Antibacterial Effectiveness of Calcium Hydroxide Combined with Chlorhexidine or Sodium Hypochlorite Vehicle Against Gram Positive and Gram Negative Bacteria in the Root Canal. Adding of CHX may enhance the antibacterial activity of Ca(OH)2 against klebsiella spp. as a gram negative bacteria, while adding of NaOCl enhance the bactericidal of Ca(OH)2 against both types of bacteria with interesting effect on streptococcus spp. as gram-negative bacteria. However, they were not able to produce complete eradication of bacteria. Till date, there is no literature investigate the antimicrobial efficacy of Ca(OH)2/CHX combination against klebsiella spp.. Intra-canal medicaments and irrigations, Dentistry/ Endodontology.


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Antibacterial Activity of Calcium Hydroxide Combined with Chlorhexidine or Sodium Hypochlorite against Gram Positive and Gram Negative Bacteria

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Dedication

To our parents
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1. Introduction

Bacteria play a fundamental role in the etiology of pulpo-periapical pathosis (Haapasalo & Qian, 2008), and non-vital teeth have been considered as foci of infection. They are sources for bacteria with their toxins which may escape from the tooth and circulate throughout the body to localize on a new tissue and cause an infection in distant part of body (Murray & Saunders, 2000 & Walsh, 1997). The polymicrobial nature of endodontic infection has been stated, where predominant microbial groups frequently isolated from infected root canal are the aerobic and facultative anaerobic organisms (Baumgartner & Falker, 1991). Among these streptococcus spp. are one of the most commonly identified bacteria penetrating the root dentinal tubules and considered as a major cause of endodontic failure ( Perez et al., 1993 & Gajan et al., 2009). It was established that the streptococci, enterococci and lactobacilli appear to survive commonly and recover following root-canal treatment of non-vital teeth with apical periodontitis (Chávez et al., 2003). Besides that, the transformation of streptococcus to enterococcus faecalis had been reported (Schleifer & Kilpper-Balz, 1984). Regarding the gram-negative bacteria, it was also reported that klebsiella has been isolated from non-vital teeth (Little, 1975) and may play a significant role in clinical condition of flaring up where it was determined as a cause ( Chaudhry et al., 1997). Klebsiella spp. Frequently causes pneumonia, urinary tract infection, wound infection, and bacteremia (Podschun & Ullmann, 1998). Klebsiella has developed resistant to chlorhexidine which could be clinically more problematic (Hammond et al., 1987 & Thomas et al., 2000).

However, endodontic treatment is mainly based on the removal of potentially noxious stimuli from the complex root canal system. This treatment is more difficult if the infection is spread through the root canal. Although the variety of endodontic instrumentations and irrigations exist, it has been reported that debris is regularly left behind. Thus, since, the maximum reduction of the
root canal microorganisms has been introduced as a major goal of endodontic therapy. It may be achieved with the aids of numerous materials and several irrigating solutions (Bystrom & Sundqvist, 1981).

During endodontic treatment, the main goal of instrumentation is to enable easy and efficient irrigation, disinfection and filling. Many researches have demonstrated that large areas of the root canal walls untouched by the endodontic files, emphasizing the significance of using chemical substances for cleaning and disinfecting all the root canal areas.

2. Intra-canal medicaments

Endodontic treatment of teeth with a vital pulp does not require intra-canal medication. However, if the treatment is not finished in one appointment, it is generally recommended that the root canal should be filled between appointments with an antibacterial dressing, for instance, Ca(OH)$_2$, to provide sterility in the canal space until a permanent root filling is placed. However, no studies available, comparing the bacteriological condition of the root canals following root canal treatment, when the canals have been left empty or filled with an antibacterial dressing.

The role of intra-canal medicaments becomes more interesting, and complex, in the treatment of apical periodontitis and pulpal necrosis. In the literature, there is overwhelming evidence that many if not most root canals contain viable microorganisms following the chemomechanical preparation completion at the end of the first appointment (Peters et al., 2002; Sathorn et al., 2007; Card et al., 2002). Thus, a variety of intra-canal medicaments have been applied between appointments to provide disinfection of the root canal system. Also, intra-canal medicaments may have other beneficial activities. For instance, Ca(OH)$_2$ neutralizes the biological action of bacterial lipopolysaccharide (Tanomaru et al., 2003). It also makes necrotic tissue more susceptible to the solubilizing activity of NaOCl at the next appointment. Another advantage in
using intra-canal medicaments may be that, a more thorough instrumentation is achieved because
of the longer overall time used for the endodontic therapy. On the other hand, high number of
appointments can also increase the risk for aseptic complications, for example, through a leaking
temporary filling and poor patient compliance (Siren et al., 1993).

Numerous studies have showed a poorer prognosis of the treatment of apical periodontitis if
viable bacteria are residing in the root canal system at the time of filling (Engstrom, 1964; Katebzadeh et al., 2002). Other studies, however, have contradicted these findings and
demonstrated no significant differences in healing between teeth filled after positive or negative
cultures from the root canal, or between treatments performed in one or two appointments (Peters
et al., 2002; Weiger et al., 2000). It has also been suggested that "intracanal sampling techniques
suffer from deficiencies that limit their predictive value." (Sathorn et al., 2007). A permanent root
filling of high quality using endodontic cements with antibacterial activity can positively and
effectively seal and entomb residual microorganisms in the canal and prevent communication
with periradicular tissues. Continued killing of the micro-organisms could take place as a result of
the antibacterial activity of the root-filling materials and unavailability of nutrients (Saleh et al.,
2004).

3. Ideal requirements of intra-canal medicaments

- It should be an effective germicide and fungicide.
- It should be not irritating to the periapical tissue.
- It should remain stable in solution.
- It should be active in the presence of blood, serum and protein
derivatives of tissues.
• It should have low surface tension and easily diffusible.
• It should not interfere with the repair of periapical tissues.
• It should not stain the tooth structure.
• It should be capable of inactivation in a culture medium.
• It should not induce a cell mediated immune response.
• It should be easy to handle (mix, place and remove).
• It should not be very expensive.

4. Calcium hydroxide

Ca (OH)\textsubscript{2} has unique properties to be considered as an ideal root canal dressing such as tissue dissolving capability, antimicrobial effect, biocompatibility and maintenance in root canal for a long time. It also promotes an alkalinizing osteogenesis through the continuous release of OH- ions (Mustafa et al., 2012). Also, it has low water solubility that is useful clinical characteristic as the long period is required for Ca(OH)\textsubscript{2} to be soluble in tissue fluids through the direct contact with the vital tissues (Farhad and Mohammadi, 2005). However, Ca(OH)\textsubscript{2} cannot be considered as a universal intra-canal medicament, since it is not equally effective against all bacteria found in the root canal. In fact, several studies have reported the failure of Ca(OH)\textsubscript{2} to eliminate enterococcus faecalis effectively as they tolerate high pH values (Gomes et al., 2002&Pinheiro et al., 2003). Ca(OH)\textsubscript{2} mixed with CHX to fulfill antimicrobial requirements of an intra-canal medicament (Valera et al., 2009&Farhad et al., 2012). Moreover, Ca (OH)\textsubscript{2} has the ability to promote the formation of hard tissues (Silveira et al., 2011).

Calcium hydroxide is a strong alkaline material with pH approximately 12.5. However, most
endodontic pathogens are not survived in a highly alkaline environment provided by Ca (OH)$_2$. As well, Ca(OH)$_2$ is a white powder without odor and low water solubility, which decreases as the temperature increase. Its antimicrobial activity is related to the release of hydroxyl ions in an aqueous medium. These ions are highly oxidant free radicals, which exhibit extreme reactivity and reacting with many biomolecules. The lethal impact of the free radicals on bacteria is probably because the damage to the bacterial cytoplasmic membrane, protein denaturation or DNA damage (Siqueira and Lopes, 1999). Figure 1 describe the possible mechanism of Ca(OH)$_2$.

Ca (OH)$_2$ in the availability of water → Calcium ions+ Hydroxyl ions

![Diagram](image)

Figure 1: A schematic view of calcium hydroxide mechanisms. Adopted from (Mohammadi et al., 2012).

1- **Damage to the bacterial cytoplasmic membrane**

Many important functions that are necessary for the bacterial cell survival are possessed by the cytoplasmic membrane, for example:

1. excretion of hydrolytic exoenzymes.
2. electronic transport and oxidative phosphorylation in aerobic species.
3. selective permeability and transport of solutes.
(4) having carrier molecules and enzymes that work in DNA biosynthesis, membrane lipids and polymers of cell wall.

(5) carrying essential elements for chemotactic and other sensory transduction systems.

Hydroxyl ions promote peroxidation of lipid leading to the destruction of phospholipids, structural components of the cellular membrane. Furthermore, hydroxyl ions eliminate hydrogen atoms from unsaturated fatty acids, producing a free lipid radical. This radical react with oxygen, giving rise to lipid peroxide radical that removes another hydrogen atom from a second fatty acid, resulting in lipid peroxide. Therefore, peroxides work as radicals and leading to more loss of unsaturated fatty acids and extensive damage to the cell membrane.

2- **Protein denaturation**

The enzymatic activities greatly control the cellular metabolism. Optimum activity and stability of enzymes are achieved in a narrow range of pH, which turns around neutrality. The high alkalinity environment provided by Ca (OH)$_2$ induce the ionic bonds breakdown that keep the tertiary structure of proteins. Consequently, the enzymes maintain their covalent structures but the polypeptide chains are randomly unraveled in variable and irregular special conformations. These frequent changes lead to the loss of enzymatic biological activities and cellular metabolism disruption. Damage to the structural proteins by hydroxyl ions might occur.

3- **DNA damage**

Splitting of DNA is promoted due to the interaction between bacterial DNA and hydroxyl ions, resulting in gene lost. Subsequently, the replication of DNA is inhibited, and the cellular activity is discharged. Lethal mutations may also be induced by the free radicals.

Scientific finding believes that three techniques may occur. Therefore, it is difficult to define in a chronological order, which is the main mechanism responsible for bacterial cell death following
exposure to a strong base. The suggestion is that the ability of calcium hydroxide to absorb carbon dioxide contributing to its antibacterial activity has been developed. However, cementum has permeability to water, ions, and small molecules. Hence, the remaining bacteria in the root canal may maintain the supply of carbon dioxide from the outside environment. Additionally, bacteria found in ramification have a direct source of carbon dioxide from the periradicular tissues. Little reasons are available to consider calcium hydroxide impedes the supply of carbon dioxide to the bacteria. A series of researches demonstrated the antimicrobial activity of Ca(OH)2 (Table 1). Leonardo et al., (2000) evaluated the antimicrobial activity of two Ca(OH)2 pastes as intra-canal medicament against seven bacterial strains, and both of them were effective for all strains. Mehrvarzfar et al., (2011) compared bioactive glass with Ca(OH)2. They found that both showed antimicrobial activity against E. faecalis with a superior disinfecting effect of Ca(OH)2. Moreover, the researchers found that Ca(OH)2 effectively inhibited the growth of all examined microorganisms following 24 hours, using agar diffusion method. However, there was a difference in the sensitivity of each microorganism, such that Streptococcus mutans was the most sensitive and E. faecalis was the least (Pavelić et al, 1991).

However, calcium hydroxide has a limited antibacterial activity that does not affect all the endodontic bacterial membranes. Moreover, the physiochemical characteristics of Ca(OH)2 may limit its effectiveness in disinfecting the entire root canal system following a short-term usage. Many studies have reported the ineffectiveness of Ca(OH)2 as an antimicrobial agent. Table (2) indicates a brief description of these studies. For instance, studies compared the antimicrobial activity of Ca(OH)2 with other agents reported a less activity of calcium hydroxide more than others (Badr et al., 2011; Mattigatti et al., 2012).

Several studies examined the antimicrobial activity of vehicles in which Ca(OH)2 was one of the ingredients. (Table 3). For example, a study found that adding certain agents to Ca(OH)2 did not
affect its antimicrobial activity (Estrela et al., 2001). However, Games et al., (2002) reported that some vehicles agents had an impact on the antimicrobial activity of Ca(OH)$_2$. For instance, adding camphorated paramonochlorophenol (CMCP) increased the effectiveness of Ca(OH)$_2$, while adding water or glycerine showed little or no effect.

Table 1: Studies reporting antimicrobial effect of Ca(OH)$_2$.

**Adopted from Kim and Kim, 2014**

<table>
<thead>
<tr>
<th>Year</th>
<th>Researchers</th>
<th>Test method</th>
<th>Microbial Strains</th>
<th>Major ingredients</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>Pavelić et al.</td>
<td>ADT</td>
<td>2 (+)</td>
<td>CH</td>
<td>1, 2 day</td>
</tr>
<tr>
<td>1992</td>
<td>Gencoglu &amp; Kulekci</td>
<td>DET</td>
<td>4</td>
<td>CH, CMCP, IKI, cresophene</td>
<td>10, 15 min</td>
</tr>
<tr>
<td>1993</td>
<td>Alaçam et al.</td>
<td>DET</td>
<td>6</td>
<td>CH, NaOCl, metronidazole</td>
<td>0 - 3 day</td>
</tr>
<tr>
<td>1993</td>
<td>Georgopoulou et al.</td>
<td>DET</td>
<td>30</td>
<td>CH, CMCP</td>
<td>3 - 60 min</td>
</tr>
<tr>
<td>1995</td>
<td>Kontakiotis et al.</td>
<td>DET</td>
<td>40</td>
<td>CH</td>
<td>3 day</td>
</tr>
<tr>
<td>1996</td>
<td>Barnard et al.</td>
<td>DET</td>
<td>1</td>
<td>CH, NaOCl</td>
<td>1 - 30 min, 7 day</td>
</tr>
<tr>
<td>1998</td>
<td>Estrela et al.</td>
<td>DET</td>
<td>6</td>
<td>CH</td>
<td>0 - 7 day</td>
</tr>
<tr>
<td>2000</td>
<td>Leonardo et al.</td>
<td>ADT</td>
<td>7 (+)</td>
<td>CH, ZnO</td>
<td>2 hr</td>
</tr>
<tr>
<td>2003</td>
<td>Morrier et al.</td>
<td>ADT</td>
<td>3 (+)</td>
<td>CH, 5 commercial</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Researchers</td>
<td>Test method</td>
<td>Microbial strains</td>
<td>Major ingredients</td>
<td>Period</td>
</tr>
<tr>
<td>------</td>
<td>----------------------</td>
<td>-------------</td>
<td>-------------------</td>
<td>----------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>2003</td>
<td>Podbielski et al.</td>
<td>DET</td>
<td>5 (+)</td>
<td>CH, ZnO</td>
<td>0 - 14 day</td>
</tr>
<tr>
<td>2006</td>
<td>Amorim Lde et al</td>
<td>DET/ADT</td>
<td>4 (+)</td>
<td>CH, Vitapex, ZOE, TC</td>
<td>0 - 3 day</td>
</tr>
<tr>
<td>2007</td>
<td>Ferreira et al.</td>
<td>BDT</td>
<td>5(+)</td>
<td>CH, 6 antibiotics</td>
<td>1 hr</td>
</tr>
<tr>
<td>2007</td>
<td>Tanomaru et al.</td>
<td>ADT</td>
<td>5</td>
<td>CH, CH + CMCP</td>
<td>2 day</td>
</tr>
<tr>
<td>2008</td>
<td>Blanscet et al.</td>
<td>ADT</td>
<td>6 (+)</td>
<td>40, 50, 60%-CH, UltraCal, Vitapex</td>
<td>2, 4 day</td>
</tr>
<tr>
<td>2011</td>
<td>Mehrvarzfar et al.</td>
<td>DET</td>
<td>1 (+)</td>
<td>CH, BAG</td>
<td>0 - 3 day</td>
</tr>
</tbody>
</table>

(+) *E. faecalis* was included as a subject of the experiment. ADT, Agar diffusion test; DET, Direct exposure test; BDT, Broth dilution test, CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide; NaOCl, Sodium hypochlorite; ZnO, Zinc oxide; ZOE, Zinc oxide eugenol; TC, Tetracycline; BAG, Bioactive glass. Vitapex, Neo-Dental Int., Federal Way, WA, USA; UltraCal, Ultradent Products Inc., South Jordan, UT, USA.

Table 2 Studies Ca(OH)2 reporting to be ineffective.

*Adopted from Kim and Kim, 2014*
<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Test</th>
<th>Time</th>
<th>Microorganisms</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>Barbosa et al.</td>
<td>DET</td>
<td>11(+)</td>
<td>CH, CHX, NaOCl, H2O2, EDTA</td>
<td>1-60min, 1week</td>
</tr>
<tr>
<td>2002</td>
<td>Ferreira et al.</td>
<td>BDT</td>
<td>4</td>
<td>CH, CHX, CMCP</td>
<td>2,4days</td>
</tr>
<tr>
<td>2002</td>
<td>Rosa et al.</td>
<td>BDT</td>
<td>3</td>
<td>CH, CHX, FC, CMCP</td>
<td>2, 4days</td>
</tr>
<tr>
<td>2007</td>
<td>Reddy &amp; Ramakrishna</td>
<td>ADT</td>
<td>26(+)</td>
<td>CH, ZOE, CP, Metapex</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Badr et al.</td>
<td>ADT/BDT</td>
<td>1(+)</td>
<td>CH, Liquorice</td>
<td>2 day</td>
</tr>
<tr>
<td>2012</td>
<td>Adl et al.</td>
<td>ADT</td>
<td>1(+)</td>
<td>CH, Antibiotics</td>
<td>7 days</td>
</tr>
<tr>
<td>2012</td>
<td>Hegde et al.</td>
<td>ADT</td>
<td>7(+)</td>
<td>ApexCal, Metapex, Endoflas, ZOE</td>
<td>1-2 day</td>
</tr>
<tr>
<td>2012</td>
<td>Mattigatti et al.</td>
<td>ADT</td>
<td>2(+)</td>
<td>CH, CHX, NaOCL, EDTA, MTAD, Propolis</td>
<td>2 day</td>
</tr>
</tbody>
</table>

(+), *E. faecalis* was included as a subject of the experiment. DET, Direct exposure test; BDT, Broth dilution test; ADT, Agar diffusion test; CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide; CHX, Chlorhexidine; NaOCl, sodium hypochlorite; H2O2, Hydrogen peroxide; EDTA, Ethylenediaminetetraacetic acid; FC, Formocresol; ZOE, Zinc oxide eugenol; CP, Camphorated phenol. Metapex, Meta Biomed Co., Ltd., Cheongju, Korea; ApexCal, Ivoclar Vivadent, Schaan, Liechtenstein; Endoflas, Sanlor Laboratory, Miami, FL, USA; MTAD, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA.
Table 3 Studies on the effect of Ca(OH)$_2$ mixed with vehicles or other agents. Adopted from Kim and Kim, 2014

<table>
<thead>
<tr>
<th>Year</th>
<th>Researchers</th>
<th>Test method</th>
<th>Microbial Strains</th>
<th>Major ingredients “Mixed”</th>
<th>Period</th>
<th>Effect (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Siqueira &amp; Uzeda</td>
<td>ADT</td>
<td>12 (+)</td>
<td>CH, CHX, CMCP</td>
<td>7 day</td>
<td>-</td>
</tr>
<tr>
<td>1998</td>
<td>Siqueira &amp; Uzeda</td>
<td>BDT</td>
<td>4 (+)</td>
<td>CH, CMCP</td>
<td>0 - 3 day</td>
<td>+</td>
</tr>
<tr>
<td>2001</td>
<td>Estrela et al.</td>
<td>DET</td>
<td>5 (+)</td>
<td>CH, CHX, CMCP</td>
<td>0 - 7 day</td>
<td>+</td>
</tr>
<tr>
<td>2001</td>
<td>Estrela et al. 49</td>
<td>DET/ADT</td>
<td>4 (+)</td>
<td>CH, PEG, CMCP</td>
<td>0 - 3 day</td>
<td>+</td>
</tr>
<tr>
<td>2002</td>
<td>Gomes et al.</td>
<td>ADT</td>
<td>11 (+)</td>
<td>CH + 7 vehicles</td>
<td>0 - 7 day</td>
<td>+/-</td>
</tr>
<tr>
<td>2002</td>
<td>Gomes et al.</td>
<td>ADT</td>
<td>11 (+)</td>
<td>CH + 7 vehicles</td>
<td>1 - 7 day</td>
<td>+/-</td>
</tr>
<tr>
<td>2003</td>
<td>Basrani et al.</td>
<td>ADT</td>
<td>1 (+)</td>
<td>CH, CHX</td>
<td>7 day</td>
<td>-</td>
</tr>
<tr>
<td>2003</td>
<td>Lin et al.</td>
<td>ADT</td>
<td>1 (+)</td>
<td>CH, CHX</td>
<td>1, 3 days</td>
<td>-</td>
</tr>
<tr>
<td>2005</td>
<td>Vianna et al.</td>
<td>BDT</td>
<td>5 (+)</td>
<td>CH + 6 vehicles</td>
<td>0 - 7 day</td>
<td>+</td>
</tr>
<tr>
<td>2006</td>
<td>Gomes et al.</td>
<td>DET/ADT</td>
<td>5 (+)</td>
<td>CH, CHX</td>
<td>1 - 2 day</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>Ballal et al.</td>
<td>ADT</td>
<td>1 (+)</td>
<td>CH, CHX</td>
<td>2, 3 days</td>
<td>+/-</td>
</tr>
<tr>
<td>2007</td>
<td>Neelakantan et al.</td>
<td>ADT</td>
<td>3 (+)</td>
<td>CH, CHX</td>
<td>0 - 3 days</td>
<td>-</td>
</tr>
<tr>
<td>2008</td>
<td>de Souza-Filho et al.</td>
<td>ADT</td>
<td>6 (+)</td>
<td>CH, CHX</td>
<td>1 - 2 day</td>
<td>+/-</td>
</tr>
<tr>
<td>2009</td>
<td>Turk et al.</td>
<td>ADT</td>
<td>1 (+)</td>
<td>CH + 7 vehicles</td>
<td>1 day</td>
<td>+/-</td>
</tr>
<tr>
<td>2010</td>
<td>Jhamb et al.</td>
<td>ADT</td>
<td>1 (+)</td>
<td>CH, CHX</td>
<td>3 day</td>
<td>-</td>
</tr>
<tr>
<td>2011</td>
<td>Gangwar</td>
<td>ADT</td>
<td>6 (+)</td>
<td>CH + 4 vehicles</td>
<td>1,4,7 day</td>
<td>+/-</td>
</tr>
<tr>
<td>2012</td>
<td>Pacios et al.</td>
<td>ADT</td>
<td>6 (+)</td>
<td>CH + 9 vehicles</td>
<td>2 day</td>
<td>+/-</td>
</tr>
</tbody>
</table>

(+), *E. faecalis* was included as a subject of the experiment; +/-, the result showed a limited effect. ADT, Agar diffusion test; BDT, Broth dilution test; DET, Direct exposure test, CH,
Calcium hydroxide; CHX, Chlorhexidine; CMCP, Camphorated paramonochlorophenol; PEG, Polyethylene glycol.

In brief the researches have differed on evaluating the antimicrobial activity of Ca(OH)$_2$ according to the culture medium, inoculum size, and age, a bacterial strain used and methodology. As well, the equivalent root canal environment could not be ensured on the condition of experiments.

**Effects of Ca(OH)$_2$ on endotoxin**

Endotoxin is present on all gram-negative bacterial. It is composed of proteins, polysaccharides and lipids and is referred as lipopolysaccharide (LPS), emphasizing its main chemical structure (Mohammadi et al., 2012). Lipid A is the zone that is responsible for toxic effects of endotoxin. On the free activity, endotoxins do not cause tissue or cell pathogenesis; they stimulate the release of chemical mediators. Thus, intrinsically, endotoxins are not toxic (Leonardo et al., 2004).

However, LPS is released during multiplication or bacterial death on the root canal treatment, leading to a series of biological impacts that produce the inflammatory reactions and peripheral bone resorptions (Yamasaki et al., 1992). High prevalence of gram-negative anaerobic bacteria disseminates throughout the root canal system in teeth with chronic peripheral lesions. The use of intra-canal medication is recommended to help in the elimination of these bacteria particularly, in the areas that are difficult to instrument and increase the possibility of clinical success (Nelson-Filho et al., 2002). Safavi and Nichols, (1993) demonstrated the ability of Ca(OH)$_2$ to hydrolyse the lipid A, which is the high toxic and damaging molecule of endotoxins. Another study indicated that Ca(OH)$_2$ transformed lipid A to nontoxic components, namely fatty acids and amino sugars (Safavi and Nichols, 1994).
5. Chlorhexidine gluconate

CHX has been used widely as an irrigant as well as an intra-canal medicament through the endodontic treatment of teeth with apical periodontitis. Chlorhexidine has broad spectrum antibacterial activity. The antimicrobial effect of CHX is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering the cell's osmotic equilibrium (Mohammadi & Abbott, 2009). Many researchers reported that CHX was the more effective than NaOCl against anaerobic bacteria (Ohara et al., 1993 & Jeansonne & White, 1994). Ferraz et al. (2001) also claimed the antimicrobial property of 2% CHX gel. When used as an intra-canal medicament, CHX was more effective than Ca(OH)_2 against enterococcus faecalis infection in dentinal tubules. Gram-negative bacteria are less susceptible to CHX than gram-positive bacteria (Gomes et al., 2001 & Hugo 1992). Many types of bacteria may develop resistance to CHX, including klebsiella (McDonnell & Russell AD, 1999). However, although CHX lack the property of tissue-dissolving, which one of useful advantage of NaOCL, it has some advantages over the NaOCL because it’s less toxicity to the host tissues and present substantivity to dentine that may lead to residual antimicrobial activity for up to seven days (Jose et al., 2007). CHX does not erode dentine structure as NaOCl does as the final irrigants following EDTA, and thus, 2% CHX solution may be a best choice to maximize the antibacterial action at the end of chemomechanical preparation (Haapasalo et al., 2010). Furthermore, it possesses lubricating characteristics, rheological activity (available in gel, keeping the debris in suspension), it is stable chemically, it inhibits the metalloproteinase, and it is odor less. CHX has been recommended as a substitute to NaOCL, particularly in conditions of root resorption, open apex, root perforation and foramen enlargement because of its biocompatibility, or in the cases of allergy related to bleaching solutions (Gomes et al., 2013). Endodontic literatures reported that 0.1-0.2% of aqueous CHX solutions are used in periodontics, while 2% concentrations have been considered as an irrigants
for root canals (Zamany et al., 2003). Currently, CHX is considered the gold standards of oral antiseptics and is with the fluoride the most extensively preventive agents in dental researchers (Fardal et al., 1986). CHX is more stable in the salts form that suffer from the poor water solubility and thus replaced by the digluconate in 1957, which is high water soluble salt. Aqueous form of CHX is more stable with pH range of 5-8. However, the antimicrobial activity of CHX is depended on pH, with a maximum range 5.5-7, within which is the pH of body tissues and surfaces (Gomes et al., 2013).

A clinical study assessed the degree of microbial reduction following chemo-mechanical preparation of root canals containing necrotic pulp tissue using two endodontic irrigating substances, 5.25% NaOCl or 2% CHX gel. Evaluation of the bacterial load was accomplished by use of real-time quantitative-polymerase chain reaction (RTQ-PCR) directed against the small subunit ribosomal DNA using the SYBRGreen and TaqMan formats. The bacterial load was reduced substantially in both groups (Over 96%). The bacterial reduction in the NaOCl-group (SYBRGreen 99.99%; TaqMan: 99.63%) was significantly greater (p<0.01) than in the CHX-group (SYBRGreen 96.62%; TaqMan: 96.60%), probably due to the differences between the mechanisms of action of NaOCl and CHX (Vianna et al., 2006).

On the other hand, Ferraz et al. (2001) demonstrated that 2% gel of CHX produced the cleanest dentin wall surfaces in comparison with other irrigants, including NaOCl. As a result of its viscosity and rheological properties that maintains the debris in suspension, the gel seems to compensate for CHX’s inability to dissolve pulp tissue, by inducing a better mechanical cleansing of the root canal and removing dentin debris and remaining tissues. The mechanical characteristics of the gel seem to be the primary factor for this difference due to the same chemical agents in the liquid form produced lower cleaning efficiency, although presenting similar antimicrobial activity.
In addition to that, a study compared the effectiveness of calcium hydroxide Ca(OH)$_2$, iodine potassium iodide (IKI) and a CHX solution in disinfection root canal systems, which were contaminated with Actinomyces israelii. The root canals were exposed to either IKI, calcium hydroxide or 2% CHX for periods of 3, 7 and 60 days. CHX was the only disinfectant that was able to remove A. israelii from all samples at all time periods, while 25% of the specimens treated with IKI and 50% of the specimens treated with Ca(OH)$_2$ still had viable A. israelii after treatment (Basson & Tait, 2001). Also, 2% CHX was more effective against enterococcus faecalis than 5.25% NaOCl (Oncag et al., 2003).

Mohammadi and Abbott, (2009) revealed that “Both the 2% gel and 2% liquid formulations of CHX eliminated Staphylococcus aureus and Candida albicans within 15 s, whereas the gel formulation killed E. faecalis within 1 min. All of the tested irrigants eliminated Porphyromonas endodontalis, Porphyromonas gingivalis, and Prevotella intermedia within 15 s. The time required for 1.0% and 2.0% CHX liquid to eliminate all microorganisms was the same as the time required for 5.25% NaOCl. These studies confirm that the antimicrobial action is related to the type, concentration and presentation form of the irrigants as well as the microbial susceptibility to the formulation used”.

Siqueira et al., (2007) compared the effectiveness of 2.5% NaOCl and 0.12% CHX as irrigants in decreasing the cultivable bacteria in infected root canal systems of teeth with apical periodontitis. They reported that the two irrigants had comparable impacts in removing bacteria, and they suggested that both could be used as irrigant solutions.

In a randomized clinical trial, Manzur et al. (2007) evaluated the antibacterial efficacy of intra-canal medication with Ca(OH)$_2$, 2% CHX gel and a combination of both Ca(OH)$_2$ and CHX in teeth with chronic apical periodontitis. Bacteriological samples were obtained from the operative field and the root canals before and after instrumentation in the first treatment session. More
samples were taken from the canals at the commencement of the second appointment one week later. They pointed out that the antibacterial efficacies of Ca(OH)$_2$, CHX and a mixture of Ca(OH)$_2$/CHX were comparable.

6. CHX and Ca(OH)$_2$

Chlorhexidine is an irrigating solution with optimal antimicrobial activity is achieved within a pH range of 5.5–7.0 (Athanassiadis et al., 2007). Thus, it is probably that alkalinizing the environment by adding Ca(OH)$_2$ to CHX will result in precipitation of the CHX molecules and thereby reduces its activity. The researchers had been demonstrated that the alkalinity of Ca(OH)$_2$ stayed unchanged during mixing with CHX. Therefore, the benefits of mixing Ca(OH)$_2$ with CHX is still controversial and unclear (Athanassiadis et al., 2007). The CHX as an intra-canal medicament was more effective than Ca(OH)$_2$ in removing E. faecalis from inside dentinal tubules (Athanassiadis et al., 2007). Almyroudi et al. (2002), reported that all of several CHX formulations tested, encompassing a CHX/ Ca(OH)$_2$ 50:50 mix, were effective in removing E. faecalis from the dentinal tubules with a 1% CHX gel acting slightly better than the other preparations. These findings were in consistent with results by Gomes et al. (2006) in bovine dentine and Schafer & Bossmann (2005) in human dentine where 2% CHX gel had a higher activity against E. faecalis, followed by CHX/ Ca(OH)$_2$ and then Ca(OH)$_2$ used alone. Using agar diffusion could not demonstrate any additional antibacterial impact by mixing Ca(OH)$_2$ powder with 0.5% CHX and the researchers reported that the CHX had a reduced antibacterial action (Haenni et al., 2003). However, Ca(OH)$_2$ maintains its antibacterial properties in such a mixture. This may be because of the deprotonation of CHX at a pH >10, which decreases its solubility and changes its interaction with bacterial surfaces as a result of the altered charge of the molecule. In an in vitro study using human teeth, Erkan et al. (2006) found that the 2% of CHX gel was the most efficient preparation against E. faecalis inside dentinal tubules, followed by a
Ca(OH)$_2$/ 2% CHX mix, while Ca(OH)$_2$ alone was completely ineffective, even after 30 days. The 2% CHX gel was also significantly more efficient than the Ca(OH)$_2$/2% CHX mix against C. albicans at 7 days, although there was no significant difference at 15 and 30 days. Ca(OH)$_2$ alone was completely ineffective against C. albicans. This finding was supported by an in vivo study using deciduous teeth, that indicated that a 1% CHX-gluconate gel, both with and without Ca(OH)$_2$, was more effective against E. faecalis than Ca (OH)$_2$ alone over a two days period (Oncag et al., 2006).

Schafer & Bossmann (2005) and Lin et al. (2003) showed that the 2% CHX gluconate was significantly more effective against E. faecalis than the Ca(OH)$_2$ used alone or a mixture of the two. However, a study by Evans et al. (2003) used bovine dentine postulated that the 2% CHX with Ca(OH)$_2$ was shown to be more effective than Ca(OH)$_2$ in water. Lindskog et al. (1998) reported that teeth dressed with CHX for four weeks had decreased the inflammatory reactions in both apical and marginal periodontium and less root resorption, in animal teeth. Furthermore, Waltimo et al. (1999) showed that the 0.5% CHX-acetate was more effective than the saturated Ca(OH)$_2$, in killing C. albicans, while the Ca(OH)$_2$ combined with the CHX was more effective than Ca(OH)$_2$ used alone. The high alkanity of Ca(OH)$_2$ was unaffected during combination with CHX in this study. Taken together, it seems that the usefulness of mixing Ca(OH)$_2$ with CHX remains unclear and controversial.

Sinha et al., (2013) compared the antimicrobial activity of calcium hydroxide, 2% CHX and a combined product of both against anaerobes and Candida species. The results showed effective antimicrobial activity of all three medicaments against obligate anaerobes microorganisms. The researchers concluded that CHX with or without Ca(OH)$_2$ was more effective than calcium hydroxide alone against all the tested microorganisms.
7. Sodium hypochlorite

NaOCl has been represented as the most popular irrigant with excellent tissue dissolving and antibacterial activities (Thomas et al., 2000). Beside their wide spectrum with non-specific killing effects on all microbes, hypochlorite preparations are sporicidal, viricidal (Weine, 1982), and show far greater tissue dissolving effects on necrotic than on vital tissues (Austin & Taylor, 1918). The antimicrobial effectiveness of NaOCl is based in its high pH (hydroxyl ion action). It interferes with the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation (Estrela et al., 2002). Sodium hypochlorite is an antimicrobial agent. Its germicidal activity is related to the hypochlorous acid (HOCl) that forms when the NaOCl contacts the organic debris (Basrani et al., 2007). At the neutral pH, chlorine remains predominantly as HOCl, while, at high pH, OCl⁻ ion is the predominant. This ion is less effective than the un-dissolved HOCl. Hypochloric acid is responsible for the antibacterial activity by disrupting many functions of microbial cell, leading to cell death (Barrettte et al., 1989).

Sodium hypochlorite is a strong antimicrobial irrigants, killing most bacteria. It is effective dissolving agent for the pulp remnants and collagen. Among the available irrigating solutions, NaOCl is the only irrigants that dissolve both vital and necrotic organic tissues. Although, NaOCl alone does not eliminate the smear layer, it affects the organic portion of smear layer facilitating its complete removal by following irrigation such as EDTA or citric acid (Haapasalo et al., 2010). In the literature, there is a great variation regarding the antimicrobial activity of NaOCl. Some articles reported the ability of hypochlorite to kill targeted bacteria in seconds, even at low concentrations, and some showed a longer time for the same bacterial species. Such difference is due to the presence of organic materials that work as confounding factors. These organic
materials affect the antibacterial activity of NaOCL by delaying its killing activity (Haapasalo et al., 2010).

Valera et al. (2009) stated that 1% NaOCl was effective in the immediate reduction of candida albicans and enterococcus faecalis counts after root canal preparation. In contrast Verrisismo et al. (2010) reported that NaOCl showed the worst performance when used alone as intra-canal medicament. This probably occurred because NaOCl loses its antimicrobial properties and becomes ineffective inside the canal after short period. Additionally, Portenier et al., (2005) concluded that 0.0001% of NaOCL is able to kill enterococcus faecalis nearly after contact, while another study reported that 0.0005% of NaOCL can eliminate these bacteria in three minutes (Zender et al, 2002). However, Retamozo et al., (2010) pointed out that the higher concentration of NaOCL and more time contact are necessary for E. faecalis elimination.

Many studies have been carried out to measure the antibacterial activity of NaOCl. A study evaluated the antibacterial effectiveness of several irrigants against different bacteria. The results showed that the antibacterial activity of 2.5 % NaOCl and 4% NaOCl was significantly higher than other tested substances (Siqueira et al., 1998).

Vianna et al, (2004) studied the antibacterial activity of five concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%) and compared the results with different concentrations of CHX (0.2%, 1%, and 2%). The findings showed that all the irrigants removed Porphyromonas gingivalis, Porphyromonas endodontalis, and Prevotella intermedia in 15 seconds. The time needed for 5.25% NaOCl to remove all microorganisms was the same required for 1% and 2% CHX. Furthermore, the study evaluated several concentrations of NaOCl (0.5%, 2.5%, and 5.25%) as intra-canal irrigants against enterococcus faecalis associated with hand and rotary instruments. The researchers found that the concentration of 5.25% was the most effective irrigants followed
by 2.5% concentration (Berber et al., 2006).

Antibacterial effectiveness of 0.5% NaOCl was assessed in vivo study, using 15 single rooted teeth. No antibacterial intra-canal treatment was used between appointments. The results showed that at the fifth appointment, no bacteria could be recovered from twelve of fifteen teeth in 0.5% NaOCl compared to eight root canals in the normal saline group (Bystrom & Sundqvist, 1983).

However, injection of sodium hypochlorite inadvertently beyond the apical foramen may occur in teeth with wide, apical foramina or teeth with destroyed apical constriction as a result of root canal preparation or resorption. As well, high pressure from the irrigation or binding of irrigated needle tip in the root canal without coronal release of irrigant may lead to the contact of a large amount of irrigant solution with apical tissues. Consequently, tissue necrosis will result due to the dissolving ability of sodium hypochlorite (Hulsmann & Hahn, 2000).

However, the calcium hydroxide is not effective against all the kinds of endodontic bacterial infections. Enterococci bacteria are presented in one-third of the patients in whom root canal treatment has failed. Since, the Ca(OH)\(_2\) is not effective against enterococci, many recent studies have confirmed the advantage of combination of Ca(OH)\(_2\) powder with antibacterial endodontic irrigating solutions to produce a wider long-lasting antimicrobial effect (Molander et al., 1998). It has been found that the association of NaOCl with Ca(OH)\(_2\) shows equal antibacterial activity to Ca(OH)\(_2\) and CHX combination. Zehnder et al. (2003) reported a quicker antimicrobial effect for Ca(OH)\(_2\) with NaOCl in comparison to Ca(OH)\(_2\) with water. Farhad et al. (2012) stated that using of CHX as a vehicle to Ca(OH)\(_2\) revealed more significant antibacterial effect than its mixture with H\(_2\)O. The antibacterial activity of Ca(OH)\(_2\) and NaOCl did not differ significantly from Ca(OH)\(_2\) and CHX or Ca(OH)\(_2\) /H\(_2\)O mixtures. Unlike NaOCl, CHX has a property the substantivity which allows prevention of microbial colonization on dentine surface for some time.
beyond the actual period of medicament application (Athanassiadis et al., 2007). On the other hand, NaOCl can dissolve remnant debris in the canal, a property which is desired from an intra-canal medicament. However, NaOCl has limited capacity to penetrate into dentinal tubules (Valera et al., 2009). Ca(OH)$_2$/NaOCl combination can dissolve the remnant debris and tissues, in addition to its antibacterial activity (Moorer & Wesselink, 1982). Furthermore, many researchers reported that the antimicrobial activity of Ca(OH)$_2$ are raised significantly when addition CHX in a paste (Evans et al., 2003). A clinical study reported that the dressing of infected canal with a mixture of CHX (2%) and Ca (OH)$_2$ was at least as effective as Ca(OH)$_2$ in the disinfection of treated canals with apical periodontitis. Following the using this medication for 7-10 days the percentage of culture negative canals was 65% (Zerella et al., 2005).

Microorganism might be infiltrated into the canal between endodontic treatment appointments via the poor temporary sealant. Thus, the use of a root canal sealing is fundamental for the good disinfection of root canal following mechanical debridement and prior to the canal obturation (El Karim et al., 2007).

8. Klebsiella bacteria and root canal failure

The main factor in endodontic treatment is identifying and eliminating the reason for the apical periodontitis development. Thus, the optimal outcome can be obtained. Bacteria and their by-products play a significant role in the pathogenic process of apical periodontitis. Therefore, the main goal in root canal treatment is to remove bacteria and sources of nutrient supply from the root canal system (Baumgartner and Falkler, 1991).

The failure of root canal treatment is because the survival of microorganisms in the apical third of root-filled tooth. The dominant microorganisms in the secondary endodontic infection are one or
few bacterial species in opposite to the primary infection, which is usually polymicrobial in nature and mainly gram-negative anaerobic rods (Baumgartner and Falkler, 1991; Hancock et al., 2001).

Topley and Wilson, (1990) stated that klebsiella is non-motile, gram negative, non-sporulating, facultative anaerobic bacilli, which is 0.3- 0.5μ by width and 2-5μ in length. It has large irregular capsule. The most prominent among Klebsiella is K pneumonia. In the oral cavity of a healthy person, Klebsiella may occur sporadically and in small numbers. In oral infections K. pneumonia, may develop under conditions, such as:

(1) nosocomial infection
(2) Poor oral hygiene
(3) Extensive dental caries
(4) High alcoholic intake
(5) Leukemia
(6) Immuno compromised patients

The teeth in which endodontic treatment has been compromised or grossly inadequate for example: repeated but unsuitable antibiotic therapy, multiple openings of root canal, inadequate periapical surgical treatment, in such instances Klebsiella species and yeast are recovered. The presence of these and other mainly non oral microorganisms suggests that a blood-borne infection of the periapical lesion may take place (Tronstad and Sunde, 2003). Antibiotic resistance has developed approximately in all classes of bacteria of pathogenic potential. Resistance in organisms of low virulence can emerge as important pathogens. Use of these in turn has the appearance of bacteria with newer modes of resistance.
In the endodontic infection, a variety of bacteria has been isolated in the past. This diversity is best seen in untreated root canals with a necrotic pulp, of symptomatic or non-symptomatic teeth. The microbial composition of the endodontic infected teeth resembles that of the subgingival flora of marginal periodontitis, and most of the microorganisms recovered are also involved. Similar to the causes of marginal periodontitis, the etiological and pathological roles of many root canal organisms is not well known, but the microbial specificity in apical periodontitis seems to be low. However, there are reasons to believe that some species or groups of microorganisms are more significant than others. This is indicated by their expression of various virulence factors (Oslen and Dahlen, 2004).

A study isolated and determined the viable bacteria on infected root canal of fifty patients based on culture. The researchers isolated eighty-one strains of bacteria with streptococcus species and klebsiella species reported the highest strains with 51 and 17 strains respectively. Klebsiella species resented 21% of the isolated species (Ufomata and Akerele, 1992).

Nowadays, Ca (OH)$_2$ combined with distilled water (DW) is the most common used mixture for intra-canal medication. This is because its characteristics such as fast ionic dissociation into alkaline ions, cost effectiveness, ease of use, and also its ability to minimize osteoclastic activity and simulate the repair (Farhad & Mohammadi, 2005).

The aim of this study is to evaluate the effect of adding CHX or NaOCl on the antibacterial activity of Ca(OH)$_2$ against gram positive and gram negative bacteria which had been isolated from primarily infected root canals.
9. Materials and Methods

9.1 Sample selection

This study was carried out in the dental clinics of College of Dentistry in University of Kufa during the period between September to December 2013. The bacterial samples were randomly selected from 40 patients. Each patient has at least one symptomatic nonvital tooth of an X-ray of periapical involvement.

9.2 Collection of specimens

The bacterial samples were taken under aseptic conditions. The involved tooth was isolated. The field was disinfected, and the accessed cavity was prepared with a sterile round bur. After gaining access to the pulp, a sterile reamer was inserted apically into the root and root canal contents were obtained for culture. Reamer containing root canal contents was placed in tubes containing brain heart infusion broth. Then all the tubes immediately transferred to the laboratory for bacteriological study (McFadden 2000).

9.3 Isolation of bacteria

Root canal contents were inoculated on blood agar and MaConkey agar then incubated at 37°C for 24 hours. Colony characteristics were noted in the case of any growth and identification of microorganisms was done according to the morphology using gram staining and by biochemical reactions (Oguntebi et al., 1982).

Culturing: the isolated bacteria test: streptococcus spp. and klebsiella spp. were subcultured onto appropriate culture media under gaseous conditions for 48 hours on sheep blood-brain heart infusion (BHI) agar plates at 37°C for 24 hours.

The tested materials were grouped into 5 groups of 20 samples for each group:
G1: sterile paste made of calcium hydroxide mixed with water at 1mg/1ml (Ca(OH)$_2$/H$_2$O)

G2: 2% chlorhexidine solution (CHX).

G3: 2.5% sodium hypochlorite solution (NaOCl).

G4: sterile paste made of calcium hydroxide mixed with 2% chlorhexidine at 1g/1ml (Ca(OH)$_2$/CHX).

G5: sterile paste made of calcium hydroxide mixed with 2.5% sodium hypochlorite at 1g/1ml (Ca(OH)$_2$/NaOCl).

Ten samples from each group had been inoculated with streptococcus spp. and the other 10 had been inoculated with klebsiella spp. Then all groups cultured under gaseous conditions on sheep blood-brain heart infusion (BHI) agar plates at 37°C for 24 hours. With respect to the 24 hours bacterial growth plates which showed no growth were further incubated up to 24 hours before deciding it as a negative result. Microbial growth was verified by gram stain and light microscope.

**9.4 Statistical analysis:**

Microbial growth was verified and numbers of plates which show growth or no growth for streptococcus spp. or klebsiella spp. were counted and analyzed statistically using Chi-square with p-value 0.05
10. Result

Table (4): Growth comparison of streptococcus and klebsiella in relation to tested materials

<table>
<thead>
<tr>
<th>Tested materials</th>
<th>Streptococcus spp.</th>
<th>Klebsiella spp.</th>
<th>Chi square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Growth</td>
<td>No.</td>
<td>Growth</td>
</tr>
<tr>
<td>Ca(OH)$_2$</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>CHX</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>NaOCl</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Ca(OH)$_2$/CHX</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Ca(OH)$_2$/NaOCl</td>
<td>10</td>
<td>2</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

Table (4) illustrates the number of plates of streptococcus ssp. and klebsiella ssp. which shows growth or no growth after treatment with tested materials. Paste of Ca(OH)$_2$ /H$_2$O had an effect against both types of bacteria with no significant statistical difference.

2% CHX appeared to be less effective with no significant statistical difference in the antibacterial effectiveness of 2% CHX against both types of bacteria.

2.5%NaOCl revealed to be effective against both types of bacteria with no significant statistical difference in the antibacterial effectiveness of 2.5%NaOCl on streptococcus spp. or klebsiella spp..

The more surprising correlation is with the adding of 2% CHX to Ca(OH)$_2$ had a synergistic effect on its antibacterial activity against both types of bacteria with significant effect on klebsiella spp. While mixing of NaOCl with Ca(OH)$_2$ affected both types with higher effect on
streptococcus spp.

Table (5): Comparison of Ca(OH)\(_2\) with Ca(OH)\(_2\)/CHX

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Ca(OH)(_2)/H(_2)O</th>
<th>Ca(OH)(_2)/CHX</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>No growth</td>
<td>Growth</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

As shown in table (5), there was no statistical significant differences between antibacterial activity of Ca(OH)\(_2\) and Ca(OH)\(_2\)/CHX.

Table (6): Comparison of Ca(OH)\(_2\)/H\(_2\)O with Ca(OH)\(_2\)/NaOCl

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>CaOH(_2)/H(_2)O</th>
<th>CaOH(_2)/NaOCl</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>No growth</td>
<td>Growth</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table (6): reveals a comparison between the antibacterial activity of Ca(OH)\(_2\)/H\(_2\)O and Ca(OH)\(_2\)/NaOCl. There are significant statistical differences with encouraging effect especially
on streptococcus spp.

Table (7): Comparison of Ca(OH)$_2$/H$_2$O with Ca(OH)$_2$/NaOCl

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Ca(OH)$_2$ /CHX</th>
<th>Ca(OH)$_2$ /NaOCl</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>Growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>8</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

According to the table (7), there was a significant difference between the Ca(OH)$_2$/CHX and Ca(OH)$_2$/NaOCl.

Table (8): Comparison of 2% CHX with Ca(OH)$_2$/CHX on streptococcus spp.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>CHX</th>
<th>Ca(OH)$_2$ /CHX</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>No growth</td>
<td>Growth</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table (8) compares the antibacterial activity of 2% CHX and Ca(OH)$_2$/CHX on streptococcus spp. There was no significant statistical difference between two tested materials.
Table (9): Comparison of 2% CHX with Ca(OH)$_2$/CHX on klebsiella spp.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>2%CHX</th>
<th>Ca(OH)$_2$ / CHX</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>No growth</td>
<td>Growth</td>
</tr>
<tr>
<td>Klebsiella ssp.</td>
<td>9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table (9) evaluates the antibacterial activity of 2%CHX and Ca(OH)$_2$+CHX on Klebsiella spp. .There was significant statistical synergistic effect.

Table (10): Comparison of 2.5% NaOCl with Ca(OH)$_2$/NaOCl

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>2.5%NaOCl</th>
<th>Ca(OH)$_2$ /NaOCl</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>No growth</td>
<td>Growth</td>
</tr>
<tr>
<td>Streptococcus ssp.</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella ssp.</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table (10) compares the antibacterial activity of 2%NaOCl and Ca(OH)$_2$/NaOCl on both types of bacteria. There was significant statistical difference with an interesting effect for Ca(OH)$_2$/NaOCl on G+ve bacteria.
11. Discussion

Shaping and cleaning of root canal is of great significance in endodontic therapy. However, the quality obturation plays an important role in the successful endodontic treatment. This is supported by the fact that 60% of endodontic failure is attributed to the incomplete obturation of root canal systems. Radical elimination of microorganisms from infected root canals is a difficult task and various measures have been recommended to reduce the numbers of endodontic microorganisms, including the use of irrigation regimens and intra-canal medicaments (Gomes et al., 1996). In addition to the chemo-mechanical debridement of intra-canal medications, they also reduce bacterial load and prevent coronal microleakage during endodontic therapy (Bystrom & Sundqvist, 1981; Spangberg & Haapasalo, 2002). These medications produce their effect either physically by preventing bacterial penetration or chemically by killing microorganisms that exist in root canals. Ca(OH)₂ has been established as antimicrobial agent and it was reported that it may be the best available inter appointment medication (Law & Messer, 2004). However, no irrigating solution can remove all the organic and inorganic materials completely and at the same time has antibacterial property (Basrani et al., 2007).

The present study revealed its effectiveness against the streptococcus and klebsiella spp. that is in agreement with the finding of Gomes et al. (2002)as they stated that, Ca(OH)₂ is equally effective against all bacteria found in the root canal.

Its high pH (approximately 12-12.5) has a destructive effect on bacterial cell membranes and protein structures (Mustafa et al., 2012). However, Ca(OH)₂ failed to eradicate all the tested bacteria. It has been suggested to mix Ca(OH)₂ powder with antimicrobial endodontic irrigants to obtain a wider antimicrobial spectrum with a long lasting effect (Waltimo et al., 1999). Different
vehicles have been added to Ca(OH)₂ in an attempt to improve its antimicrobial activity, biocompatibility, speed of ionic dissociation, and diffusion (Fava & Saunders, 1999).

NaOCl and CHX are antimicrobial agents frequently used in root canal therapy as irrigant as well as intra-canal medicament (Zehnder et al., 2003). Efficacy of CHX is because of the interaction of the positive charge of its molecules with the negatively charged phosphate groups on microbial cell walls (Mohammadi & Abbott, 2009). CHX has wide spectrum antimicrobial activity and prolonged action. Cervone et al. (1990) demonstrated that CHX has inhibitory effects on bacteria commonly found in endodontic infections while other researcher stated that gram-negative bacteria are less susceptible to CHX than gram-positive bacteria (McDonnell & Russell, 1999). However, in the present research it appeared to be less effective against both types of bacteria.

The combination of Ca(OH)₂ and CHX has been used with an encouraging result (Mohammadi & Abbott, 2009; Gomes et al., 2006; Evans et al., 2003). The aim of combining Ca(OH)₂ and CHX is to increase the antimicrobial properties of Ca(OH)₂ and act as an adjunct to destroy bacteria due to its antimicrobial activity and substantivity (Gomes et al., 2002). A number of studies using in-vitro or in-vivo models have stated that the antimicrobial efficacy of CHX/Ca(OH)₂ against enterococcus. faecalis is more than Ca(OH)₂ alone (Cervone et al., 1990; Ercan et al., 2006). Another study revealed that the combination of Ca(OH)₂ and CHX resulted in 100% antibacterial activity against enterococcus faecalis after one or two days, but this activity is reduced between 7 and 15 days. The researchers also reported that chlorhexidine gel alone completely inhibited the bacterial growth, while Ca(OH)₂ allowed microbial growth during all the experiment time (Gomes et al., 2002). Other studies using different study designs have not found the same results (Schafer & Bossmann, 2005 & Zerella et al., 2005). We didn't find the research has investigated the antimicrobial effect of Ca(OH)₂/CHX combination against klebsiella spp.
However, the methods of evaluating the antibacterial impact of different materials can produce conflicting findings. Estrella et al. (2003) compared the antibacterial activity of NaOCl and CHX utilizing agar diffusion and direct exposure techniques. The researchers reported that NaOCl exhibited more antibacterial activity in direct exposure technique and CHX showed more antibacterial effect in agar diffusion method. Agar diffusion technique produces an inhibition zone around the discs containing the agent. In this method, the size of the microbial inhibition zone relies to a large extent on the solubility and infusibility of the test materials and thus may not express its full potentiality (Farhad et al., 2012).

This study illustrated the support of the CHX to the bacteriocidal effect of Ca(OH)$_2$ with interesting effect on klebsiella. Regarding the effect of this mixture against streptococcus , our result was in disagreement with the finding of Manzur et al. (2007) who assessed the antibacterial efficacy of intra-canal medication with Ca(OH)$_2$, 2%CHX gel and a combination of both Ca(OH)$_2$ and CHX in teeth with chronic apical periodontitis. They concluded that the antibacterial efficacies of Ca(OH)$_2$, CHX and mixture of Ca(OH)$_2$/CHX were comparable. The finding of this study is comparable with the result of Silvera et al. (2011) as they assessed antibacterial activity of four formulations of Ca(OH)$_2$ pastes against Streptococcus as one of the tested bacteria. They found that the association of Ca(OH)$_2$ and CHX showed a better performance than Ca(OH)$_2$ alone.

The application of CHX is based on its ability to change the osmotic equilibrium of bacterial cells. Haenni et al. (2003) showed that the antimicrobial action of CHX is lowered when combined with Ca (OH)$_2$, but calcium hydroxide did not lose its antimicrobial activity in such a mixture. Ercan et al. (2006) reported that 2% CHX gel was the most effective material against E.faecalis inside dentinal tubules followed by a Ca (OH)$_2$/2% CHX, and Ca (OH)$_2$ alone was
totally ineffective even after 30 days. Nevertheless, Ca (OH)$_2$ has unique properties such as capability of tissue dissolving, antimicrobial activity, maintenance in root canal for a long time and biocompatibility, which cannot be ignored (Sjogren et al., 1997). The mixing of Ca(OH)$_2$ with CHX can fulfill substantial antimicrobial requirements of an intracanal medicament such as the ability to remove E. faecalis, the most commonly isolated species from root canals of teeth with failed endodontic treatment (Valera et al., 2009).

The present study has revealed the effectiveness of NaOCl against both bacteria. The effectiveness of NaOCl as an irrigant solution has been confirmed by several researches. It is widely used in endodontic, and 2.5% NaOCl concentration was selected because this is a commonly used in endodontic treatment. It can preserve a sufficient amount of chlorine to eliminate a significant amount of bacterial, comparable with the effect of a higher concentration (Siquiria et al., 2000).

The present study revealed that the NaOCl had stronger antibacterial effect than the CHX. This is supported by finding of Sprat et al. (2001). They studied the effectiveness of 2.25%NaOCl, 0.2%CHX and 10% povidone iodine against five root canals isolated bacteria including P.intermedia, streptococcus intermedius, peptostreptococcusmiros, fusobacteriumnucleatum and enterococcus faecalis . They reported that NaOCl was the most effective antimicrobial agent followed by iodine solution.

Valera et al. (2009) showed that immediately following endodontic treatment, 1% of NaOCl was effective in reducing E.faecalis and C.albicans counts. However, the study of Verrisimo et al. (2010) revealed that the NaOCl had the worst performance as an intracanal medicament when used alone. The authors revealed this probably due to the NaOCl becomes ineffective inside the canal through a short period and loses its antimicrobial activity. It has been found that the mixture
of NaOCl and Ca(OH)$_2$ shows equal antibacterial activity to Ca (OH)$_2$/CHX (Zehnder et al., 2003). This result was similar to the findings of the study of Farhad et al., (2012).

On the other hand, our finding was disagreed with the finding of Sequeira et al. (2007). They compared the effectiveness of 2.5%NaOCL and 0.12%CHX. They found that both irrigants had the ability to reduce the cultivable bacteria in infected root canals. Furthermore, Ercan et al. (2004) finding didn’t in consistent with our research, when they evaluated the antibacterial efficacy of 2% CHX and 5.25% NaOCl in infected root canals of incisor and premolars. They concluded that both CHX and NaOCl were effective irrigants for reducing the number of microorganisms in teeth with necrotic pulp and/or periapical pathosis.

In comparison of Ca(OH)$_2$, CHX and NaOCl, the present study has illustrated that there was no significant difference between the effect of Ca(OH)$_2$ and NaOCl on streptococcus spp. and klebsiella spp. with antibacterial stronger than CHX. This is in disagreement with the statement of Elca et al. (2005). They conducted a study to evaluate their antibacterial effects against streptococcus pyogenes, streptococcus mitis, streptococcus bovis, streptococcus anginosus, enterococcus faecalis, staphylococcus aureus. Ca(OH)$_2$ had the strongest impact in direct contact with the studied microorganisms, followed by 0.1% CHX and 3% NaOCl.

Unlike CHX, NaOCl can dissolve remnant debris in the canal, a property that is desired from an intra-canal medicament. However, NaOCl has limited penetration capacity into dentinal tubules (Valera et al., 2009). On the other hand, CHX has a substantivity property, which allows prevention of microbial colonization on dentine surface for some time beyond the actual period of time of medicament application (Athanassiadis et al., 2007).

Our research verified that when Ca(OH)$_2$ is mixed with NaOCl, the antimicrobial efficacy of the mixture is greater than Ca(OH)$_2$ by itself with significant effect on streptococcus. To some
extent, this finding is comparable to result of Farhad et al., (2012) as they compared the antibacterial effectiveness of Ca(OH)$_2$ in combination with H$_2$O, CHX, or NaOCl against enterococcus faecalis. Their findings indicated that the antibacterial potency of Ca(OH)$_2$ can be enhanced by preparing it with antibacterial irrigants such as CHX or NaOCl.
12. Conclusion

Within the limitation of this experimental study, involving only two bacterial species, it can be concluded that adding of CHX may enhance the antibacterial activity of Ca(OH)$_2$ against klebsiella spp. as a gram negative bacteria, while adding of NaOCl enhance the bactericidal of Ca(OH)$_2$ against both types of bacteria with interesting effect on streptococcus spp. as gram-negative bacteria. However, they were not able to produce complete eradication of bacteria.

Till date, there is no literature investigate the antimicrobial efficacy of Ca(OH)$_2$/CHX combination against klebsiella spp.
13. Suggestion

1-Further research is strongly recommended to investigate the antimicrobial effect of the combinations against other types of microorganisms and using different study methodology.

2-Further studies should be done to secure the biocompatibility of Ca(OH)$_2$ combination as an introduction for a clinical application as intra-canal medicament, periodontal pack and infected wound dressing.

3-Further studies should also evaluate the possible chemical interactions between different irrigating solutions and the effect of the reaction on the irrigant properties.
14. References


Elka Radeva , B Indjov, R Vacheva.(2005). Antibacterial activity of intracanal medicaments against bacterial isolates in cases of acute periapical periodontitis (non-exudative form). J of IMAB - annual proceeding (scientific papers); 11(2) : 35-38.


- Gomes BP, Ferraz CC, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB, Souza-Filho FJ. (2002). In vitro antimicrobial activity of calcium hydroxide pastes and their vehicles against selected...


Moorer WR, Wesselink PR. (1982). Factors promoting the tissue dissolving capability of sodium


-Siquira JF,RJcas IN, favueriA, lima KC.(2000). chemomechanical reduction of bactriocidal
population in root canal after instrumentation and irrigation with 1, 2.5, 5.25% of sodium hypochlorite. *J Endod* ; 26(6):331-334.


- Topley & Wilson’s, *Principles of Bacteriology, Virology and Immunity* - 8 Edn 1990 vol-II


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