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ORIGINAL RESEARCH

Comparative Evaluation of Effectiveness of Sodium Dichloroisocyanurate and Calcium Hydroxide against *Candida albicans*

Jacob Kurian, Nagesh Bolla, Bhargavi Damaraju

ABSTRACT

Introduction: *Candida albicans* is the most commonly isolated fungi from the oral cavity. It is the most infective to various intracanal medicaments and is considered as invasive yeast. Sodium dichloroisocyanurate (NaDCC) which is used as a disinfectant and as a biocide in treating *potable* water has similar action to that of sodium hypochlorite against microbes. The aim of the present study is to compare the effectiveness of calcium hydroxide and NaDCC against *Candida albicans*.

Materials and methods: After obtaining the stock cultures of *Candida*, the isolates were divided into six groups which were exposed to different concentrations of NaDCC and calcium hydroxide $Ca(OH)_2$. Group 1 consisted of the isolates which were subdivided into three groups, subjected to three different concentrations of NaDCC. Group 2 also consisted of three subgroups exposed to three different concentrations of $Ca(OH)_2$. Group three consisted of three subgroups which were exposed to three different concentrations of combinations of both NaDCC and $Ca(OH)_2$.

Results: The results of the present study show that calcium hydroxide was totally ineffective at all concentrations and NaDCC was effective and also the combination of both was shown to be effective.

Conclusion: NaDCC alone was effective at all concentrations and the combination with $Ca(OH)_2$ was found to be less effective. $Ca(OH)_2$ was totally ineffective.

Keywords: *Candida albicans*, Sodium dichloroisocyanurate, Calcium hydroxide, Agar diffusion test.

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INTRODUCTION

Candida albicans is the most commonly isolated fungi from the oral cavity of both healthy and medically compromised individuals. It is the most infective and invasive yeast among the *Candida* species.[1] The incidence of *Candida albicans* in the infected root canals has been shown to vary between 7 and 55%. It has been reported that *Candida* can use dentin as the nutrient source because of its collagenolytic activity.[2]

The presence of *Candida albicans* in the infected root canal space and periradicular areas has been demonstrated through the use of light and electron microscopy as well as using culture techniques.[3] *Candida* is versatile and can adapt to a range of pH change, gene expression in response to environmental cues, adhere to a variety of surfaces, produce degradative enzymes and change morphologic forms to evade the immune system.[4] Effectiveness of intracanal irrigants and medicaments on *Candida albicans* were studied and stated that direct contact of calcium hydroxide [$Ca(OH)_2$] paste is effective than the other forms.[5]

Waltimo et al evaluated different oral *Candida* species including *Candida albicans* in the presence of calcium hydroxide *in vitro*.[6] They reported that *Candida* species were resistant to calcium hydroxide and concluded that there is a need for supplementary agents to effectively treat persistent apical periodontitis.

Sodium dichloroisocyanurate (NaDCC) which is used as a disinfectant and as a biocide in treating potable water has similar action to that of sodium hypochlorite against microbes. It releases hypochlorous acid which acts on the microbial cell wall and causes its deterioration.[7] It would therefore be of value in determining the effectiveness of this agent against *Candida albicans*. The purpose of the present study was to evaluate the effectiveness of NaDCC and calcium hydroxide against *Candida albicans*.

MATERIALS AND METHODS

Stock cultures of *Candida albicans* obtained from Microbial Type Culture Collection (MTCC) were maintained on

Sabourand's dextrose agar plates (Himedia, India). The cultures were incubated at 37°C for 24 hours. Staining was done with Gram's stain and the growth of *Candida albicans* was confirmed by germ tube production which is the characteristic feature of *Candida albicans*. The test solutions were prepared at three different concentrations of 0.15, 0.6 and 0.9% of both NaDCC and $Ca(OH)_2$. The isolates obtained were divided into three groups each which were subjected to three different concentrations of the solutions.

- Group 1 consisted of three subgroups based on the level of exposure to different concentrations of NaDCC, N1 (0.15%); N2 (0.6%); N3 (0.9%)
- Group 2 is divided into three subgroups based on the level of exposure to different concentrations of $Ca(OH)_2$, C1 (0.15%); C2 (0.6%); C3 (0.9%)
- Group 3 has three groups exposed to three different concentrations of combinations of NaDCC and $Ca(OH)_2$.

This study was carried out following the method described by Kirby Bauer (1966).[7] In this method the disk diffusion susceptibility test for antifungal resistance is detected by challenging fungal isolates with antifungal agents placed on the surface of an agar plate seeded with a lawn of fungi. When a known concentration of antimicrobial agent is placed on the surface of a freshly inoculated plate, the agent immediately begins to diffuse and establish a concentration gradient around the agent. The highest concentration is closest to the agent and upon incubation the fungi grow on the surface of the plate except where the antifungal agent concentration in the gradient is sufficiently high to inhibit growth.

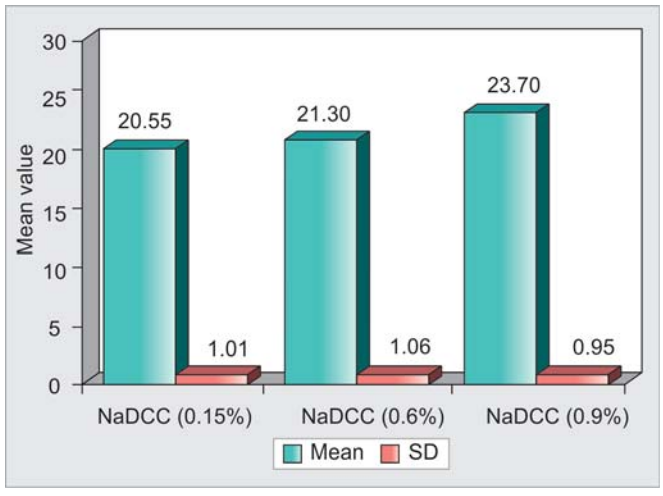
Candida albicans, obtained from the microbial test culture collection, was cultured on the Sabouraud's dextrose agar media and the plate was incubated 37°C in ambient atmosphere for a 24 hours period in ten 15 × 100 mm petri dishes. The plates were placed into a UV laminar flow chamber to obtain a lawn culture.

In this agar diffusion method, wells of 6 mm depth and 2 mm diameter were created with a punch (punch well method). The test solutions, NaDCC (Ecolabs, Garforth, UK), available as tablet form, calcium hydroxide (Deepashree products, Ratnagiri, India), available as powder form, were diluted to the concentrations noted above and 50 ml of each concentration was transferred into the wells. The fungal growth inhibition was calculated by using zone scale (Himedia, India) to measure the inhibition zones from the outer end of the well to the point where the zone ended in millimeters. The point of abrupt diminution of growth, which corresponds to the point of complete inhibition of growth, was taken as zone edge.

STATISTICAL ANALYSIS

Comparison of diameter in three groups, i.e. NaDCC with 0.15, 0.6 and 0.9% with respect to zone diameter by one-way ANOVA reveals that, there is a significant difference was observed between three groups, i.e. NaDCC with 0.15, 0.6 and 0.9% with respect to zone diameter scores ($F = 26.6550$, $p < 0.05$) at 5% level of significance (Graph 1, Table 1). It means that, the mean zone diameter score is different in different groups (Table 2). If F is significant, to know the pairwise comparison of three groups, i.e. NaDCC with 0.15, 0.6 and 0.9% with respect to zone diameter, the Tukeys multiple post-hoc procedure was applied results of the above table reveals that (Table 3):

1. NaDCC (0.15 and 0.6%) groups do not differ with respect to zone diameter scores at 5% level of significance. It means that, the mean zone diameter score is similar in NaDCC (0.15% and 0.6%) groups.
2. NaDCC (0.15 and 0.9%) groups differ with respect to zone diameter scores at 5% level of significance. It means that, the mean zone diameter score is higher in



Graph 1: Comparison of three groups, i.e. NaDCC with 0.15, 0.6 and 0.9% with respect to zone diameter

Table 1: Summary statistics of zone diameter in NaDCC with 0.15, 0.6 and 0.9%

Groups	*N*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*	*Std. error*
NaDCC (0.15%)	10	19	22	20.55	1.01	0.32
NaDCC (0.6%)	10	20	23	21.30	1.06	0.34
NaDCC (0.9%)	10	22	25	23.70	0.95	0.30

The results of the above table represent the summary statistics of zone diameter in NaDCC with 0.15, 0.6 and 0.9%

Table 2: Comparison of diameter in three groups, i.e. NaDCC with 0.15, 0.6 and 0.9% with respect to zone diameter by one-way ANOVA

Source of variation	*df*	*Sum of squares*	*Mean square*	*F-value*	*Sig.*
Between groups	2	54.15	27.08	26.6550	0.0000*
Within groups	27	27.43	1.02		
Total	29	81.58			

*p < 0.05

Table 3: Pairwise comparison of three groups, i.e. NaDCC with 0.15, 0.6 and 0.9% with respect to zone diameter by Tukeys multiple post-hoc procedures

Groups	*NaDCC (0.15%)*	*NaDCC (0.6%)*	*NaDCC (0.9%)*
Mean	20.55	21.30	23.70
SD	1.01	1.06	0.95
NaDCC (0.15%)	p = 1.0000	–	–
NaDCC (0.6%)	p = 0.2373	p = 1.0000	–
NaDCC (0.9%)	p = 0.0001*	p = 0.0002*	p = 1.0000

*p < 0.05

The mean and SD scores of zone diameter is also presented in the following table

NaDCC (0.6%) group as compared to NaDCC (0.15%) group.

3. NaDCC (0.6 and 0.9%) groups differ with respect to zone diameter scores at 5% level of significance. It means that, the mean zone diameter score is higher in NaDCC (0.9%) group as compared to NaDCC (0.6%) group.

   Similarly, pairwise comparison of three groups, i.e. NaDCC and Ca $(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter by Tukeys multiple post-hoc procedures reveals that,

4. Combination of NaDCC and $Ca(OH)_2$ (0.15%) and (0.6%) groups do not differ with respect to zone diameter scores at 5% level of significance. It means that the mean zone diameter score is less in NaDCC (0.15 and 0.6%) groups.
5. Combination of NaDCC and $Ca(OH)_2$ (0.15 and 0.9%) groups differ with respect to zone diameter scores at 5% level of significance. It means that, the mean zone diameter score is higher in (0.9%) group as compared to (0.15%) group.
6. Combination of NaDCC and $Ca(OH)_2$ (0.6 and 0.9%) groups differ with respect to zone diameter scores at 5% level of significance. It means that, the mean zone diameter score is higher in (0.9%) group as compared to (0.6%) group.

RESULTS

Statistically the antimicrobial effect of NaDCC (Fig. 1) alone with a mean 23.70 mm (see Fig. 1) was significantly better at all three concentrations when compared with the combination of this agent with calcium hydroxide and calcium hydroxide used alone (Fig. 2, Table 4 and Graph 2). The combination of NaDCC and calcium hydroxide was effective at the highest concentration of 0.9% with a mean zone diameter 14 mm but there were no statistically significant differences when compared to the lower concentrations (Tables 5 and 6, Graph 2).

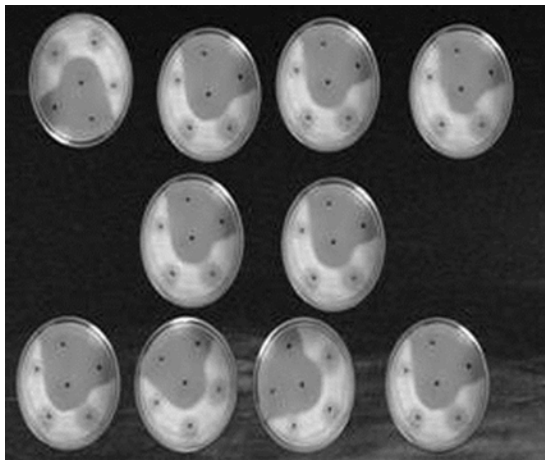


Fig. 1: Inhibition zones of sodium dichloroisocyanurate and calcium hydroxide

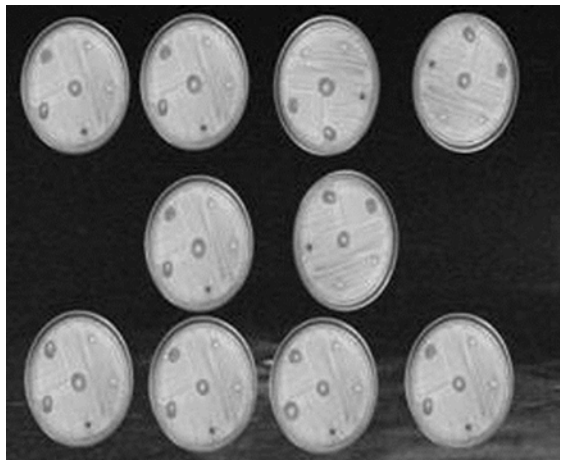


Fig. 2: Inhibition zones of combination of sodium dichloroisocyanurate and calcium hydroxide

Table 4: Summary statistics of zone diameter in NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9%

Groups	*N*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*	*Std. error*
NaDCC and $Ca(OH)_2$ (0.15%)	10	4	7	5.70	0.95	0.30
NaDCC and $Ca(OH)_2$ (0.6%)	10	5	7	5.90	0.88	0.28
NaDCC and $Ca(OH)_2$ (0.9%)	10	12	15	14.00	0.94	0.30

Table 5: Comparison of three groups, i.e. NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter by one-way ANOVA

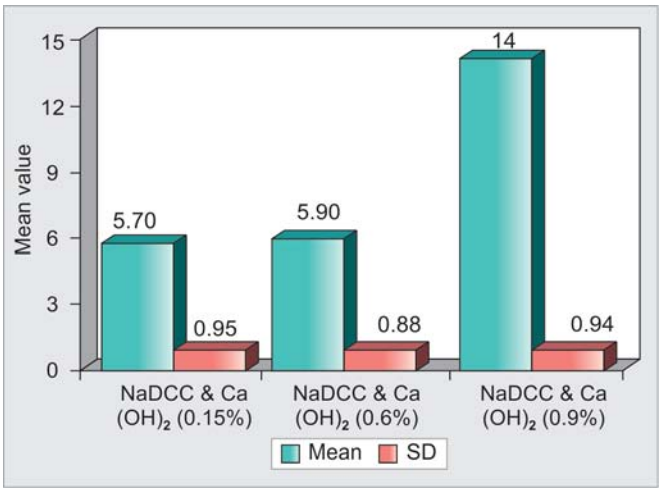
Source of variation	*df*	*Sum of squares*	*Mean square*	*F-value*	*Sig.*
Between groups	2	448.47	224.23	263.2300	0.0000*
Within groups	27	23.00	0.85	–	–
Total	29	471.47	–	–	–

*p < 0.05

Table 6: Pairwise comparison of three groups, i.e. NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter by Tukeys multiple post-hoc procedures

Groups	*NaDCC and $Ca(OH)_2$ (0.15%)*	*NaDCC and $Ca(OH)_2$ (0.6%)*	*NaDCC and $Ca(OH)_2$ (0.9%)*
Mean	5.70	5.90	14.00
SD	0.95	0.88	0.94
NaDCC and $Ca(OH)_2$ (0.15%)	p = 1.0000	–	–
NaDCC and $Ca(OH)_2$ (0.6%)	p = 0.8793	p = 1.0000	–
NaDCC and $Ca(OH)_2$ (0.9%)	p = 0.0001*	p = 0.0001*	p = 1.0000

*p < 0.05



Graph 2: Comparison of three groups, i.e. NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter

Calcium hydroxide was totally ineffective against *Candida albicans* after 24 hours (Tables 7 and 8 and Graph 3) exposure as there was no inhibition of growth of the *Candida* (Tables 9 and 10).

DISCUSSION

The method used to evaluate the inhibition of fungal growth in this study was agar diffusion test which is the most effective method of evaluating the antifungal as well as antibacterial properties of any restorative material and solution.[8,9] This method allowed direct contact between *Candida albicans* and the test solutions. The growth of *Candida* is confirmed by the presence of turbidity when a tube dilution test is used and by growth covering the agar plate when the diffusion test is used.[6] Absence of turbidity and zones of inhibition indicate that the test material or solution is active against *Candida*.[5]

NaDCC was used in this experiment as the primary test agent. It is sodium salt of dichloroisocyanuric acid and is also known as sodium troclosene and sodium dichloro-s-triazinetrione.[9,10] This granular product, containing 64.5% of available chlorine, is an effective chlorine-releasing agent (CRA) like other CRA's, such as sodium hypochlorite and the chloramines.[11-13]

NaDCC releases free available chlorine into water in the form of hypochlorous acid (HOCl) which, in turn, dissociates into H^+ and OCL^-. The hypochlorite ion has about one hundredth the biocidal potency of undissociated HOCL. HOCL has similar chemical structure to that of water and is electrically neutral. These factors enable the compound to penetrate the cell wall in a similar way to that of water.[13] The action of HOCL is mainly on DNA where it disrupts DNA synthesis.[14] On the other hand hypochlorite ion has a different structure and is electrically charged making it more difficult to diffuse through the cell wall thereby reducing its biocidal potential by about one hundred.[14,15]

NaDCC releases free available chlorine in seconds and when needed this chlorinated isosyanurate species behaves

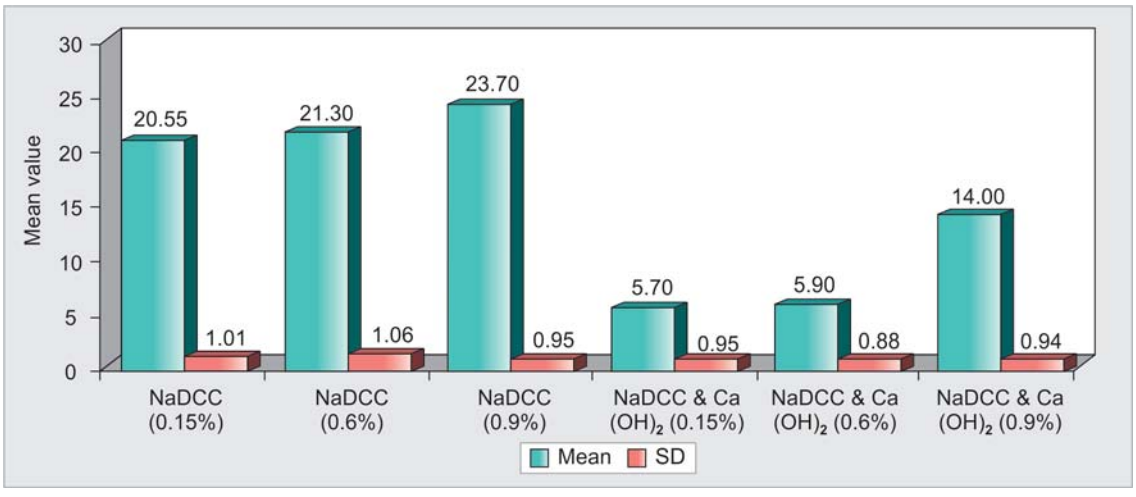
Table 7: Summary statistics of zone diameter in NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% groups

Groups	*N*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*	*Std. error*
NaDCC (0.15%)	10	19	22	20.55	1.01	0.32
NaDCC (0.6%)	10	20	23	21.30	1.06	0.34
NaDCC (0.9%)	10	22	25	23.70	0.95	0.30
NaDCC and $Ca(OH)_2$ (0.15%)	10	4	7	5.70	0.95	0.30
NaDCC and $Ca(OH)_2$ (0.6%)	10	5	7	5.90	0.88	0.28
NaDCC and $Ca(OH)_2$ (0.9%)	10	12	15	14.00	0.94	0.30

Table 8: Comparison of six groups, i.e. NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter by one-way ANOVA

Source of variation	*df*	*Sum of squares*	*Mean square*	*F-value*	*Sig.*
Between groups	5	3162.62	632.52	677.3680	0.0000
Within groups	54	50.43	0.93		
Total	59	3213.05			

*p < 0.05



Graph 3: Comparison of six groups, i.e. NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter

Table 9: Pairwise comparison of six groups, i.e. NaDCC and $Ca(OH)_2$ with 0.15, 0.6 and 0.9% with respect to zone diameter by Tukeys multiple post-hoc procedures

Groups	*NaDCC (0.15%)*	*NaDCC (0.6%)*	*NaDCC (0.9%)*	*NaDCC and $Ca(OH)_2$ (0.15%)*	*NaDCC and $Ca(OH)_2$ (0.6%)*	*NaDCC and $Ca(OH)_2$ (0.9%)*
Mean	20.55	21.30	23.70	5.70	5.90	14.00
SD	1.01	1.06	0.95	0.95	0.88	0.94
NaDCC (0.15%)	p = 1.0000	–	–	–	–	–
NaDCC (0.6%)	p = 0.5152	p = 1.0000	–	–	–	–
NaDCC (0.9%)	p = 0.0001	p = 0.0001	p = 1.0000	–	–	–
NaDCC and $Ca(OH)_2$ (0.15%)	p = 0.0001*	p = 0.0001*	p = 0.0001*	p = 1.0000	–	–
NaDCC and $Ca(OH)_2$ (0.6%)	p = 0.0001*	p = 0.0001*	p = 0.0001*	p = 0.9972	p = 1.0000	–
NaDCC and Ca (OH)2 (0.9%)	p = 0.0001*	p = 0.0001*	p = 0.0001*	p = 0.0001*	p = 0.0001*	p = 1.0000

*p < 0.05

Table 10: $Ca(OH)_2$ used alone

C1—0.15%	10	Nil
C2—0.6%	10	Nil
C3—0.9%	10	Nil

as a 'reservoir' of freely available chlorine.[13,16] The release of 'reservoir' chlorine provides the greater biocidal capacities found with NaDCC when compared to other chlorine donors and explains why NaDCC is less inactivated by organic matter. NaDCC is as effective as NaOCl in its organic tissue dissolving property and its antimicrobial action.[12,13]

The other agent used in this study was calcium hydroxide whose antibacterial property depends on its long lasting alkalinity and antimicrobial activity of the calcium hydroxide is related to the release of hydroxyl ions into an

aqueous environment.[17] These hydroxyl ions are highly oxidant free radicals that show extreme reactivity with several biomolecules.[18,19] Several studies have reported that *Candida* species were resistant to calcium hydroxide when used along with other irrigants,[19-21] but in our study the combination of NaDCC and calcium hydroxide was found to be effective because of higher concentration of NaDCC at 0.9%.

CONCLUSION

With in the limitations of the present study, the results show that NaDCC alone was effective at all concentrations in inhibiting the growth of *Candida albicans*. The combination of NaDCC with calcium hydroxide was found to be less effective and calcium hydroxide was totally ineffective. Further studies are needed to determine the biocompatibility of this material and to be used as an root canal irrigant.

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