

In Vitro Study of the Antibacterial Activity of Calcium Hydroxide on Candida Albicans

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Abstract: The aim of this in vitro study is to evaluate the antimicrobial activity of calcium hydroxide used in eliminating *Candida albicans*. In the present study we used clinical isolates of *Candida albicans* from primary infected root canals with opened pulp chamber and acute apical periodontitis. An agar-diffusion method was used to investigate the antimicrobial activity of Calcium hydroxide against *Candida albicans*. Calcium hydroxide was used mixed with different additives: Calcium hydroxide and destilated water (mixed ex tempore) Calcium hydroxide and 2% Chlorhexidine gluconate (mixed ex tempore), Calcium hydroxide and Iodoform (Metapex paste). In each plate three cavities were filled with one of the calcium pastes and one cavity- control agent. The zones of inhibition around the used medicaments in the cavities were measured in millimeters after being incubated in the period at 37°C for the period of 24 - 48 hours. The data was input and processed using the statistical package SPSS 15.0.1. The level of significance for rejecting the null hypothesis was fixed at $p < 0.05$. The results showed that the largest zone of inhibition was achieved by the combination of $\text{Ca(OH)}_2 + 2\% \text{CHX}$ with average mean – 12.75 mm, followed by Ca(OH)_2 + destilated water – 11.00 mm. The least impact was achieved by the Calcium hydroxide + Iodoform (Metapex paste) – 8.50mm. This in vitro study showed that the addition of drugs to Ca(OH)_2 leads to increased antimicrobial activity. However, in vivo studies this antibacterial activity decreases and it is not sufficient to eliminate *Candida albicans*.

Keywords: calcium hydroxide, *Candida albicans*, intracanal medicaments

1. Introduction

The success of endodontic treatment depends on the reduction or elimination of bacteria present in the root canal system [1, 11].

Candida albicans is the most commonly isolated yeast from infected root canals. Various studies have reported isolating it from primary endodontic infections [2, 12, 14]. Fungi can also be found in teeth that have undergone unsuccessful endodontic treatment [7, 12, 14].

C. albicans is also part of the normal oral microflora. It is possible to isolate yeasts from plaque, caries, subgingival microflora and active periodontal pocket [3, 10]. *Candida* spp. are gram-positive yeast-like fungi. These fungi are oval or round in shape (fig. 1). They develop optimally at a temperature of 37°C and in a slightly acidic medium pH - 6-6.5.

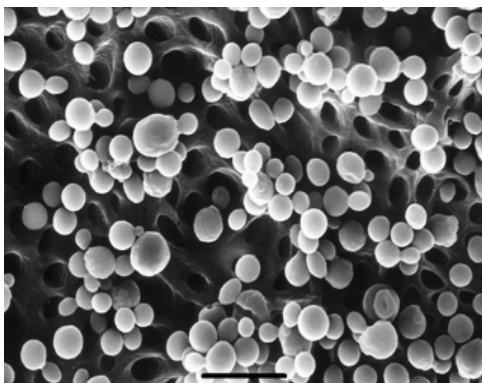


Figure 1: Scanning electron micrograph of yeast cells in pure culture on root canal wall. (bar remarks 10 mm).

Candida albicans can survive in the root canals even after thorough mechanical instrumentation and irrigation procedures.

The multiple experimental studies show that there are constant attempts to find more effective medicaments and combinations to eliminate *Candida albicans* from infected root canals [6, 12, 14].

The aim of this in vitro study is to evaluate the antimicrobial activity of calcium hydroxide used in eliminating *Candida albicans*.

2. Material and Methods

The study used clinical isolates of *Candida albicans* from primary infected root canals with opened pulp chamber and acute apical periodontitis.

An agar-diffusion method was used to investigate the antimicrobial activity of Calcium hydroxide against *Candida albicans*. Calcium hydroxide was used mixed with different additives:

- Calcium hydroxide and destilated water (mixed ex tempore);
- Calcium hydroxide and 2% Chlorhexidine gluconate (mixed ex tempore);
- Calcium hydroxide and Iodoform (Metapex paste).

The agar used was Mueller-Hinton, with 5% defibrinated sheep blood (Bul Bio, National center of Infectious and Parasitic diseases-Sofia). The petri dishes were inoculated with a suspension from the respective strains of *Candida albicans*, prepared in a sterile physiological solution of 0.5 McFarland density.

In each plate three cavities were filled with one of the calcium pastes and one cavity- control agent. The zones of inhibition around the used medicaments in the cavities were measured in millimeters after being incubated at 37°C for the period of 24 - 48 hours.

The data was input and processed using the statistical package SPSS 17.0.1. The level of significance for rejecting the null hypothesis was fixed at $p < 0.05$. The following methods were applied: descriptive analysis, analysis of variance, graphical analysis, Shapiro-Wilk test,

nonparametric test of Kruskal-Wallis, Student's parametric test, Mann-Whitney nonparametric test.

3. Results and Discussion

The results showed that the largest zone of inhibition was achieved by the combination of $\text{Ca(OH)}_2 + 2\% \text{CHX}$ with average mean – 12.75 mm, followed by $\text{Ca(OH)}_2 + \text{destilated water}$ – 11.00 mm. The least impact was achieved by the Calcium hydroxide + Iodoform (Metapex paste) – 8.50 mm (fig. 2).

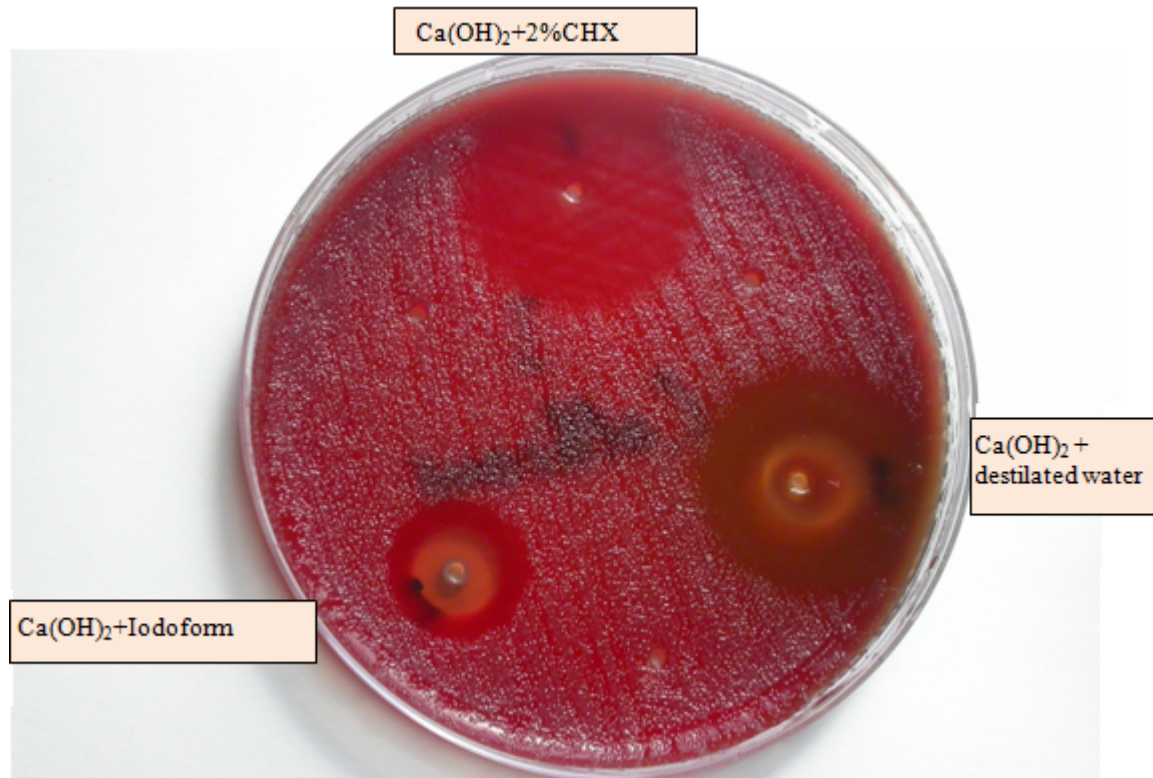


Figure 2: Zones of bacterial growth suppression

The difference between the used medicaments is statistically significant (table 1 and fig. 3).

Table 1: Comparative analysis of the zone diameter of bacterial growth inhibition according to the used medicaments

Medicaments	N	X	SD	Min	Max
$\text{Ca(OH)}_2 + \text{destilated water}$	16	11.00 ^a	0.93	10.00	12.00
$\text{Ca(OH)}_2 + 2\% \text{CHX}$	16	12.75 ^b	0.62	12.00	14.00
$\text{Ca(OH)}_2 + \text{Iodoform}$	16	8.50 ^c	0.53	8.00	9.00

*The different letters show that there is a significant difference ($p < 0.05$)

N-number of plates

X-arithmetic mean of the diameter of the zone of activity

SD – standard deviation

Min – minimal diameter of the zones in millimeters

Max – maximum diameter of the zones in millimeters

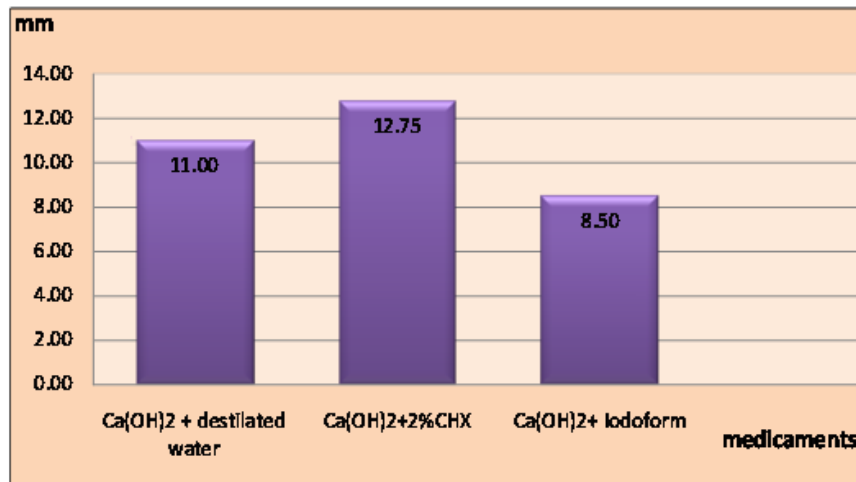


Figure 3: Arithmetic mean values of the zone diameter of bacterial growth inhibition according to the used medicaments

The endodontic infection is polymicrobial and not all medicaments are equally effective on all microorganisms in the infected root canal [6, 12, 15]. Calcium hydroxide (Ca(OH)_2) has been shown by scientific evidence to be the best therapeutic option as intracanal dressing. The basic principle action of calcium hydroxide involves the ionic dissociation into hydroxyl ions and calcium ions and its positive effect on microorganisms and tissue healing process. Calcium hydroxide has high pH around (12.5). Its high pH has a damaging effect on cell membranes and protein structure on bacterial cells [4, 5, 8].

Chlorhexidine (CHX) has antiseptic properties. It displays affinity to dentine, forms depots and has longer antimicrobial activity. Usually 2% solution of chlorhexidine is used in endodontics. In the present study, when CHX combine with Ca(OH)_2 its activity increases to 12.75 mm which is still higher than the activity of Ca(OH)_2 used with destilated water – 11.00 mm. Other researchers report similar results [9, 12, 13].

Ca(OH)_2 and Iodoform paste presented less antimicrobial effectiveness for the agar-diffusion test on *Candida albicans*. Our results of this finding correspondence to results of other authors [5]. Their results demonstrate that iodoform did not increase the antimicrobial effect of calcium hydroxide paste.

Endodontic infections are polymicrobial. The medicament that is effective against single microbe in vitro may not necessarily be effective against the same microbe in vivo since the root canal consist multiple microorganisms.

4. Conclusions

The present in vitro study shows that the addition of 2% Chlorhexidine gluconate leads to increased antimicrobial activity of Ca(OH)_2 . Iodoform did not increase the antimicrobial effect of calcium hydroxide paste.

However, in vivo studies this antibacterial activity of calcium hydroxide decreases and it is not sufficient to eliminate *Candida albicans*.

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Dr. Elka Radeva, DMD, PhD has been an assistant professor in the Department of Conservative dentistry, Faculty of Dental medicine, Medical University-Sofia, Bulgaria since 1993. Since then, she has been teaching students in preclinical and clinical courses in endodontics and operative dentistry. She has PhD thesis related to Endodontology: “Therapeutic treatment of microflora and pain in the initial phase of acute apical periodontitis without exudation in the root canal”. Since 2002, she has been a scientific secretary of the Journal of Dental Medicine – an edition of the Bulgarian Scientific Dental Association. Dr. Radeva is an accredited lecturer of the Bulgarian Dental Association. She gives lectures and manages practical courses in endodontics for postgraduate education in Bulgaria. Dr. Radeva is a co-author of books on pharmacology for medicine and dental medicine students.