

**EVALUATION OF ANTIFUNGAL EFFICACY OF VARIOUS ROOT CANAL IRRIGANTS AGAINST CANDIDA ALBICANS - AN IN VITRO STUDY**Priti Desai<sup>1\*</sup>, Saikat Chatterjee<sup>2</sup>, Niladri Maiti<sup>3</sup> and Shibendu Biswas<sup>4</sup>

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**ABSTRACT**

**Aim & Objective:** The study was designed to evaluate and compare the antifungal efficacy of 3% Sodium Hypochlorite, 0.2% Chlorhexidine, 20% Citric acid against *Candida Albicans* using Agar diffusion method. **Methodology:** The four irrigating material divided in to group(n =10) GROUP I- Normal saline (control), GROUP II- 3% Sodium Hypochlorite(ICPA), GROUP III-0.2% Chlorhexidine, GROUP IV- 20% Citric acid(Primadent). To test antifungal activity with agar diffusion method, sterile muller hinton plates were prepared first (n=10 per group) and then the broth culture of *Candida albicans* were swabbed on these plates with sterile swabs. The two wells per plate were cut into agar plates with the help of sterile cork-borer. 50 µl of test samples will be added to the wells respectively according to groups. The plates were incubated at 37°C for 24 hrs. The antifungal activity was evaluated by measuring the diameter of inhibition zone. **Results:** Group IV shown 15.4 mm inhibition zone, group III shown 11.2 mm inhibition zone, Group –II shown 4.4 mm inhibition zone and group I shown 0.00 mm inhibition zone. **Conclusion:** 20% Citric acid shows greater antifungal efficacy than 0.2% Chlorhexidine and 3% Sodium Hypochlorite against *Candida albicans*. While normal saline does not have antifungal activity so use of citric acid as irrigating solution is helpful in eradicating fungi from root canal.

**KEYWORDS:** Agar diffusion, Mueller-Hinton, *Candida albicans*, Sodium hypochlorite Chlorhexidine gluconate, Citric acid.

**INTRODUCTION**

The primary goal of endodontic treatment is to optimize root canal disinfection and to prevent recurrence of infection.<sup>[1]</sup> This can be achieved by chemomechanical preparation means mechanically removal of infected debris and chemical preparation to kill remaining microorganism using intracanal medicaments and irrigating solution. Chemical disinfection involves thorough cleaning by flushing of debris from root canal system.<sup>[2]</sup> Different microorganisms have been isolated from the infected root canals like *Prevotella* species, *Porphyromonas* species, *Tannerella forsythia*, *Dialister* species, *Fusobacterium*, *Spirochetes*, Gram positive anaerobic rods, Gram positive bacteria like *Streptococci*, *Enterococcus faecalis* etc. The role of fungi has been well documented in infections of the pulp, root canals and dentinal tubules.<sup>[3]</sup> Fungi have been reported to be present in 7-55% of infected root canals. *Candida*

*albicans* is the most common fungi involved in endodontic infections.<sup>[4]</sup>

The incidence of candidiasis in the oral cavity with predominant *C. albicans* isolation has been reported to be 45% in neonates,<sup>[5]</sup> 45-65% in children,<sup>[6]</sup> 30-45% of healthy adults,<sup>[7]</sup> 50-65% in cases of long-term denture wearers,<sup>[8]</sup> 65-88% in those residing in acute and long-term facilities,<sup>[9]</sup> 90% in patients with acute leukemia undergoing chemotherapy,<sup>[10]</sup> and 95% of patients with HIV infection.<sup>[11]</sup>

*Candida* is versatile and can adapt to a range of pH, change gene expression in response to environmental conditions, adhere to a variety of surfaces, produce degradative enzymes, and change morphologic forms to evade the immune system. Clinically important *Candida* species grow well in vitro over a pH range of 3.0-8.0.<sup>[12]</sup> So, it is a challenge for endodontists to eliminate *Candida*

albicans from root canal system to avoid recurrent root canal infection. Residual pulpal tissue, bacteria, fungi and dentine debris may persist in their regularities of root canal systems, even after meticulous mechanical preparation. Therefore, irrigant solutions with antibacterial efficacy should be used in combination with canal preparation.<sup>[13]</sup>

Intra-canal irrigating solutions with strong antimicrobial properties are required and they must be able to dissolve organic residues, disinfect the intra-canal space, and remove debris from the prepared canals. Also they should have a lubricating effect with no cytotoxicity in peri-radicular tissues if extruded beyond root apex.<sup>[14]</sup>

Among all irrigating solution used today, no one fulfill the ideal requirement of irrigating solution. commonly used irrigating solution are Normal saline, Sodium hypochlorite (NaOCl), Chlorhexidine gluconate, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and recently introduced Q Mix, MTAD, Citric acid (20%).

Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigating solution. However, which does not have all the properties of an ideal irrigating solution<sup>[15]</sup> Though NaOCl commonly used irrigating agent it has many undesirable effect when extruded below periapical region causing- pain, swelling, ecchymosis, hemorrhage, and allergic reactions.<sup>[16]</sup> Chlorhexidine gluconate is acknowledged as a wide spectrum antimicrobial agent. It is unique in its ability to bind to oral tissues for extended periods from which it is released slowly; this property is called 'Substantivity'. It is relatively nontoxic but does not have the capacity to dissolve organic tissues (Kuruville Jr, 1998).<sup>[17]</sup>

Chlorhexidine gluconate solution used in dentistry in various concentration like 2%, 0.2% w/w etc. Delany *et al.* evaluated the 0.2% CHX gluconate on infected root canals. Bacteriologic samples were obtained before, during, immediately after and 24 hours after instrumentation, irrigation, and medication either with CHX gluconate or with sterile saline. There was a highly reduction in microorganisms in the CHX-treated specimens after the instrumentation and irrigation procedures.<sup>[18]</sup>

Table-1.

<b>MEAN GROWTH INHIBITION ZONE IN MM AFTER 24HR INCUBATION AT 37<sup>0</sup>C</b>				
<b>Descriptive Statistics</b>	<b>Group-I Control (normal saline) (in mm)</b>	<b>Group-II 3% Sodium hypochlorite (in mm)</b>	<b>Group -III 0.2% Chlorhexidine (in mm)</b>	<b>Group -IV 20% Citric acid (in mm)</b>
Mean±s.d.	0.00±0.00	4.40±0.97	11.20±1.55	15.40±0.97
Median	0.00	4.50	11.00	15.50
Range	0 - 0	3 - 6	9 - 14	14 - 17

There was no growth inhibition zone in normal saline. The mean growth inhibition zone was highest of all in

Recently citric acid is used as irrigating agent. Citric acid is a mild acidic material, effective at dissolving and cleaning calcium hydroxide from the canal. It also helps in removal of smear layer from dentinal walls, helps in conditioning of canal for better instrumentation.

Here with we have done the study to examine antifungal efficacy of three irrigating solutions 3% NaOCl, 0.2% Chlorhexidine and 20% citric acid and comparison of their antifungal efficacy.

#### MATERIAL AND METHOD

There are four groups GROUP I- Normal saline (control), GROUP II- 3% Sodium Hypochlorite (ICPA), GROUP III- 0.2% Chlorhexidine, GROUP IV- 20% Citric acid (Primadent). *Candida Albicans* (MTCC227) was cultured in Nutrient Agar broth. Muller Hinton Agar was prepared by mixing 3.8gms of Muller Hinton powder and 2 grams of Agar in 100ml distilled water. The mixture was autoclaved for 30 minutes and poured in 4 petri dishes and solidified at room temperature. 2 wells (6mm diameter and 4 mm deep) per plate were cut into agar plates with the help of sterile cork-borer. In one well 50 µl of test samples were added. The plates were incubated at 37°C for 24 hrs.

The antifungal activity was evaluated by measuring the diameter of growth inhibition zone in plates after 24 hour.

#### RESULTS

Statistical Analysis was performed with help of Epi Info (TM) 7.2.2.2 EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC).

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviation (s.d.).

Also One Way Analysis of variance (ANOVA) followed by post hoc Tukey's Test was performed with the help of Critical Difference (CD) at 5% and 1% level of significance to compare the mean values.

$p < 0.05$  was taken to be statistically significant.

20% Citric acid followed by in 0.2% Chlorhexidine and 3% Sodium hypochlorite.

Graph -1

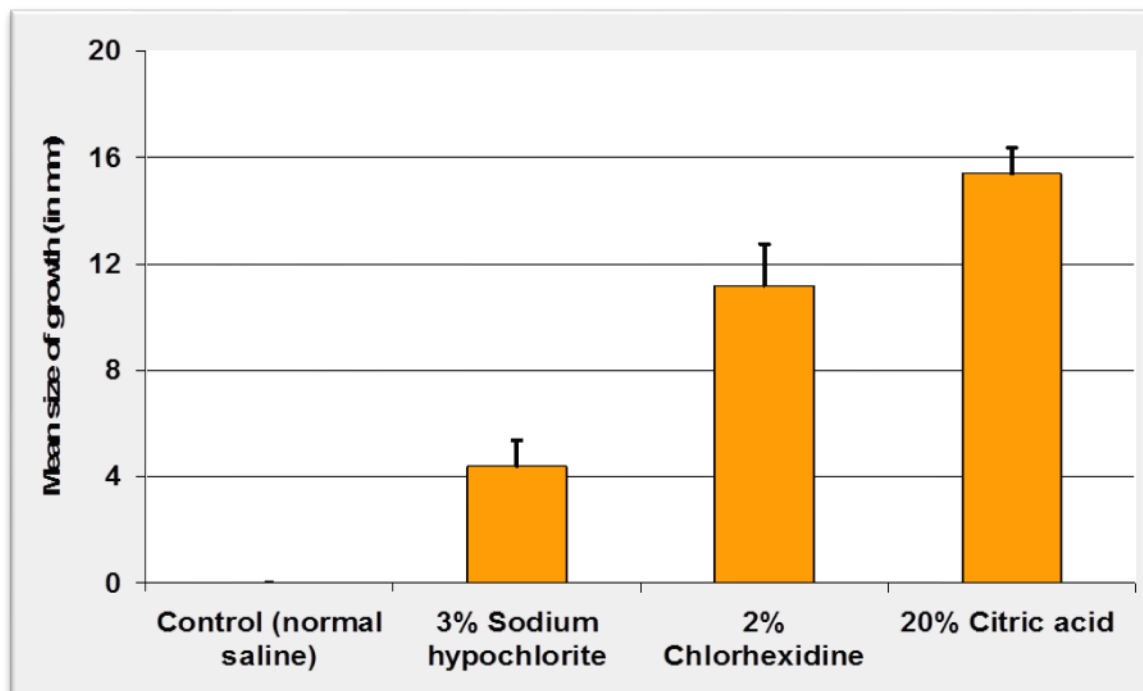


Table 3.

Analysis of variance to compare the growth inhibition zone with various irrigating agents				F	p-value
Source of Variation	SS	df	MS		
Between Groups	1417.1	3	472.36	442.84	<0.0001*
Within Groups	38.4	36	1.0666		
Total	1455.5	39			

One way ANOVA showed that there was significant difference in mean growth with various irrigating agents ( $F_{3,36} = 442.84; p < 0.0001$ ).

As per Tukey's Critical Difference (CD) the mean growth was significantly highest of all in 20% Citric acid followed by in 0.2% Chlorhexidine and 3% Sodium hypochlorite.

## DISCUSSION

Microbiological investigations of apical periodontitis associated with root canal failure cases during the past 50 years have revealed that yeasts can be isolated from infected root canals. (Grossman, 1952; Slack, 1953, 1957; Macdonald et al., 1957; Hobson, 1959; Margaret I. Wilson et al. (1968); Goldman and Pearson, 1969; Matusow, 1981;).

*Candida albicans* is the most frequently isolated variety. Other isolates include: *C. glabrata*, *C. guilliermondii*, *C. inconspicua*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. crusei*.<sup>[19]</sup>

*Candida albicans* has a series of features that allow them to survive in the root canals like a) Resistance to drugs in the canal, b) Ability to form biofilm, c) Invasion to dentinal tubules & long survival without substrate..

In this study 20% citric acid irrigating agent shows highest antifungal efficacy followed by 0.2% chlorhexidin gluconate and least with 3% sodium hypochlorite but control group normal saline shown 0% efficacy which indicate to eradicate *Candida* from canal irrigating agent with antifungal efficacy are useful than only normal saline.

The choice of culture media in the present study was Mueller-Hinton Broth, as it is readily available and commonly used for *C. albicans*.

The agar diffusion test, used in the present study, because it is a worldwide accepted procedure for determining the in vitro sensitivity under routine laboratory conditions. This method is simple, standardized, and reproducible, being used in several studies, not only to test the antimicrobial properties of irrigants<sup>[20]</sup>, but also of intracanal medicaments and root canal sealers.<sup>[21]</sup>

Sodium hypochlorite (NaOCl) is the most common root canal irrigant with both antimicrobial and tissue-dissolving properties. It has been demonstrated that 0.5% NaOCl kills *C. albicans* during a 10-sec contact time.<sup>[22]</sup> However, one of the major drawbacks of NaOCl in the root canal system is its high surface tension, which limits

its penetration into dentinal tubules and irregularities of the root canal system.<sup>[23]</sup>

Chlorhexidine (CHX) is a cationic bisguanide with a wide antimicrobial spectrum which is effective against both Gram-positive and Gram-negative bacteria as well as fungi.<sup>[24]</sup>

According to Beena Shino et al(2016). 2% chlorhexidine showed significant antimicrobial activity against *C. albicans*.<sup>[25]</sup> And in our study also we found significance better antifungal effect with CHX.

Vahdaty et al evaluated in vitro the antibacterial efficiency of 2% and 0.2% chlorhexidine, comparing them with NaOCl in the same concentrations. These cleansers were used in the infected dentin tubules. The results indicated that both substances reduced the number of bacteria in the superficial layers of the dentin tubules.<sup>[26]</sup>

Citric acid solutions had antimicrobial activity against *Staphylococcus aureus*, *Streptococcus sanguis*, *Lactobacillus casei*, and *Actinomyces naeslundii*. *Peptococcus niger*, *Peptostreptococcus anaerobius*, *Bifidobacterium bifidum*, *Eubacterium lentum*, *Propionibacterium avidum*, *Veillonella parvula*, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum*.<sup>[27]</sup>

The use of 25% citric acid was found to be ineffective in eradication of biofilms of *E. faecalis* after 1, 5, and 10 mins of exposure is proven by Moliz MT et al<sup>[28]</sup> While using citric acid following precautions should be taken using of rubber dam, preventing direct contact with eye and soft tissue and Washing the canal with water if it goes beyond apex to reduce its irritating effect.

## CONCLUSION

20% Citric acid shows greater antifungal efficacy than 0.2% Chlorhexidine and 3% Sodium Hypochlorite against *Candida albicans*.

Further in vivo and in vitro studies are required to draw a definitive irrigation plan to treat fungally infected root canal system.

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