



بسم الله الرحمن الرحيم

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Antibacterial And Cytotoxic Effects of Cysteamine Alone And in Combination With Various Intracanal Medications (In Vitro Study)

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by

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ
أَنْتَ الْعَلِيمُ الْحَكِيمُ}
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This work is dedicated to....

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List of abbreviations

Abbreviation	Full-term
RC	Root canal
NaOCl	Sodium hypochlorite
CHX	Chlorhexidine
EDTA	Ethylenediaminetetraacetic acid
CaOH	Calcium hydroxide
Cys	Cysteamine
CFU	Colony-forming unit
EF	E faecalis
BHI	Brain heart infusion
SEM	Scan electron microscope
OD	Optical density
UI	Microlitre
VBNC	Viable but non-cultivable

Introduction

During the last years, biofilm formation inside root canals and its role in apical periodontitis was supported. So, biofilm eradication and canal disinfection became a great priority in endodontic treatment. And 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⁽¹⁾.

The success of root canal treatment depends mainly on eliminating microbial contamination from the root canal system or decreasing their level to the extent that allows the immunity to deal with the remaining bacteria. The main reason for endodontic failure is the presence of some species that remain in root canals such as *Enterococcus faecalis*, which are more resistant to disinfectants, causing a persistent intra or extra-radicular infection⁽²⁾.

There are many types of intracanal disinfectants with variable degrees of effectiveness. Also, cytotoxicity level differs from one medicament to another, so further research is made to find the most effective one with the least adverse effects. Intracanal medications and their degradation products must be biocompatible with Periapical tissues as they are in close contact. Otherwise, these degradation products can

cause high levels of inflammation, stimulated by several mediators which can induce tissue destruction. Therefore, these drugs should provide the ability to induce repair in the injured area without interfering with cementum and bone formation⁽³⁾.

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. The pKa value of it 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 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. It is used as a resistant breaker for various antibiotics, so it's considered valuable material in the anti-bacteriology field.

Our hypothesis of this study is to assess its antibacterial effect against *E. Faecalis* and compare the improvement effect to its combination with various other intra-canal medicaments against *E. faecalis* inside the root canal, and to detect the cytotoxic effect of Cysteamine and its combinations on fibroblast cells to detect its level of acceptance to be used.

Literature review

I- Microbiology in endodontics and *E. faecalis* infection:

Endodontic microbiology has been studied for many years to reach observations that have an impact on the type of treatment and medication used. *E. faecalis* is gram-positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen and can grow in harsh environments as in extreme alkaline pH =9.6. Our challenge; as endodontic specialists, is to find methods to eradicate this microorganism during and after root canal treatment. At that time, using good aseptic technique, increasing apical preparation sizes, and inclusion of full-strength sodium hypochlorite and 2% chlorhexidine irrigants were the most effective methods to eliminate *E. faecalis*.

Stuart et al. ⁽⁵⁾ showed that the prevalence of *E. faecalis* is low in primary endodontic infections and high in persistent infections. They are more commonly associated with asymptomatic cases than with symptomatic ones.

Arias-Moliz et al. ⁽⁶⁾ studied the minimal film eradication concentration of different medications at different time intervals on *E. faecalis* biofilm and the results showed that NaOCl was the most effective after one minute at 0.00625% concentration and CHX was effective after five minutes at 2% concentration. Other medications such as EDTA, citric, and phosphoric acid were not effective at the tested time and concentrations.

Saber & El-Hady⁽⁷⁾ made a study to develop a mature biofilm of *Enterococcus faecalis* inside the root canal system and to test its susceptibility to some antimicrobial agents. Biofilm formation and maturation were monitored using SEM. Biofilms of bacteria were exposed to Amoxicillin +clavulanate, Ciprofloxacin, Clindamycin, Doxycycline, and calcium hydroxide as intracanal medications for one week. Results showed that SEM examination confirmed the formation of a mature biofilm at the end of the incubation period. All the chemotherapeutic agents used were significantly better than Calcium hydroxide in the elimination of biofilm bacteria. So, they concluded that the method used for bacterial biofilm development and maturation is reliable and can be used to assess the anti-bacterial potential of endodontic materials.

Alghamdi & Shakir⁽⁸⁾ discussed the effect of *Enterococcus faecalis* on endodontics treatment and the available treatment options to decrease the amount of *E. faecalis* during root canal treatment. They concluded that the role of *E. faecalis* in the failure of endodontic treatment is confirmed. Furthermore, *E. faecalis* has specific characteristics that enable it to escape chemo mechanical instrumentation during root endodontic treatment by having the ability to form biofilms and colonize in remote unreachable areas away from the main canals, such as accessory canals, apical deltas, and isthmuses. In addition, *E. faecalis* uses different mechanisms to survive in harsh environments, as activating some survival genes, using alternative metabolic pathways, living in an area with high sources of nutrients, and possessing bacterial synergism and aggregation capacity.