated for 7 days. The percentage reduction in colony counts (%RCC) after 7 days was used to measure the antibacterial effectiveness of different intracanal medications. The percentage reduction in bacterial counts in the three groups were compared statistically using the One-way ANOVA test.

Results: Activated charcoal and calcium Hydroxide/activated charcoal groups showed statistically significantly higher mean percentage of reduction in bacterial counts than Calcium Hydroxide group..

Conclusion: Activated charcoal and Calcium Hydroxide/Activated charcoal were effective against Enterococcus faecalis than calcium hydroxide.

KEY WORDS: Calcium Hydroxide; Activated charcoal; Enterococcus faecalis

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INTRODUCTION

Intracanal medication is considered mandatory in management of cases with resistant infection with continuous pain and exudate [1]. Various substances have been utilized as intracanal medicaments, but none of them possesses the ideal material's characteristics. However, calcium hydroxide [$Ca(OH)_2$] is still considered the gold standard of intracanal medicament due to its antibacterial activity, organic tissue dissolving action [2] and its ability to inactivate bacterial endotoxins [3].

However, its high pH is neutralized by buffering effect of dentin [4], so that not all microbes, including Enterococcus faecalis, are susceptible to it. [5].

Activated charcoal is a natural product that has been used as an emergency anti-poison treatment since the early 1800s, as it can bind to a wide variety of drugs, reducing their effects[6]. It has been also reported that activated charcoal has ability to disinfect water systems [7].

Activated charcoal based oral products are a recent progress in the oral healthcare market, with society trending toward using "natural" products [8].

Up to date, according to literature, there is no study that evaluated the efficacy of activated charcoal against Enterococcus faecalis when used as intracanal medications after root canal preparation, so the aim of the present study was to evaluate efficacy of activated charcoal and activated charcoal/ calcium hydroxide combination versus calcium hydroxide as intracanal medication against Enterococcus faecalis

MATERIALS AND METHODS

The Faculty of Dentistry, Minia University 's Research Ethics Committee gave the study the stamp of approval; (Committee No 91, Decision No 664)

Sample size calculation

According to the findings of a pilot study with three samples per group, the mean values were 82.8, 88.7 and 84.3 %, respectively. The standard deviation within group was anticipated to be 5%. The effect size (f) was 0.805. With a power of 80% and an alpha (α) level of 5% and Beta (β) level of (20%), the minimum predicted sample size was a total of 42 samples (14 samples per group). This power analysis used percentage reduction in bacterial counts as the primary outcome. G*Power Version 3.1.9.2 was used to calculate sample size.

Samples collection

42 extracted human mandibular premolar teeth with intact mature single roots which were extracted for reasons unrelated to the present study were considered for the study. Following extraction, the teeth were kept in 5.25% sodium hypochlorite for 30 minutes. After carefully cleaning teeth under running water, the ultrasonic scaler was used to plan the root surfaces and remove any remaining soft tissues or hard tissues. The teeth were then kept in saline till use.

Preparation of samples:

All the teeth were decoronated at cemento-enamel junction (CEJ) using a water-cooled low speed diamond disk to obtain a standard root with length of 14 mm.

The samples were mechanically prepared shorter than root length by 1mm using Protaper next up to X3 (Dentsply Maillefer, Ballaigues, Switzerland)

Samples were irrigated using 3 mL of 2.5% NaOCl after each file change. Then, the samples were irrigated with 17% EDTA (PREVEST Dent Pro, India) for 1 min followed by final irrigation with saline.

Root surfaces except the apical 3 mm were sealed by nail varnish to ensure a good bacterial seal. The apical 3mm of the root of each sample was

etched, bonded, and sealed with composite resin in order to prevent bacterial leakage and to retain the irrigation solution within the canals to simulate the in-vivo apical counter pressure.

Samples were sterilized by autoclave at temperature 121°C and pressure 2 bars for 20 minutes in a sterile tubes containing phosphate buffered saline (PBS), to preserve the samples dehydration. To confirm sterilization, samples were incubated for 24 hours at 37°C in brain-heart infusion broth (BHI, Merck, Darmstadt, Germany). After that, a sterile culture medium was placed inside the root canals, then some culture media were collected from each sample to confirm samples sterilization.

Enterococcus faecalis culturing and transfering into the samples

The frozen bacteria were defrosted before being incubated at 37°C with aerobic conditions on Brain-Heart Infusion Agar (BHIA) culture media (Merck, Darmstadt, Germany). Isolated colonies were collected and suspended in 5 ml of BHI in a sterile falcon glass tube. The bacterial culture was adjusted to 0.5 McFarland turbidity standards which is equivalent to 1.5 x 108 CFU/ml [9].

Each sample was filled with a total of 100 µL of the bacterial culture using a micropipette. The samples were then cultured for 21 days at 37°C..

Primary bacterial counts

To calculate the primary bacterial counts, a plastic syringe with a side-vented needle was used to inject sterile saline solution into the canal lumen. As a sampling technique, a sterile Protaper next X3 paper point (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the root canal for 1 minute, and then transferred into a test tube containing sterile normal saline solution. The test tube was then placed on a vortex mixer for 1 minute, and from the resulting solution, a solution with a dilution of $1:10^7$ was prepared. 1 mL of this solution was then added to BHIA culture media. For 24 hours, the culture media were incubated at 37°C. The number of colonies was used to gauge any bacterial growth (Figure 1; A). Repeating these steps with three paper points was done for all the samples before application of intracanal medicament.

Application of intracanal dressing and final bacterial counts

The samples were randomly allocated to three major groups. (14 samples for each group) using the Randomize List software program according to the used medication as follows:

Group I: Ca $(OH)_2$ (Cerkamed, Poland) mixed with distilled water, Group II: activated charcoal (Eucarbon Sedico, Cairo, Egypt) /Ca $(OH)_2$ mixed with distilled water, Group III: activated charcoal mixed with distilled water

The pastes were introduced into the canals using Lentulo spirals (Mani Corp., Tachigi-ken, Japan), and packed with a hand plugger (FANTA dental materials, Shanghai China).

All samples were sealed with temporary filling applied directly to the canal orifice and incubated at temperature 37°C for 7 days. The canals were reopened and the medication were removed using size #30 Hedstrom files (Dentsply Maillefer) and 5 ml sterile saline solution.

Similar to the primary bacterial count determination procedures, final bacterial counts were calculated. (Figure 1; B).

The percentage reductions in colony counts (RCC%) between the first and second stages were used to calculate the antibacterial capabilities of the materials using the following formula [10]:

(Primary colony-final colony count) / primary colony count, where the bacterial colony count before applying the intracanal dressing was used to compute the primary colony count, and the bacterial colony count after applying the intracanal dressing was used to calculate the final colony count.

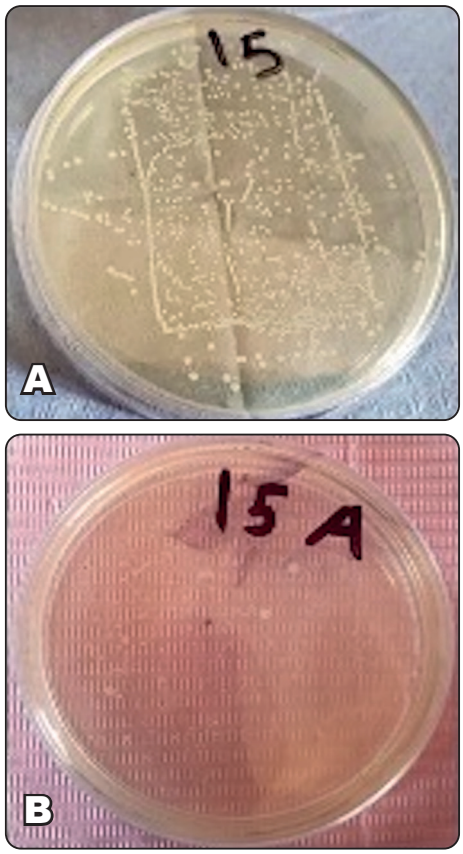

Fig. (1); Bacterial colony counts on BHIA culture media A; initial, B; final

Statistical Analysis

By examining the distribution of the data and applying normality tests, numerical data were examined for normalcy (Kolmogorov-Smirnov and Shapiro-Wilk tests). Due to the wide range of bacterial counts, logarithmic transformation of the data was carried out. The mean and standard deviation (SD) values of the data were displayed. The three groups' respective percentage reductions in bacterial counts were compared using a one-way ANOVA test. The cut off for significance was chosen at $P \leq 0.05$.

RESULTS

Table 1 shows that before medication application, there was no statistically significant difference between Log_{10} colony forming unit (CFU) of bacterial counts in the three groups. After medication , there was a statistically significant difference between Log_{10} CFU of bacterial counts in the three groups. Pair-wise comparisons between groups revealed that calcium hydroxide group showed the statistically significantly highest mean Log_{10} CFU of bacterial counts. There was no statistically significant difference between calcium hydroxide/activated charcoal and activated charcoal groups; both showed statistically significantly lower mean values.

Table 2 shows percentage reductions in the colony counts (RCC %) in the three groups, there was no statistically significant difference between calcium hydroxide/activated charcoal and activated charcoal groups; both showed statistically significantly higher mean percentage reduction in the colony counts than calcium hydroxide group.

TABLE (1): Descriptive statistics and results of repeated measures ANOVA test for comparison between Log_{10} CFU of bacterial counts in the three groups and changes within each group

Medication application	Calcium Hydroxide (n = 5)		Calcium Hydroxide/Activated charcoal (n = 5)		Activated charcoal (n = 5)		*P*-value	Effect size (*Partial Eta squared*)
	Mean Log_{10}	SD	Mean Log_{10}	SD	Mean Log_{10}	SD		
Before application	2.72	0.04	2.72	0.04	2.72	0.04	1	0
After application	2.18 ^{A}	0.04	1.73 ^{B}	0.08	1.56 ^{B}	0.22	<0.001*	0.813
P-value	<0.001*		<0.001*		<0.001*			
Effect size (*Partial Eta squared*)	0.845		0.949		0.961			

: Significant at P ≤ 0.05, Different superscripts indicate statistically significant difference between groups

TABLE (2): Descriptive statistics and results of one-way ANOVA test for comparison between percentage reductions in bacterial counts in the three groups

Calcium Hydroxide (n = 14)		Calcium Hydroxide/Activated charcoal (n = 14)		Activated charcoal (n = 14)		*P*-value	Effect size (*Eta squared*)
Mean %	SD	Mean %	SD	Mean %	SD		
76.1 ^{B}	8.4	89.8 ^{A}	7.7	92.2 ^{A}	10.4	<0.001*	0.903

: Significant at P ≤ 0.05, Different superscripts indicate statistically significant difference between groups

DISCUSSION

Calcium hydroxide is considered the most popular intracanal medication [11], to increase its efficacy, combination of Ca $(OH)_2$ with other antimicrobial agents, has been suggested [12].

Activated charcoal is a natural product with antibacterial efficacy that is recently used in many dental products[13]. Enterococcus faecalis was chosen because it is the most frequent bacteria detected in persistent infection and treatment failures [14,15].

The bacteria was able to spread throughout the entire root canal system over the 21-day incubation period [16]. Ca $(OH)_2$ has been reported to require a minimum of 7 days of contact time in order to completely get rid of any bacteria that might have survived the chemo-mechanical preparation [17].

In this in vitro study we compared the efficacy of activated charcoal and activated charcoal/ calcium hydroxide combination versus calcium hydroxide as intracanal medicament against Enterococcus faecalis. Regarding the findings of the current study, there was a statistically significant decrease in bacterial counts in all groups after medicament application for one week. Calcium hydroxide has reduced E. faecalis count from the root canal lumen after 7 days in previous studies [18-21]. This may be attributed to its alkalinity and its ability to destroy bacterial lipo-polysaccharides, denaturate bacterial proteins, damage bacterial DNA and tissue solving ability [22].

However, in comparison to other groups, it showed the lowest percentage in reduction of bacterial count which explained by reduction of calcium hydroxide PH due to buffering effect of dentin [23], It is well known that E. faecalis may endure in environments with pH values as high as 11.5 [5,24], so with the reduced pH value of Ca $(OH)_2$, the E. faecalis in the dentinal tubules could not be effectively eradicated.

In the present study activated charcoal had the highest percentage of reduction of E. faecalis count which may be attributed to its natural ability to absorb toxins, poisons, and noxious gases [25], in which microorganisms adhere to activated carbon particles by powerful Lifshitz van der Waals forces [26].

Activated charcoal/ calcium hydroxide combination group wasn't statistically significant different than activated charcoal group but had statistically significant higher percentage of reduction in E. faecalis count than calcium hydroxide group, as it has been shown that a synergistic antibacterial action would be possible against E. faecalis by adding antimicrobial agents to calcium hydroxide. [27].

The effect of activated charcoal on radicular dentin needs to be assessed in future research also its antibacterial efficacy should be evaluated in clinical studies to judge its effect in root canal treatment outcome.

CONCLUSION

Considering the limitations of the current investigation, it could be concluded that activated charcoal was more effective against E. faecalis than calcium hydroxide as an intracanal medication.Also, addition of activated charcoal to calcium hydroxide, increased its effectiveness against E. faecalis.

Conflict of interest

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

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