

# Effectiveness of various intracanal medicaments on *Candida albicans*: An *in vitro* study

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## ABSTRACT


**Background:** Microbiological studies have shown that *Candida albicans* is the most commonly found fungi, ranging from 7% to 18% of the root canal infections of tooth. Various intracanal medicaments were analyzed to know the best among them. **Objectives:** The study objective was to evaluate and compare the antifungal efficacy of various intracanal medicaments, i.e., calcium hydroxide, Odontopaste<sup>®</sup>, Ledermix<sup>®</sup> paste, Pulpdent<sup>®</sup> paste, tri-antibiotic paste, and Coconut<sup>®</sup> oil cream on *C. albicans*. **Materials and Methods:** Seventy freshly extracted human permanent maxillary and mandibular anterior teeth extracted for various therapeutic reasons were selected for the study. A standard strain of *C. albicans* (10,231) was selected and suspended in 20.0 ml of Sabouraud Dextrose broth, and the suspension was adjusted to match the turbidity equivalent to 0.5 McFarland standards. The dentinal blocks were inoculated with *C. albicans* strains. The seventy dentinal blocks were then divided into seven groups according to the intracanal medicaments used in the study. Each group comprised of ten specimens. Each group was further divided into two subgroups according to the experimental periods, i.e., day 1 and day 7. The data were tabulated and statistically analyzed using nonparametric Wilcoxon signed-rank test and Kruskal–Wallis test to compare the mean and median reduction of *C. albicans* between intracanal medicaments.  $P < 0.05$  was considered statistically significant. **Results:** Among the tested medicaments, Odontopaste<sup>®</sup> and Ledermix<sup>®</sup> paste showed the maximum effectiveness throughout the experimental period followed by Pulpdent<sup>®</sup> paste. **Conclusion:** The medicaments used in this study showed antimicrobial activity even though complete elimination was not obtained with any of the medicaments. The present study also showed that Odontopaste<sup>®</sup> and Ledermix<sup>®</sup> paste were the most effective antimicrobial medicaments against *C. albicans*. More researches are necessary for improving the antifungal efficacy of the medication used nowadays as intracanal dressing.

**Key words:** Calcium hydroxide, *Candida albicans*, coconut oil, intracanal medicaments, Ledermix<sup>®</sup> paste, Odontopaste<sup>®</sup>, Pulpdent<sup>®</sup> paste

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## INTRODUCTION

Microorganisms play a fundamental role in the etiology of pulpal and periapical disease, while primary endodontic infections are polymicrobial in nature.<sup>[1]</sup> Certain microorganisms are resistant to routine therapy and the infection may persist even after the treatment.<sup>[2]</sup> Microbiological studies have shown that certain fungi such as *Candida albicans* can survive in the root canals even after thorough mechanical instrumentation and irrigation.<sup>[3]</sup>

*C. albicans* strains are believed to be involved in the pathogenesis due to factors such as adaptability to environmental conditions, surface adhesion, production of hydrolytic enzymes, morphogenetic transition, bio-film formation, and by host defense immunomodulation.<sup>[4,5]</sup> Hence, there is a necessity for intracanal medication in cases where infection was challenging the success of usual treatment and also where the outcome of endodontic therapy was compromised.<sup>[6]</sup> It is very important to ensure that interappointment medicament used in root canal treatment should be biocompatible, should have effective antimicrobial properties that may help in complete eradication of microorganism from the root canal system, and may considerably increase the success of root canal treatment.<sup>[7]</sup> Interappointment medication can be used in decreasing the remaining microbial flora found even after biomechanical preparation and can create an environment which is conducive to periapical tissue repair.<sup>[8]</sup> Therefore, use of intracanal medicament is needed especially in cases when the infection is resistant to the regular treatment and outcome of endodontic therapy.

Despite a plethora of materials available as intracanal medicament, obtaining complete or near-complete elimination of the microorganism from root canal is a challenge, hence there is a need to identify the best intracanal medicaments available. Therefore a study was done to evaluate the antifungal effectiveness of various intracanal medicaments such as calcium hydroxide, tri-antibiotic paste, Odontopaste<sup>®</sup>, Ledermix<sup>®</sup> paste, Pulpdent<sup>®</sup> paste, and coconut oil against *C. albicans*.

## MATERIALS AND METHODS

After clearance from the institutional ethical committee, a total of seventy freshly extracted human permanent maxillary and mandibular anterior teeth with complete root formation, extracted for various therapeutic reasons, were taken for the study. The tooth was sectioned below the cemento-enamel junction and the apical part of the root to obtain 6 mm of the middle third of the root, and dentin blocks were made. The root canal was enlarged to an internal diameter of 0.9 mm using Pecho Reamer, and the dentin blocks were subjected to ultrasonic irrigation using 5.25% sodium hypochlorite and then 17% ethylenediaminetetraacetic acid for 1 min

to remove smear layer. Thereafter, the dentin blocks were exposed to sterilization by autoclave for 20 min at 121°C.

The external surfaces of specimen were covered with nail varnish to prevent the attachment of *C. albicans* and intracanal medicaments to the external surface of the specimen. Twelve Petri dishes containing wax with a flat surface were prepared, and the surface was subjected to sterilization using 70% ethanol and air dried in a sterile cabinet before use. The apical ends were covered with thin sterile plastic strips to prevent the softened wax entering the canal area.

*C. albicans* was suspended in 20.0 ml of Sabouraud Dextrose Broth, and the suspension was adjusted to match turbidity equivalent to 0.5 McFarland standards. The dentinal blocks were inoculated with *C. albicans* strains with the use of sterile 5.0-mL syringes with 30G needles under sterile laminar flow hood, and the coronal part of the dentin blocks was sealed with the help of paraffin wax and subjected for incubation for 37°C for 21 days, with a renewal of *C. albicans* every 3<sup>rd</sup> day done throughout the inoculation period.

After inoculation period, the canal of dentine blocks was irrigated with sterile saline and dried with sterile paper points to create the exact environment for the placement of intracanal medicaments. The seventy dentin blocks were divided into the following seven groups according to the intracanal medicaments used in the study. Group I: calcium hydroxide, Group II: Odontopaste<sup>®</sup>, Group III: Ledermix<sup>®</sup> paste, Group IV: Pulpdent<sup>®</sup> paste, Group V: tri-antibiotic paste, Group VI: Virgin coconut oil, and Group VII: normal saline as control group. Each group was further divided into two subgroups according to the experimental periods, i.e. day 1 and day 7.

The intracanal medicaments were placed in the canal with the help of sterile 5.0 mL syringe. Following the placement of medicaments inside the canal, the coronal orifices were sealed with paraffin wax and the specimens were incubated at 37°C upto the experimental period.

At the end of the experimental period, the dentine blocks were removed from the Petri dishes and the canal was cleaned and dried with sterile paper points to ensure complete removal of medicaments. Specimens of dentinal shavings were collected after day 1 and after day 7. Dentinal shavings were collected using Pecho reamer size no: 6 (1.7-mm diameter). This size of Pecho reamer allows a collection of dentinal shavings up to a depth of 400 µm; the dentinal shavings were collected with the help of Eppendorf tubes and centrifuged and separated. *Candida* broth was transferred immediately into a microcentrifuge tube containing 1.0 ml sterile SD broth.

A sterile microtip was used to take 0.1 ml of broth containing dentinal shavings, which were spread evenly and duplicated

to know the individual specimen variability. These plates were incubated for 24 h at 37°C, colonies were counted, and readings were tabulated using a digital colony counter and colony-forming unit (CFU) count was expressed in CFUs/microliter.

## RESULTS

The results were tabulated and statistically analyzed with SPSS Inc Version 17.0. (Chicago:USA). Wilcoxon signed-rank test and Kruskal–Wallis tests were done to compare the mean and median reduction of *C. albicans* between different intracanal medicaments and to compare the CFU based on intracanal medicaments on day 1 and day 7.

Table 1 shows the growth of *C. albicans* on day 1; when compared to the normal saline, all medicaments showed statistically significant fungal growth ( $P < 0.01$ ) at 400 μm dental tubule depth. Odontopaste® and Ledermix® paste were found to be the best in terms of antifungal properties with a highly significant difference compared to the other four medicaments. This was followed by Pulpdent® paste, tri-antibiotic paste, Coconut® oil, and calcium hydroxide in decreasing order of antifungal efficacy.

Table 2 shows the growth of *C. albicans* on day 7; Odontopaste® and Ledermix® paste were found to be the best in terms of antifungal properties with statistically significant difference compared to the other four medicaments used ( $P < 0.01$ ), this was followed by Pulpdent® paste, calcium hydroxide, tri-antibiotic paste, and Coconut® oil in decreasing order of antifungal efficacy.

Table 3 shows the comparison between the mean and median values in day 1 and day 7. Calcium hydroxide showed  $262.6 \pm 1.8 \times 10^4$  CFUs on day 1 and  $17.2 \pm 0.7 \times 10^4$  on day 7, which was statistically significant ( $P < 0.05$ ). No growth was observed on day 1 and minimal growth on day 7 with regard to Odontopaste® and Ledermix® paste. A statistically significant increase in antifungal effectiveness was observed on day 7 for Pulpdent® but for tri-antibiotic paste and Coconut oil, there was decrease in the antifungal effectiveness on day 7. Significant reduction of bacterial growth was seen on normal saline from day 1 to day 7 CFUs.

Figure 1 shows the comparison of calcium hydroxide with other medicaments used on day 1. Calcium hydroxide had shown antifungal effectiveness on *C. albicans* at 400 μm dental tubule depth. Odontopaste® and Ledermix® paste showed high antifungal effectiveness on *C. albicans* followed by Pulpdent® paste, tri-antibiotic paste, and Coconut® oil.

Figure 2 shows the comparison of calcium hydroxide with other medicaments used on day 7. Odontopaste®, Ledermix® paste, and Pulpdent® paste showed high antifungal

**Table 1: Comparison of colony-forming unit based on intracanal medicaments on day 1**

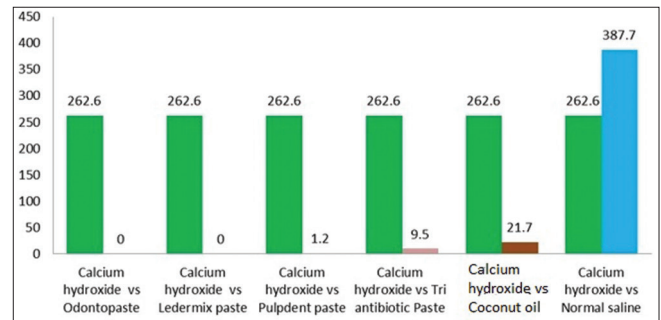
Intracanal medicament	Mean (10 <sup>4</sup> )	SD	Median (10 <sup>4</sup> )	Z <sup>§</sup>	P
Calcium hydroxide	262.6	1.8	263.0	33.52**	0.000
Odontopaste®	0	0	0.0		
Ledermix® paste	0	0	0.0		
Pulpdent® paste	1.2	0.1	1.2		
Tri-antibiotic paste	9.5	0.5	9.5		
Coconut® oil	21.7	0.5	21.8		
Normal saline	387.7	5.7	388.6		

§Kruskal-Wallis test, \*\*Significant at 0.01 level. SD: Standard deviation

**Table 2: Comparison of colony-forming unit based on intracanal medicaments on day 7**

Intracanal medicament	Mean (10 <sup>4</sup> )	SD	Median (10 <sup>4</sup> )	Z <sup>§</sup>	P
Calcium hydroxide	17.2	0.7	17.0	32.76**	0.000
Odontopaste®	2.7	0.3	2.7		
Ledermix® paste	2.8	0.5	2.8		
Pulpdent® paste	9.6	0.6	9.4		
Tri-antibiotic paste	102.4	0.6	102.4		
Coconut® oil	142.7	0.6	142.8		
Normal saline	192.7	1.6	193.2		

§Kruskal-Wallis test, \*\*Significant at 0.01 level. SD: Standard deviation



**Figure 1: Comparison of calcium hydroxide with different medicaments on day 1**

effectiveness, whereas Pulpdent® paste and tri-antibiotic paste and Coconut® oil showed less antifungal activity on day 7 compared with that of calcium hydroxide.

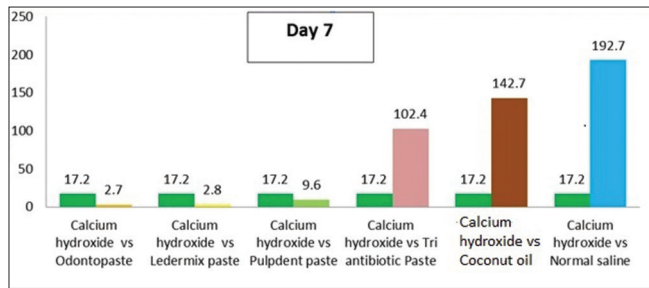
## DISCUSSION

Numerous medicaments have been attempted in root canals of teeth with their particular merit/demerits assessed. Nonetheless, calcium hydroxide remained the “gold standard” endodontic medicament used widely to eliminate microbial flora.<sup>[9,10]</sup> The phenotypic alteration of *C. albicans* helps to adapt the ecologically unfavorable conditions such as high alkaline environment. These virulence factors make *C. albicans* to be resistant to calcium hydroxide.<sup>[11-14]</sup> This limitation of calcium hydroxide made way to different intracanal medicaments into the market, which includes calcium hydroxide-based medicaments and other newer combinations aiming for complete sterilization of the root canal system.

**Table 3: Comparison of colony-forming unit based on intracanal medicaments on day 1 and day 7**

Intracanal medicament	Day 1		Day 7		Wilcoxon signed-rank test	P
	Mean±SD (10 <sup>4</sup> )	Median (10 <sup>4</sup> )	Mean±SD (10 <sup>4</sup> )	Median (10 <sup>4</sup> )		
Calcium hydroxide	262.6±1.8	263.0	17.2±0.7	17.0	2.02*	0.043
Odontopaste®	0±0	0.0	2.7±0.3	2.7	2.02*	0.043
Ledermix® paste	0±0	0.0	2.8±0.5	2.8	2.02*	0.043
Pulpdent® paste	1.2±0.1	1.2	9.6±0.6	9.4	2.03*	0.042
Tri-antibiotic paste	9.5±0.5	9.5	102.4±0.6	102.4	2.02*	0.043
Coconut® oil	21.7±0.5	21.8	142.7±0.6	142.8	2.02*	0.043
Normal saline	387.7±5.7	388.6	192.7±1.6	193.2	2.02*	0.043

\*Significant at 0.05 level. Wilcoxon signed-rank test



**Figure 2: Comparison of calcium hydroxide with different medicaments on day 7**

In this study, the possible reasons for antifungal effectiveness of Odontopaste against *C. albicans* could be because of its contents, which are mainly clindamycin hydrochloride, triamcinolone acetonide, zinc oxide, and calcium hydroxide. Clindamycin hydrochloride has a relative antimicrobial property on *C. albicans* and with the presence of triamcinolone acetonide, it provides anti-inflammatory effect also. The presence of calcium hydroxide helps in protein denaturation and damage to DNA, thereby resulting in the death of *C. albicans*, leading to maximum antifungal effectiveness. Although there was an added advantage of Zinc oxide present in Odontopaste slows down antibiotic release, hence helps in sustaining a larger concentration within the root canal.<sup>[15-18]</sup>

The Ledermix® paste has been recommended for endodontic use due to its anti-inflammatory property, which helps in reducing postendodontic pain,<sup>[19]</sup> and is also known for its highly effective antimicrobial property due to the presence of demeclocycline hydroxide.<sup>[18]</sup> The reason for developing Ledermix® paste was based on the use of corticosteroids to control pain and inflammation, which was associated with pulp and periapical diseases.<sup>[7]</sup> Although adding the antibiotic component along with steroid component was to compensate corticoid-induced reduction in the host immune response, within the peripheral part of dentine and periradicular tissues. The concentration achieved through diffusion was not sufficient to inactivate microorganisms especially over time.

The main difference between Odontopaste and Ledermix® paste is in their composition wherein Clindamycin hydrochloride in Odontopaste replaces Demeclocycline hydrochloride in Ledermix® paste.<sup>[18,20]</sup> Due to the

tetracycline content in Ledermix® paste, it can result in minimal staining on teeth. No posttreatment bleaching is required with the use of Odontopaste, as it does not stain teeth. Hence Odontopaste is said to have a better clinical advantage over Ledermix paste.<sup>[21,22]</sup>

Pulpdent paste was the original premixed calcium hydroxide methylcellulose pulpal dressing medicament which has a pH > 12 and provides all benefits of calcium hydroxide.<sup>[23-25]</sup> In the present study, the reason for mild antifungal activity of calcium hydroxide initially could be due to the fact that it does not inhibit the growth of *C. albicans* and also *Candidas* have the ability to penetrate the dentinal tubules and adapt to the changing environment. Late antifungal effectiveness observed in this study may be attributed to the continuous sustained release of hydroxyl ions and also by enhancing the release of calcium ions. This in turn leads to the inhibition of candidal growth by regulating *C. albicans* morphogenesis and inhibiting the mycelial growth.<sup>[26,27]</sup> The results of our study are in accordance to the study by Holmes *et al.*,<sup>[28]</sup> in which diminished candidal growth was observed on day 7.

Tri-antibiotic paste has metronidazole which has a wide spectrum of antimicrobial activity and eliminates anaerobic organisms. In addition, components such as minocycline and ciprofloxacin have been added to tri-antibiotic paste to possess strong antimicrobial property, leading to complete sterilization of root canal system. These three antibiotics also result in synergistic effect against microorganisms.<sup>[29-31]</sup>

The antifungal effectiveness of coconut oil could be due to the fact that coconut oil contains medium-chain fatty acid which has the capacity to alter microbial cell walls, penetrate and disrupt cell membranes, and inhibit enzyme involved in energy production and nutrient transfer, leading to the death of microorganism.<sup>[31]</sup> The decreasing antifungal effectiveness of coconut oil in later periods in this study might be due to the deprivation of medium-chain fatty acids. Additionally, hydrogenation increases the trans-fat content of the coconut oil, which was known to decrease the antimicrobial effectiveness of coconut oil.<sup>[32-34]</sup>

**Limitation of the study**

The major limitation of this *in vitro* study is that a single microorganism was used to infect the root canal in contrast to

clinical situations with polymicrobial endodontic infections. The medicaments which are effective in *in vitro* situations may not necessarily be effective against the same microbe in *in vivo* situations.

## CONCLUSION

The study substantiates the evidence of antifungal effectiveness of calcium hydroxide, tri-antibiotic paste, Odontopaste, Ledermix® paste, Pulpdent® paste, and coconut oil against *C. albicans*. All the medicaments used in this study showed antimicrobial activity even though complete elimination was not obtained with any of the medicaments. The present study showed that Odontopaste and Ledermix® paste were the most effective medicaments against *C. albicans*.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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