



## Evaluating the Effectiveness of Iranian and Korean Injectable Intracanal Calcium Hydroxide on *Candida albicans*, *In vitro*

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### ABSTRACT

**Background:** *Candida albicans* is one of the mouth normal flora which may cause failures in endodontics. The resistance of *C. albicans* to intracanal medicaments such as calcium hydroxide could reduce success rate root canal treatments.

**Objectives:** Due to recivening some reports regarding resistance of *C. albicans* to calcium hydroxide from different parts of the world, the aim of this study was to evaluate anti candidal effects of Iranian and Korean made injectable calcium hydroxide and to compare the results.

**Materials and Methods:** In the present research, the antifungal effects of calcium hydroxide on seven clinical isolates and one standard strain of *C. albicans* were evaluated. For this evaluation, two methods were used including: inhibition zone and colony count. In all experiments distilled water and clotrimazole were used as negative and positive controls, respectively. In order to evaluate the effects of exposure time of calcium hydroxide on *C. albicans* growth, 30', 5', 1 and 24 hours of incubation periods were applied. In addition, to evaluate role the effect of calcium hydroxide concentration samples with saturated, 1/10, 1/100 and 1/1000 dilutions and also a saturated one were used.

**Results:** According to inhibition zone method, the mean diameters of *C. albicans* for Iranian and Korean made calcium hydroxide and clotrimazole were 17, 13 and 22 mm, respectively. Iranian and Korean calcium hydroxide did not show any anti candidal effects. By colony counting method, it was found that in longer exposure time, Iranian and Korean calcium hydroxide have more anti candidal effects, but no significant difference was observed between the two. Saturated and all other dilutions of calcium hydroxide base material indicated a significant statistical difference in anti candidal effect after 24 hours exposure in comparison with other periods.

**Conclusions:** The Current study, confirmed that the inhibitory effect of Iranian and Korean calcium hydroxide on *C. albicans*, up to 24 hours is within low range. Higher concentrations of base calcium hydroxide, showed greater inhibition zone on *C. albicans*.

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### ► Implication for health policy/practice/research/medical education:

The results of present study indicated uncomplete compatibility of susceptibility of *C. albicans* strains to calcium hydroxide, therefore, it seems continuous evaluation is necessary in different geographical region.

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## 1. Background

Yeasts are opportunistic microorganisms of oral cavity which may be exposed to root canal in aseptic situation (1, 2). Isolation and identification of yeasts from failed teeth root canals in which previous treatment has failed is notable (1, 3). Yeasts consist of different *Candida* spp but, *Candida albicans* is the most common yeast isolated from skin, superficial mucosal and oral cavity of either healthy or medically compromised cases (3). *C. albicans* have been isolated from dental plaque, subgingival flora, dental caries, and root canals. These oral flora organisms may become pathogenic and cause disease in the presence of predisposing factors such as, different types of malignancies, HIV infection, poor oral hygiene, diabetes and immunosuppressing situations (3-5). Calcium hydroxide has been widely used for its anti-inflammatory and antimicrobial effects in endodontic therapies in order to stimulate apexification, repair perforations, promote healing by hard tissue formation in root fractures, and stop inflammatory root resorption (1-9). Calcium hydroxide is also one of the main components of some root canal sealers and several pastes which are used as intracanal dressings in periapical lesions (10).

There is an indistinct role for oral yeasts in the etiology and pathogenesis of periodontal inflammations. However, *C. albicans* has been isolated from oral cavities of patients with severe periodontal inflammation, but recent studies have shown that *C. albicans* can colonize the periodontal pockets and is significantly associated with oral mucosal inflammation in females (11).

Despite its excellent bacteriocidal and anticandidal activities, it has been observed that colonization of oral *C. albicans* is markedly increased in some patients which is caused by resistance species. Different brands of calcium hydroxide pastes, have various chemical compositions (12) which may have different effects on candidal infections. Injectable types of calcium hydroxide as intracanal medicament are also commercially available. Recently an Iranian calcium hydroxide formula was registered (13). To make calcium hydroxide injectable, other materials as a vehicle should be added to it. This procedure may affect its anti-candidal effectiveness. On the other hand, there is a controversy regarding susceptibility of *C. albicans* to calcium hydroxide, therefore current study was conducted to evaluate in vitro effectiveness of Iranian and Korean intracanal calcium hydroxide vials on *C. albicans*.

## 2. Materials and Methods

The current research was conducted as an experimental study to evaluate the following items in vitro conditions

### 2.1. Microorganisms and Culture Media

In current study seven *C. albicans* strains isolated from patients referred to dental clinic of Ahvaz Jundishapur University of Medical Sciences. To this purpose swab samples were taken from oral cavity and cultured on Sabouraud's dextrose agar plate, incubated overnight at 37°C. *C. albicans* characterization and identification were confirmed by germ tube formation, chlamydoconidia formation on cornmeal agar and growth prohibition at 45°C. Reference strain was obtained from industrial and research standard institute PTCC5027. Sabouraud dextrose agar and tryptic-soy-broth were used for the primary isolation of yeasts (Merck company).

### 2.2. *Candida albicans* Suspension Preparation

To get final concentration as  $\sim 10^8$  c.f.u. ml<sup>-1</sup> *C. albicans* were suspended in distilled water, according to 0.5 McFarland turbidity standard (3).

### 2.3. Preparation of Medicaments

Korean sample of calcium hydroxide intracanal paste, which was used in the present study, is a product of Meta Biomed, made by a South Korean company. The Iranian sample was obtained from Pharmacy Faculty of Ahvaz Jundishapur University of Medical Sciences. The calcium hydroxide was purchased from Fluka-Chemika, Germany (14). In addition 1% clotrimazole and distilled water were used as positive and negative controls, respectively.

### 2.4. Inhibition Zone Method

Holes (5 mm in depth, 6 mm in diameter) were punched into the Sabouraud's dextrose agar plates. A loop of each *C. albicans* strain suspension was cultured on Sabouraud-dextrose agar. Holes were filled with calcium hydroxide, and clotrimazole as control and then incubated at 37°C for 24 hours. Then the diameter of inhibition growth zones were measured for each sample.

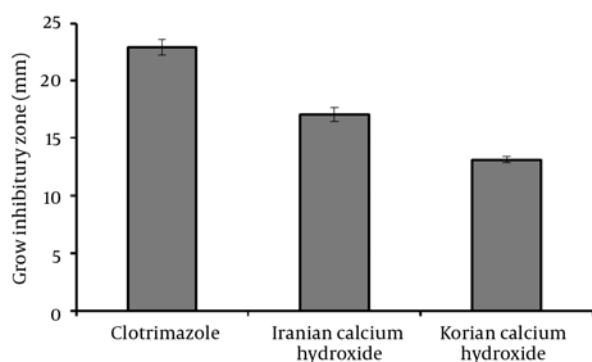
### 2.5. Colony Count Method

10 µl of *C. albicans* suspension was added to all test tubes, those containing one ml of medicaments, control positive and negative agents and then incubated at 37°C for periods of 5 seconds, 1 minute, 1 and 24 hours. Consequently, 10 µl of each suspension was inoculated on Sabouraud's dextrose agar plates and incubated at 37°C for 24 hours. The total numbers of colony forming units were calculated. The data were analyzed using a one way analysis of variances, repeated measures analysis of variances and tukey test.

### 3. Results

#### 3.1. Evaluation of Anti candidal Effects With Inhibition Zone Method

Evaluation of anti *C. albicans* effects of Iranian and Korean calcium hydroxide indicated an inhibitory growth zone range from 13-20 mm. Mean diameter zones of 17 mm and 13mm were obtained for Iranian and Korean medicaments samples, respectively. Clotrimazole which was used as positive control positive showed an inhibition growth zone of 22 mm diameter (Figure 1).



**Figure 1.** Evaluation of Anti *Candida albicans* Effectiveness of Iranian and Korean Calcium Hydroxide Using Inhibitory Growth Zone Method (Mean  $\pm$  SE).

#### 3.2. Evaluation of Anti-Candida Effects With Colony Count Method

In this experiment both calcium hydroxides indicated an anticandidal effect within 30 second, 5 minute, 1 and 24 hours period of exposure. Anti-*C. albicans* effect of both medicaments indicated significant correlation with time of exposure, therefore more anticandidal effects were obtained with longer time of incubation with cal-

cium hydroxide ( $P$  value  $<$  0.05) (Table 1).

#### 3.3. Comparison of Anti candida Effects of Both Calcium Hydroxides

Statistical analysis showed that there was significant correlation inhibition growth zone of in all experiments and Iranian sample was more effective than the Korean one ( $P$  value  $<$  0.05). Although the results obtained from colony count method was not the same, the mean colony formation in Iranian sample was highly lower than the Korean one was less lower, which may indicate more anti candidal effect of Iranian medicament (Table 2).

### 4. Discussion

is elimination of microorganisms and prevention of reinfection in root canal treatments is an important considerations of endodontical treatments (14-17). *C. albicans* has been reported as one of the resistant microorganisms to intracanal antiseptic agents (6, 14-19). The results of present study revealed that all tested *C. albicans* strains were susceptible to calcium hydroxide. In recent years, complete or partial resistance to calcium hydroxide have been reported (2-5, 8). On the other hand, most experiments showed susceptibility of *C. albicans* strains to calcium hydroxide which is inconformity with our present finding (20-23). Although there is no clear explanation regarding these controversies, but it may be a result of differences among distributed *C. albicans* strains in different regions. Additionally, regular and prolonged application of calcium hydroxide may induce resistance strain of *C. albicans*.

Our research showed that there is a significant differences between the average 0 inhibition growth zone made by Iranian and Korean calcium hydroxide samples after

**Table 1.** Evaluation of Effectiveness of Different Dilutions of Iranian Calcium Hydroxide on *Candida albicans* Regarding Different Time of Exposure

	30 Second	5 Minute	1 Hour	24 Hour
	Average (Colony Count/ml), Mean $\pm$ SD	Average (Colony Count/ml), Mean $\pm$ SD	Average (Colony Count/ml), Mean $\pm$ SD	Average (Colony Count/ml), Mean $\pm$ SD
Saturated solution	6275 $\pm$ 619	6262.5 $\pm$ 610	6737 $\pm$ 887	4100 $\pm$ 406
1/10	11662 $\pm$ 1375	11525 $\pm$ 1392	10812 $\pm$ 1364	6312 $\pm$ 1588
1/100	13000 $\pm$ 943	12862 $\pm$ 918	11875 $\pm$ 882	6362 $\pm$ 1194
1/1000	15812 $\pm$ 1352	1576 $\pm$ 1354	14250 $\pm$ 1335	9600 $\pm$ 1455
Control	2340 $\pm$ 236	23262 $\pm$ 265	22600 $\pm$ 474	22588 $\pm$ 457

**Table 2.** Evaluation of Anti *Candida albicans* Effect of Iranian and Korean Calcium Hydroxide According to Exposure Time

	30 Second	5 Minute	1 Hour	24 Hour
	Average (Colony Count/ml), Mean $\pm$ SD	Average (Colony Count/ml), Mean $\pm$ SD	Average (Colony Count/ml), Mean $\pm$ SD	Average (Colony Count/ml), Mean $\pm$ SD
Iranian	13750 $\pm$ 4385	13512 $\pm$ 4404	7162 $\pm$ 2097	2087 $\pm$ 1039
Korean	16113 $\pm$ 1612	16000 $\pm$ 1608	11162 $\pm$ 1893	5037 $\pm$ 1209
Negative control	19462 $\pm$ 470	19638 $\pm$ 373	19588 $\pm$ 390	19675 $\pm$ 391

24 hours, and Iranian samples showed more anticandidal effects, with 17 mm inhibition growth zone. Balal *et al* reported an inhibition growth zone of 21 mm diameter which is close to our findings (24). According to the obtained results from colony count method, both medicaments showed acceptable anti candidal effects.

In the present study, both medicaments were tested by colony count formation method, showed anti candidal effects in different periods of exposures, but there were significant differences between 1 and 24 hours incubation time of exposure to calcium hydroxide. Therefore it seems that the Iranian samples showed more anti-candidal effects in both applied. Al-Nazhan reported no anticandidal effects of calcium hydroxide after 1 hour incubation, but complete inhibitory growth after 24 and 72 hours exposure were observed (18). In another experiment, Balal *et al* reported anti-candidal effect of calcium hydroxide after 24 hours, but surprisingly this effectiveness was reduced after a 72 hours incubation (24).

Among 16 *C. albicans* strains, tested for calcium hydroxide susceptibility, only 3 strains have revealed anti-candidal effects after 20 minutes, after 1-3 hours of incubation 7 strains, and after 3-6 hours of exposure to calcium hydroxide, 6 strains showed inhibitory growth zone (3). Fabiane reported complete *C. albicans* resistance to calcium hydroxide after 24, 48, and 72 hours of exposure (5). In contrast, Barbosa results were similar to our findings, which indicated acceptable anti-candidal effect of calcium hydroxide after a 5 minutes exposure (17). According to the current study and most previous trials, calcium hydroxide indicated acceptable anti-candidal effect in short time exposure and highest effectiveness obtained after 24 hours of exposure.

In conclusion, present study indicated anti *C. albicans* effects of calcium hydroxide even in on short term exposure and sometimes better effectiveness were observed in Iranian samples. Hence, due to incomplete compatibility and even controversy regarding susceptibility and resistancy of different *C. albicans* strains to calcium hydroxide, it seems that continuous evaluation is necessary in different geographical regions.

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