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THE ANTIBACTERIAL EFFECT OF CYSTEAMINE AND ITS COMBINATIONS WITH VARIOUS INTRACANAL MEDICATIONS AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Background: The microbiological ecosystem is considered the main obstacle in endodontics. Therefore, canal disinfection became a great priority.

Aim of the study: This study was established to assess cysteamine's antibacterial effect against E. Faecalis and compare the improvement effect to its combination with various intracanal medicaments.

Methods and Materials: Cleaning and shaping were performed on human single-rooted teeth then inoculated by E. faecalis biofilm. Samples were divided randomly into three groups: Group I to verify the maturation of E. faecalis biofilm inside the root canal using SEM. Group II were divided into four subgroups according to the intracanal medication used. Subgroup A was treated with Cysteamine. Subgroup B was treated with Chlorhexidine Cysteamine combination. Subgroup C was treated with Calcium hydroxide (CaOH) Cysteamine combination. Subgroup D was treated with Triple antibiotic paste Cysteamine combination. Group III were divided into two subgroups treated with plain gel: positive control and negative control. Samples collected from root canals were used for viable count (CFU/ml). Log transformation of the data was carried out. The statistical significance level was set at $P < 0.05$.

Results: There was a significant difference between different groups ($p<0.001$). Post hoc pairwise comparisons showed statistically significant difference between subgroups except for the CaOH Cysteamine combination subgroup and TAP Cysteamine combination subgroup.

Conclusion: Cysteamine usage provides a synergistic antibacterial effect with other intracanal medications. CHX Cysteamine combination was the most effective antibacterial drug that provided effectiveness against E. faecalis.

KEYWORDS: Cysteamine, Intracanal medication, TAP, Chlorhexidine, Calcium hydroxide, Antibacterial.

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INTRODUCTION

During the last years, biofilm formation inside root canals and its role in apical periodontitis was supported. Therefore, biofilm eradication and canal disinfection became a great priority in endodontic treatment[1,2]. This is achieved by compensatory techniques such as instrumentation to different canal sizes, irrigation protocols, and disinfectants that cooperate to eradicate endodontic infection. Although mechanical instrumentation of root canals can reduce bacterial population, effective elimination of bacteria can't be achieved without the use of antimicrobial root canal irrigation and medication[3]. There are many types of intracanal disinfectants with variable degrees of effectiveness. Further research is made to find the most effective one with the least adverse effects.

One of the medicaments under research is cysteamine which is used in many other fields of medicine. It is derived from cysteine and is the simplest amino thiol. Its pKa value is 9.42. It deprotonates in an alkaline environment and forms thiolate ions which are responsible for breaking the disulfide bond of bacterial proteins, by the active thiol group, due to which proteins are denatured and bacteria lose their structural integrity[4]. One of its advantages in endodontics is its mucolytic property, making it highly effective against different types of bacteria present in root canal biofilm. Breaking of mucopolysaccharides by cysteamine will disrupt the structural integrity of biofilm[5]. It is used as a resistant breaker for various antibiotics, so it's considered valuable material in the anti-bacteriology field.

Aim of the study

The objective of this study was to assess cysteamine's antibacterial effect against E. Faecalis and compare the improvement effect to its combination with various other intra-canal medicaments inside the root canal.

MATERIALS AND METHODS

Sample preparation

Extracted human teeth were collected and examined visually and radiographically according to definite criteria. Only single-rooted teeth devoid of caries with a single canal patent root canals devoid of root defects and fractures with mature apices were included. Roots with internal/external resorption, dilacerated roots, calcification, teeth with previous endodontic treatment, and immature ones were excluded. A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there was no difference between tested groups regarding antibacterial efficacy. Four teeth will be added and not included in the groups to evaluate microbial biofilm formation microscopically. So, all included samples were 44.

A total number of forty-four samples were randomly divided into three main groups: **Group I (observational group):** samples used to verify maturation of E. faecalis biofilm inside root canal (not medicated), n= four. **Group II (experimental group):** randomly selected samples were divided into four subgroups according to the intracanal medication used to investigate the antibacterial effect on E, faecalis biofilm, n= 32, **Subgroup A**, eight samples treated with Cysteamine. **Subgroup B**, eight samples treated with Chlorhexidine Cysteamine combination. **Subgroup C**, eight samples treated with Calcium hydroxide Cysteamine combination. **Subgroup D**, eight samples treated with Triple antibiotic paste Cysteamine combination. **Group III (control group):** eight samples were divided into two subgroups treated with plain gel (placebo) as follows: **Positive control subgroup:** Four infected samples by E. faecalis biofilm by immersion in infected saline with E. faecalis bacterial species for a 30-day incubation period. **Negative control subgroup:** Four Sterile samples, not infected by E. faecalis biofilm, by immersion and incubation in sterile saline for a 30-day incubation period.

Preparation of intracanal medications [6]

Cysteamine drug (Cysteamine 98%Rt, Fluka, Switzerland) was divided into small portions under nitrogen conditions using a nitrogen chamber to avoid oxidation during dispensing the drug. Fresh solutions were prepared to have maximized effect[7–9]. The powders of tested medications were measured and dispensed using sensitive balance. Liquids' volumes were measured using a graduated pipette. Every preparation after mixing was collected in a sterile 3ml plastic syringe, to be ready for direct delivery inside root canals.

Cysteamine preparation (subgroup A): was prepared by dissolving the powder in distilled water at a concentration of 200mg/ml.[6,10]. **Chlorhexidine Cysteamine combination (subgroup B):** A combination of Chlorhexidine 2% with cysteamine was prepared by dissolving 10 mg/ml cysteamine in CHX. **Calcium hydroxide Cysteamine combination (subgroup C):** fresh saturated solution of CaOH was prepared by mixing with distilled water at a concentration of 300mg/ml. A combination of cysteamine and CaOH was prepared by dissolving 10 mg/mL of cysteamine in CaOH solution [6]. **Triple antibiotic paste Cysteamine combination (Subgroup C):** TAP powder was prepared from three antibiotics in a tablet form as follows: Ciprofloxacin 250mg (Ciprobay500mg tablets, Hikma pharma S.A.E. Egypt), Metronidazole 500mg (Flagyl 500 mg tablets; Sanofi Aventis Pharma, Cairo, Egypt), and Doxycycline 100 mg(Doxycost 200 mg, Penta Pharma Egypt) [11]. The ratio for drug concentration was 1:1:1[12]. This concentration ratio was mixed with 2 ml distilled water till have a paste consistency. The total amount of powder used was 4.25 g and the total amount of water was 10 ml. A combination of cysteamine and TAP was done by dissolving 10 mg/mL of Cysteamine in TAP [6].

To prepare the gel form of medicaments, methylcellulose was used as an inert carrier [13] as follows: 20mg/ml of methylcellulose powder was added to the previously prepared solutions and to distilled water to form a plain gel (placebo) for the control group[13]. Except for the Triple antibiotic paste Cysteamine combination, 10mg/ml of methylcellulose powder was added to adjust the accepted consistency.

Preparation for all samples

For disinfection and detachment of tissue remnants, all extracted teeth included in our study were washed with 5% sodium hypochlorite and inspected for any remaining calculus or bony tissue. The crown of the tested samples was removed using a diamond disc and standardized to 15 mm in length [14]**.** Then the samples were stored in saline till usage. All teeth were explored using size 10 K-file, for checking the patency of the canal. After that, the canals were shaped using AF ONE rotary file system till tip size 25 and taper 0.06%. Continued apical preparation was done using manual files to size 50 K-file. 2 ml 2.5% NaOCl was used as an irrigant between each file[14]. The following protocol was used for the removal of the smear layer from root canal walls: 5 % sodium hypochlorite and 17% Ethylenediaminetetraacetic acid (EDTA) were respectively used for one minute, and normal physiological saline was introduced into the canals between the two previous irrigants. For removal of the remnants of EDTA and NaOCl, each tooth was rinsed with 10 mL of normal physiological saline[13]. Dryness with paper points was done. Longitudinal grooves were cut along the entire length in the observational group (group I) on mesial and distal surfaces. The apical foramen was closed with composite resin and exposed root surfaces were sealed with nail polish, except for the coronal access cavity. Finally, specimens were autoclaved at 121°C for 20 min[13].

E. faecalis biofilm inoculation in root canals *[15]*:

All procedures were done in aseptic conditions.

E. faecalis were cultured for 24 hours at 37°C in brain-heart infusion Brain heart infusion (BHI) broth. E. faecalis were collected by sterile loop and suspended in sterile Brain heart infusion (BHI) broth. E. Faecalis suspension with a standard concentration of No. 1 MacFarland turbidity standard was adjusted[16]. E. faecalis suspension was prepared in a jar for infection and inoculation of the observational group, experimental group, and positive control subgroup. 40 samples were transferred to the jar. Fresh E. faecalis suspension was used regularly every 48-h adjusted to the No. 1 MacFarland turbidity standard to replenish the broth to clear dead cells, ensure bacterial viability, and prevent sample dehydration. A sample was taken every three days and was cultivated on BHI agar plates and incubated for 24 hours at 37°C to confirm the purity of E. faecalis. Four samples acted as a negative control. To ensure absolute asepsis during the procedure, they were exposed to the same procedures for the previous 40 samples except that the suspension used was sterile in a separate sterile jar without infection with E. Faecalis and replenished every 48 hours with sterile saline. After that, all 44 samples were incubated in an incubator in a humid environment.

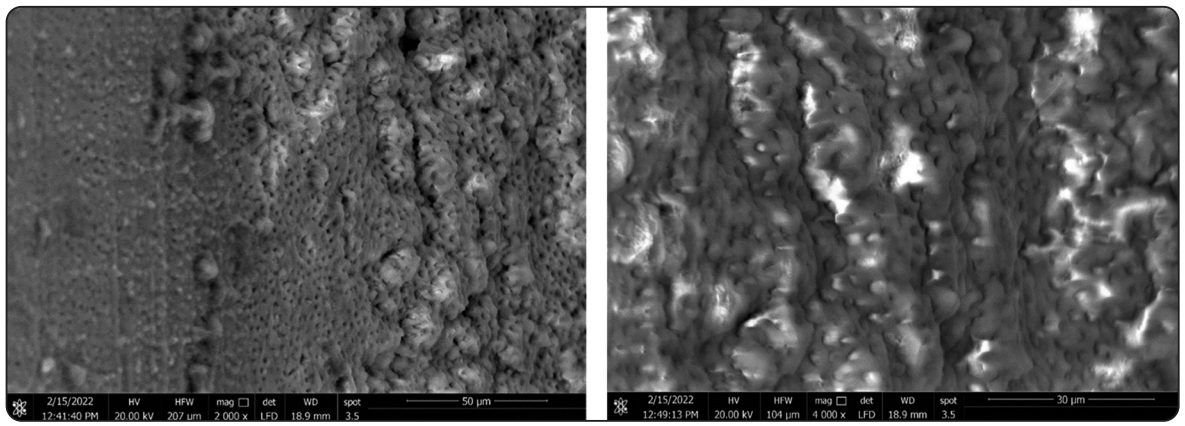

Fig. (1): SEM (2000X & 4000X) image of the root canal at 30 days inoculation with E. Faecalis, showing the extracellular polymeric matrix coating the dentinal surface.

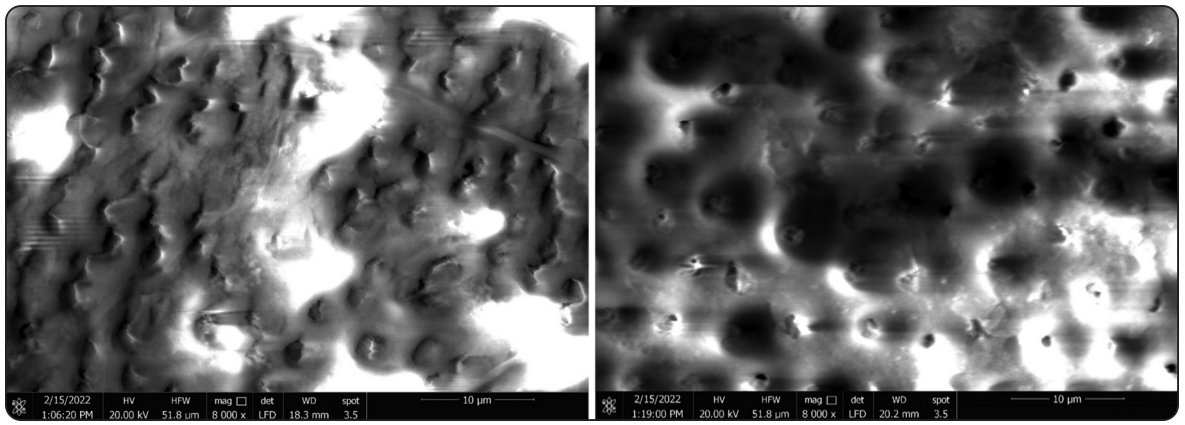

Fig. (2): SEM (8000X) images of root canals at 30 days inoculation with E. Faecalis showing bacterial aggregations and the dentinal tubule invasion.

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution and using the Shapiro-Wilk test. Bacterial count data showed non-parametric distribution and extreme positive skewness. Log transformation of the data was carried out to correct for the skewness. Leven's test showed a violation of variance homogeneity assumption so robust one-way ANOVA followed by Games Howell post hoc test was used for the analysis.

RESULTS

All samples were submitted for culture analysis on BHI agar. All samples exhibited CFU on culture plates. Logarithmic transformation (Log10 transformation) of CFU count was performed due to the high range of bacterial count. Mean and Standard deviation (SD) values for log bacterial count (CFU) for different groups were presented in **table (1)** and **figure (3).**

The highest values of Log10 CFU count were found in the positive control subgroup (12.55±0.08), and the lowest values of Log10 CFU count were found in the negative control subgroup (0.00±0.00). The Cysteamine subgroup showed the highest values of Log10 CFU count in the experimental group with Mean ± SD (10.15±0.24), followed by the CaOH Cysteamine combination subgroup with Mean ± SD (7.37±0.26). Lower results of Log10 CFU count were detected in the TAP Cysteamine combination subgroup with Mean ± SD (6.40±0.47) and CHX Cysteamine combination subgroup (3.37±2.81), which showed the lowest result in the experimental group. There was a statistically significant difference between different groups ($p<0.001$). Post hoc pairwise comparisons showed different groups to have significantly different values from each other except for the CaOH Cysteamine combination subgroup and TAP Cysteamine combination subgroup.

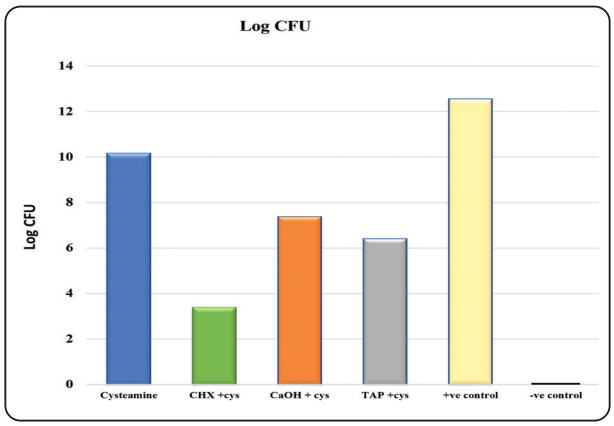

Fig. (3): Bar chart showing average log bacterial count, colony-forming unit (CFU) for different groups.

DISCUSSION

The microbiological ecosystem is considered the main obstacle in endodontics. Due to its variability and defending mechanisms, it has a big role in causing many endodontic and periodontal diseases. Microorganisms other than bacteria have been detected in association with endodontic infections. However, bacteria can be regarded as the major

TABLE (1): Mean and Standard deviation (SD) values for log bacterial count (CFU) for different groups.

Log bacterial count (CFU) (Mean±SD)						P-value
G II				G III		
Cys*	CHX** +Cys	CaOH*** + Cys	TAP**** +Cys	+ve control	-ve control	
$10.15±0.24^{B}$	$3.37±2.81^{D}$	$7.37±0.26^{C}$	$6.40±0.47^{C}$	$12.55±0.08^{A}$	$0.00±0.00^{E}$	<0.001*

Means with different superscript letters within the same horizontal row are significantly different *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$). *Cysteamine, **Chlorhexidine, *** Calcium hydroxide, ****Triple antibiotic paste.

microorganisms implicated in the pathogenesis of apical periodontitis due to their high prevalence, dominance, organization, and pathogenicity. In advanced stages of the endodontic infectious process, bacterial structures resembling biofilms are commonly observed adhering to the canal walls[18].

One of the disinfection protocols which are widely used is intracanal medications. They were having great importance as a disinfection protocol between endodontic visits in the past. Nowadays, intracanal medication usage becomes limited after following a single visit protocol in many procedures depending on the entomb theory described by **Peters et al.** [19]. Therefore, its usage shifts toward more specific conditions such as immature apices, trauma, inflammatory root resorption, and remains to have a great role as a treatment procedure for persistent diseases after root canal treatment, especially in retreatment cases with apical periodontitis.

Researchers regularly discover new intracanal medications which can fulfill the most requirements to be the safest and most effective disinfectants in the root canal system. Cysteamine material was selected due to its approval by FDA to be used in the medical field and it has many medical applications. It is an aminothiol endogenously synthesized by human body cells during the coenzyme A metabolism cycle. It is a well-tolerated compound demonstrating non-mutagenic and non-carcinogenic criteria. It also has an antibacterial effect [9].

The current study was carried out to test the antibacterial effect against E. faecalis using cysteamine material as a single protocol and in combination with chlorhexidine, triple antibiotic paste, and calcium hydroxide against E. faecalis inside the root canal. E. faecalis biofilm model was chosen due to its role in resistant periodontitis in retreatment cases because of its escaping mechanisms to resist standard disinfection protocols. E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells and alter host responses[20]. It also can share these virulence traits among species, increasing its chance for survival and causing disease[21]. E. faecalis can live in unfavorable conditions, such as growing in low oxygen, at high pH, at a wide range of temperatures between 10° and 60°, at high salinity, or in a poor nutrient environment. E. faecalis can use fluid in the periodontal ligament as nourishment[22].

The methodology followed for inoculation of mature E. faecalis biofilm in the root canal was as described by **Saber & El-Hady** [15] and the time selected for E. faecalis biofilm formation was 30 days to confirm biofilm formation on dentin substrate. E. faecalis suspension's concentration used for bacterial inoculation inside the root canal was adjusted on No. 1 McFarland turbidity standard. The McFarland Standard is a way used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate. When shaken well, the turbidity of a McFarland Standard is visually comparable to a bacterial suspension of known concentration [16].

E. faecalis can develop antibiotic resistance, especially to erythromycin and azithromycin[23]. So, it may be interesting to find that cysteamine material may reverse this resistance to azithromycin as described by **Fraser-Pitt et al.** [24] who found that cysteamine can act as a resistance breaker and can be used as a broad spectrum potentiator of antibiotics. Therefore, this may be helpful in E. faecalis resistance to any previously valuable antibiotics used. Cysteamine also has an antibacterial effect, as it deprotonates and forms thiolate anions which

disrupt both intramolecular and intermolecular disulfide bonds of bacterial proteins causing denaturation of key bacteria enzymes important for their metabolism and survival and weakening the structural integrity of the biofilm[4,6]. **Huang et al.** [25] combined cysteamine with copper nanoparticles to increase its antibacterial spectrum but there was no significant change as still had a quite limited effect on gram-negative bacteria. In the dental field, **Feldman et al.** [26] compared cysteamine antibacterial effect with NaOCl in different concentrations against E. faecalis and Fusobacterium nucleatum. They found comparable results with 8.25% NaOCl and that put cysteamine on the way to be considered a promising and interesting intracanal disinfectant that needs more research.

A pilot study was done to assess the antimicrobial effect of cysteamine against E. faecalis due to less use of it in the literature of endodontics and to exclude any storage problems that may affect its needed effect in our study. Three brain heart infusion agar plates were prepared. E. Faecalis was coated on two plates using a sterile swab and one plate was used as a negative control. A cup area in the agar was created and filled with Cysteamine at 200mg/ml concentration. Plates were incubated at 37°C and a zone of inhibition was detected after 24 hours to monitor its antibacterial effect [6]. Results of the pilot study confirmed the antibacterial effect against E. faecalis by the development of a zone of inhibition around the drug in the test plate and no change had occurred in other plates.

In our study, the Cysteamine drug was divided into small portions under nitrogen to avoid oxidation during dispensing the drug. Fresh solutions were prepared to have maximized effect, as cysteamine stability in high pH and normal atmospheric conditions is difficult due to quick oxidation reaction[7–9]. Complete eradication of bacterial biofilm was obtained at 200 mg/mL of Cysteamine. So, this concentration was selected in proportioning Cysteamine as an intracanal medication as described by **Guo et al.** [10]. In triple antibiotic paste, doxycycline was used instead of minocycline and Ciprofloxacin was in 250 mg concentration as described by **Abdelrahman et al**[11].

During gel formation, particle sizes of TAP after grinding were very large so the consistency of the mix was thick. Therefore, less amount of methylcellulose was used in TAP cysteamine gel. Methylcellulose, which is considered an inert material, was used to adjust medications' consistency in a more packable form to be maintained inside the root canal without leaching out and maintained drug contact inside the root canal for seven days [13]. 20mg/ml of methylcellulose powder was added to each of the following drugs: Cysteamine, Chlorhexidine Cysteamine combination, and Calcium hydroxide Cysteamine combination. For the Triple antibiotic paste Cysteamine combination, 10mg/ml of methylcellulose powder was added to adjust the accepted consistency.

Palin gel was formed by methylcellulose and distilled water to act as a placebo drug for the control group. The placebo-controlled trial is widely used as the gold standard for testing the efficacy of new treatments[27]. It allows proper randomization and controls bias. There is no fear of ethical conditions as the study is not directed at diseased human beings or animals. The dentin block model was used to standardize the in vitro testing of the effectiveness of root canal disinfecting agents, and it was followed by many authors in the literature. Preparations and irrigation of teeth samples were done as described by **Mozayeni et al. and Abbaszadegan et al**.[13,14].

The amount of saline used to remove remnants of sodium hypochlorite and EDTA was 10 ml to avoid their antibacterial properties affecting biofilm formation or causing bias in the antibacterial results of tested medications. The outer side (cement side) of the semi-cylindrical dentin pieces was closed by nail varnish before exposure to the antibacterial

solutions to simulate the difficulty these solutions have in vivo when penetrating dentin[28].

The time of application of intracanal medication inside the root canal was selected to be seven days as described by **Saber & El-Hady and Heidar et al**. [15,17]. This time interval was selected according to the time needed between visits for healing as it was found that the next appointment to fill non-vital cases should be scheduled approximately one week after instrumentation to maximize the antimicrobial effect of the intracanal medication when calcium hydroxide is used and to allow recovery of periapical tissue after cleaning and shaping[18].

Sampling collection after application of intracanal medication was done by irrigating the canals with 20 ml of saline to remove remnants of intracanal medications. Then, root canals were filled with sterile saline to act as transport fluid. Scrapping the dentin surface was done to have samples from deeper dentin levels. Paper points were used to absorb the transport fluid with dentin chips formed [15,29,30].

A culture-based method was followed in our study as many antibacterial detection studies on intracanal medications in literature as **Arias-Moliz et al., Saber & El-Hady, Pereira et al., and Punathil et al.**[15,31–33]. Culture-based studies and plate counting are considered the gold standard protocol for bacterial detection especially if the bacterial isolate is identified and no multi-microbial species are included in the study. It allows measuring viable bacterial cells as colony-forming units. Plate counting is considered the traditional approach to quantify bacteria in routine labs. It is very sensitive if plating conditions are optimal (theoretically, a single cell can be detected), and it allows for inspection and identification of the organism counted. It was easier to use than microscopic count using confocal microscopy (CLSM), as LIVE/DEAD staining combined with CLSM analysis was cost-intensive and needed special settings[34].

Despite, no problems were detected in the results of the culture method in detecting E. faecalis in our study, culturing methods can't be considered the most reliable way due to the state of viable but non-cultivable (VBNC) which occurred to E. faecalis under unfavorable conditions. They lose the ability to grow in culture media while maintaining viability and pathogenic effects, then they can resume cell division in optimal environmental conditions[18].To avoid VBNC, SEM was done for confirmation of biofilm formation. SEM is an excellent, highly descriptive observation method for any type of biofilm. Results of SEM showed mature biofilm formation of E. faecalis covering all the dentine surfaces as described by Saber & El-Hady and showed the survival strategy of E. faecalis through biofilm formation, aggregation, and invasion of dentinal tubules[15]. Also, Positive and negative control groups helped to avoid bias during antibacterial results.

In our study, Cysteamine intracanal medication showed the least antibacterial effect in comparison to other tested medications. This was in coordination with **Pandey et al.**'s results[6]. They disagreed with **Guo et al.** in that a 200 mg/ml concentration of cysteamine caused complete eradication of E. faecalis and this may be due to the difference in E. faecalis incubation period as they used 7-day old E. faecalis and our study used 30-day old biofilm[10].

Kim & Kim showed that Calcium hydroxide has less effect against Enterococcus faecalis and Candida Albicans and this agreed with **Pandey et al**. [6,35,36]. They showed that the least effect on E. faecalis was with Calcium hydroxide. The improvement effect of Cysteamine to antibacterial action Calcium hydroxide was supported by our results and others. In our study, the Calcium hydroxide Cysteamine combination effect was applied on 30-day E. faecalis biofilm and showed accepted results in comparison to positive control and showed a non-significant difference in

comparison to TAP Cysteamine combination. **Guo et al.** [10] also showed a synergistic antibacterial effect between CaOH and Cysteamine. Even in the presence of CaOH, Cysteamine deprotonates and disrupts the bacterial enzymes and membranes. They found that the combination of CaOH and 10 mg/mL Cysteamine was sufficient to completely eradicate seven-day E. faecalis biofilm on dentin. They also found that the antimicrobial properties of Cysteamine and CaOH remained unaffected by dentin even though the exposure of CaOH to dentin led to a significant decrease in its pH from 13 to 9. They interpreted that Cysteamine still deprotonates and forms thiolate anions at a pH of 9. Therefore, synergism between both drugs was still valid in the tooth model used in our study. This was also supported by **Pandey et al.** [6].

Ballal et al. & Jhamb et al. [37,38] confirmed that 2% CHX was much better in antibacterial effect than its combination with CaOH. While comparing the CaOH Cysteamine combination and the CaOH CHX combination, **Pandey et al.** [6] showed a non-significant difference in bacterial eradication. **Guo et al**. [10] agreed with them during applying the antibacterial test in the absence of dentin powder, but there was a significant improvement in the antibacterial effect of CaOH Cysteamine combination in presence of dentin powder. Controversial results were reported regarding the potency of both CHX and TAP, as TAP showed the highest antibacterial effect when compared with other intracanal medications as described by **Kim & Kim and Devaraj et al.**[36,39]. **Lakhani et al.** [40] found that 2% CHX gel was the most effective medicament against E. faecalis, as it showed significant differences with normal saline, calcium hydroxide, Moxifloxacin, or triple antibiotic paste at all time intervals. **Valverde et al.** [41] found that CHX was as effective as DAP and TAP as short-term intracanal medicaments. Moreover, long periods of intracanal medication would not be required to achieve effective disinfection of the root canals.

In our study, the CHX Cysteamine combination showed the highest antibacterial results followed by the TAP Cysteamine combination. No trials were found showing the effect of the Cysteamine and CHX combination. Our results were compared with studies using both drugs. There was disagreement with **Iglesias** [42] who compared different cysteamine concentrations with 2% CHX against E. faecalis and Candida Albicans and they found no significant difference between them. In our study, cysteamine showed the lowest antibacterial effect. This change in results could be due to many reasons as E. faecalis was inoculated in teeth, not discs, and formed a 30-day old biofilm. CHX was combined with cysteamine so this may be made it more potent than cysteamine and CHX if used alone. Therefore, this combination made a more potent intracanal medication.

TAP Cysteamine combination results showed a high antibacterial effect as agreed by **Pandey et al.** [6] who showed that the efficacy of TAP was enhanced by combining it with cysteamine. This may be attributed to the fact that cysteamine caused the breakdown of bacterial integrity which was further severed by the antimicrobial effect of the mixture of the other three antibiotics.

In our study, there was a non-significant difference between the antibacterial effect of the TAP Cysteamine combination and CaOH Cysteamine combination. This result disagreed with **Pandey et al.** [6] but supported the enhancement in the antibacterial effect of Cysteamine on other intracanal medications. The exact reason for the enhancement effect of Cysteamine on the antibacterial action of intracanal medicaments is not completely understood. It may be explained that cysteamine not just only denatures key bacterial enzymes which are important for their metabolism and survival but also causes the destruction of disulfide bonds of proteins acting synergistically with the intra-canal medicament.

CONCLUSION

Under the circumstances of this study, it can be concluded that Cysteamine usage provided a synergistic antibacterial effect with other intracanal medications. CHX Cysteamine combination and TAP Cysteamine combination were the most effective antibacterial drugs that provide effectiveness against E. faecalis.

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