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Short Communication

Determination of minimal inhibitory concentration of citric acid as a root canal irrigant against *E. faecalis* and *C. albicans*

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ABSTRACT

The clinical success of primary teeth endodontic therapy depends strongly on attaining prepared root canals with sufficient disinfection levels. Therefore, using natural or chemical cleaning agents to properly clean the root canals is becoming more and more important. With little to no impact on the organic components, citric acid is effective in dissolving the smear layer and the inorganic components of root dentine. Study included the custom preparation of citric acid to 3 different concentrations-6,8, and 10%. MTCC strains of *C. albicans* and *E. faecalis* were cultured on Sabouraud Dextrose agar plates and blood agar respectively. Three wells each were prepared on cultured plates and the solutions of citric acid was inoculated into them. The zone of inhibition was noted after 24 hours in each plate. The greatest zone of inhibition will be determined in each of the plates and the concentration was recorded with *E. Faecalis* and *C. Albicans*. The lowest concentration of citric acid that is capable of inhibiting bacterial growth will be taken as the minimum inhibitory concentration of citric acid against the respective organism. 8% concentration of citric acid can be used as an irrigant and gives a greater inhibition zone almost as much as that of 10 % citric acid.

Keywords: Citric acid, *C. albicans*, *E. faecalis*, Root canal irrigant

INTRODUCTION

The clinical success of pulpectomy treatment in primary teeth depends strongly on attaining prepared root canals with sufficient disinfection levels.[1] The clinical evidence revealed that mechanical preparation of root canal with hand or rotary instruments has a narrow disinfectant effect as it leaves behind marked number of infected debris or necrotic tissues inside the root canals.[2] Hence, there is an increased need for employing chemical or natural agents to effectively clean the pathogenic microorganisms from the infected root canals.[3]

During chemo mechanical preparation, either by hand or rotary instruments, results in producing considerable quantities of debris and smear layer.[4,5] Smear layer is composed of organic and inorganic components like vital or necrotic pulp tissue, micro-organisms, saliva, blood cells, and tooth structure.[5] Irrigating solutions are used for removal of this smear layer and also for different purposes like lubrication, removal of debris, and anti-bacterial effects.

With little to no impact on the organic components, citric acid is effective in dissolving the smear layer and the inorganic components of root dentine. Citric acid has proven to be beneficial in regenerative endodontic procedures due to its property of higher TGF-β1 release.[6] It also has lower cytotoxicity when compared to other irrigants as its toxicity is dose-dependent.[7]

Citric acid has proven to be effective in smear layer removal, showing better results in coronal and middle root thirds, improving its effect when combined with

manual dynamic activation.[8] It has been applied on root surfaces altered by periodontal diseases. Also, it has been proposed as a conditioning agent for dental hard tissues.[9] It has good chemical stability with anti-microbial effects against the facultative and obligative anaerobes.[10]

E. Faecalis is a potent microorganism based on their marked role in the failure of the endodontically treated teeth. It has a significant role in the incidence and persistence of the periapical lesion even after endodontic treatment.[11]

Despite the fact that endodontic infections are multi microbial, the *E. faecalis* bacterial strain is a tracer indicator regarding recurring infections and treatments. It is extensively utilized to test the effectiveness of disinfecting agents in endodontics; and, lastly, it can invade and colonize dentinal tubules in depth, thus tolerating centrifugation.[12]

The role of *Candida albicans* in the pathogenesis of endodontic infections is critical. The yeast, being a microaerophilic eukaryote, possesses the metabolic harmony necessary to survive within the harsh and barren ecosystem of the root canal. It is shown that C. albicans could use dentin itself as a source of nutrition in vitro, in the absence of other extraneous food supplements, and colonize the canal walls as well as the dentinal tubules.[13-15]

The penetration of *C. albicans* into dentinal tubules in vivo was shown to be facilitated by the presence of a smear layer, produced by instrumentation.[16] Though citric acid has been found to be effective as an irrigating solution its MIC has not been determined to date. Hence, this study was undertaken to determine the minimum inhibitory concentration (MIC) of citric acid against the two potent microorganisms present in the root canals, posing a threat to its treatment failure.

Objective

To determine the minimal inhibitory concentration of citric acid as a root canal irrigant against *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. albicans).*

METHODS

Inclusion criteria

Children with parental consent. Children aged between 4 to 7 years. Children with deep caries with pulpal exposures include no history of antibiotics in the past 3 months. Children having teeth with symptoms and signs of irreversible pulpitis.

Exclusion criteria

Tooth that is indicated for extraction. Root resorption more than 2/3rd of radiographic length. No history of any physical, mental disabilities or systemic diseases.

Ethical approval

Ethical approval was obtained from the institutional research committee for the study. All the participants were informed about the study and written consents were obtained from the parents.

This experimental study was conducted from the research wing of Mahe Institute of Dental Science and hospitals in a time period from August 2024 to October 2024.

Preparation of citric acid

40% solution of citric acid that is commercially available for root canal irrigation was mixed with distilled water at room temperature for obtaining concentrations of 6%, 8% and 10% citric acid.

To 0.85 ml of citric acid 9.15 ml of distilled water was added to get a 6% citric acid solution. To 1.25 ml of citric acid 8.75 ml of distilled water was added to get an 8% solution. To 1.6 ml of citric acid 8.4 ml of distilled water was added to get a 10% solution.

Preparation of samples

The samples consisted of two groups. Group 1 E. faecalis and Group 2 Candida albicans. They were further sub grouped into 3 groups accordingly.

Group 1 *E. feacalis* (a) 6% citric acid, (b) 8% citric acid, (c) 10% citric acid. Group 2 *Candida albicans* (a) 6% citric acid, (b) 8% citric acid, (c) 10% citric acid.

Preparation of test inoculum

MTCC strains of the test organisms were swabbed on Mueller–Hinton broth. For routine antimicrobial sustainability tests, Mueller–Hinton broth is considered the best medium because it has good reproducibility and allows the fast growth of most, pathogenic bacteria.

Microbiological preparation

Strains of *C. albicans* and *E. Faecalis* were sub cultured by streaking on sabouraud dextrose broth and blood agar plates respectively and they were incubated for 24 hours at 37℃. Three plates each of *C. albicans* and *E. faecalis* were taken for the study. Three wells each were prepared on each of the cultured plates of *C. albicans* and *E. faecalis* (Figure 1 and 2). Different concentrations of 6%, 8%,10% citric acid was introduced into the wells and incubated for 24-48 hours. The zone of inhibition in the plates were determined around each well in each of the three plates containing *C. albicans* and *E. faecalis*

Statistical analysis

Descriptive statistics were used to report the zone of inhibition in terms of the mean/median (Mdn) (central tendency) and standard deviations (SD)/inter-quartile

range (IQR) (measures of dispersion) along with the minimum and maximum values, to report range.

RESULTS

The mean zone of inhibition was determined in each of the three plates of *E. faecalis* and *C. albicans* and the concentrations were recorded. Results were recorded based on the diameter of the zones.

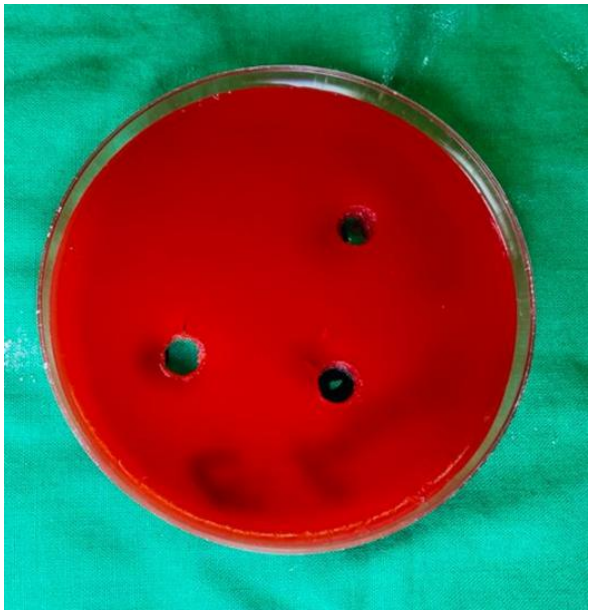

Figure 1: Well, prepared on blood agar.

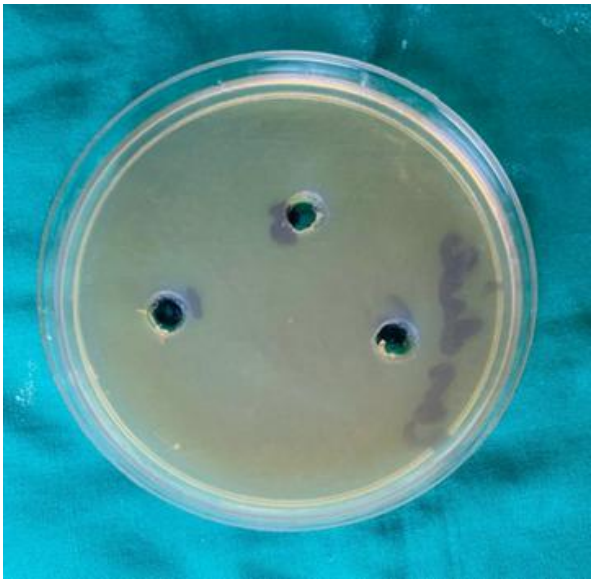

Figure 2: Well prepared on Sabouraud dextrose agar.

Table 2 and Figure 5 presents the descriptive statistics for the zone of inhibition (in mm) observed across different concentrations of citric acid. At a 10% concentration, the mean zone of inhibition was 23.0 mm (±1.00), with a median value of 23.0 mm (IQR: 22.0–24.0) and a range of 22.0–24.0 mm.



Figure 3: (a, b) Zone of inhibition on blood agar plates of *E. faecalis.*

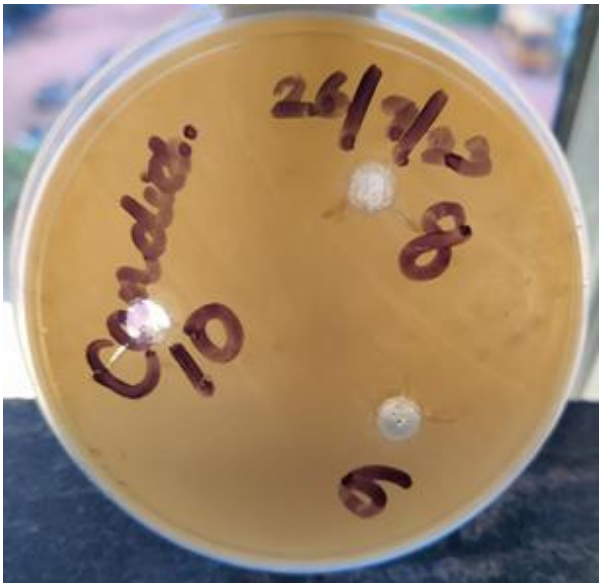

Figure 4: Cultured plate of *Candida albicans* showing no zone of inhibition.

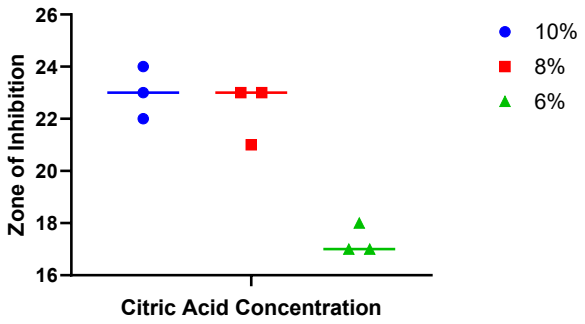

Figure 5: Interleaved scatter plot showing the individual values for the Zone of Inhibition (in mm) for the specimens across different concentrations of citric acid with mean marked as bar.

For the 8% concentration, the mean zone of inhibition was slightly lower at 22.3 mm (±1.15), with a median of 23.0 mm (IQR: 21.0–23.0) and a range of 21.0–23.0 mm. At the 6% concentration, the mean zone of inhibition was notably smaller at 17.3 mm (±0.577), with a median of 17.0 mm (IQR: 17.0–18.0) and a range of 17.0–18.0 mm. These values demonstrate a decrease in the zone of inhibition with lower concentrations of citric acid (Figure 3 (a, b)).

Table 1: Zone of inhibition of *E. faecalis* in blood agar medium.

Concentration of citric acid	Sample 1 one of inhibition	Sample 2 zone of inhibition	Sample 3 zone of inhibition
10%	23 mm	22 mm	24 mm
8%	23 mm	21 mm	23 mm
6%	17 mm	18 mm	17 mm

Table 2: Descriptive statistics of the zone of inhibition (in mm) for the specimens across different concentrations of citric acid.

Descriptive statistics/ citric acid concentrations	10%	8% (n=3)	6% (n=3)
Mean±SD	23.0±1.00	22.3±1.15	17.3±0.577
Median (Q1-Q3)	23.0 (22.0-24.0)	23.0 (21.0–23.0)	17.0 (17.0–18.0)
Min-Max	22.0–24.0	21.0–23.0	17.0–18.0

n:sample size per group, SD:Standard deviation; Q1:First Quartile; Q3:Third Quartile; Q1-Q3:Inter-quartile Range; Min-Minimum value; Max:maximum value

DISCUSSION

The present study was done to evaluate the minimal inhibitory concentration (MIC) of citric acid in different concentration against *E. faecalis* and *C. albicans* used as an irrigant in primary dentition. Various studies have shown that the choice of 10% citric acid as an irrigating solution is due to its efficiency in removing the smear layer and antimicrobial action on oral biofilms and anaerobic bacteria because of its acidic pH when compared to EDTA.[17] However, the MIC of citric acid has not been determined in any of the previous studies on primary teeth.

The MIC of citric acid is essential to be determined because it has been found that the toxicity of citric acid is dose dependent and the least concentration effective as an irrigating solution should be used in root canals to avoid it and also to prevent injury to the underlying permanent tooth bud. In our study we used the two organisms such as *E. faecalis* and *C. albicans* because they have been found to be the most prominent organisms present in the recurrent root canal infections after treatment.[18]

Blood agar is an enriched medium with trypticase soy agar base enriched with 5% sheep blood. Murad CF et al stated survival of E. faecalis in very harsh environments, with poor nutrient supply and high alkaline pH reaching up to 11.5.[19] The capacity of *E. faecalis* to grow as a biofilm, on root canal walls and as mono-infection in treated canals without synergistic support from other bacteria makes it the very resistant pathogen in root canal treatment. Study done by Stuart et al, added to the characteristics of *E. faecalis*, the capacity of this microorganism to use serum from dentin and the periodontal ligament (PDL) as a source of nutrition.[20] Here, we used blood agar for the growth of *E. faecalis*.

Yeasts can be detected in 7–18% of infected root canals, of which *C. albicans* is the most prevalent. They are commonly associated with persistent cases of apical periodontitis and can also be isolated in primary apical periodontitis. Selective culture media such as Sabouraud dextrose agar combined with cultivation from undiluted sample is used for primary isolation of yeasts in endodontic infections.[21] A variety of virulence factors enable *C. albicans* to adhere to and penetrate into dentine. They can tolerate harsh ecological conditions including high alkalinity.

A study by Egan et al, aimed at evaluating the relationship between the presence of fungi in the saliva and in the root canals of teeth with apical periodontitis revealed that *C. albicans* were 13.8 times more prevalent in the root canals when they were also present in the salivary samples of the subjects.[22,23]

Citric acid (CA) is made available in various concentrations from 1 to 50%, although 10% CA is more commonly used as it is effective in removal of the smear layer.[24] Studies by Gotze Gda et al have shown that irrigation with 6% CA for 15 or 30 seconds is quite effective in removing all the components of the smear layer of the primary teeth which was in accordance with our study, whereas peritubular dentin destruction was observed with higher concentration of CA when used as an irrigating solution.[25] Yamaguchi et al, investigated

various properties of citric acid as decalcifying and cleansing agents in root canal irrigation and antibacterial effects on powdered dentin-resin mixtures for evaluating the decalcifying effect of citric acid and EDTA solution.

Citric acid solution showed antibacterial effects on root canal bacteria.[26] Gutmann et al in their study concluded that 10% citric acid is better for smear layer removal from the root end cavities.[27] In a recent study, Machado-Silveiro et al conducted a study to measure the demineralization capability of 10% citric acid, 10% sodium citrate and 17% EDTA on root canal dentine, and showed that 10% citric acid, is effective in dentine demineralization.[28]

In our study, greater zone of inhibition was seen around 8% and 10% citric acid solution on plates of E faecalis. 23mm of zone of inhibition was seen for 10% and 8% of citric acid. Two samples of 8% citric acid showed 23 mm of inhibition zone, which is as effective as 10% of citric acid. Most of the studies in literature have been done with citric acid using 10% of the solution for irrigating the root canals. We found no studies in literature which has been done with 8% citric acid. Our study found that the zone of inhibition with 8% citric acid solution was almost equal to that of 10% solution.

The zone of inhibition of 17 mm was noted with 6% of citric acid in our study, which was below the average of the other two concentrations of 8% and 10% on plates of *E faecalis*. Studies done by Neetu et al, with the use of 6% citric acid found definitely good result in rapid elimination of pain, resolution of peri-radicular radiolucency, better obturation quality in terms of less voids and void areas.[29] Takeda et al also observed no differences between 17 % EDTA and 6% citric acid in their study with hand instrumentation and photomicrographs.[30]

Wayman BE et al, reported 6% citric acid solution applied for 60 sec removed the smear layer and smear plugs in the tubules.[31] Studies showed that *E. faecalis* biofilms were not eradicated by either citric or phosphoric acids at any dilution or time of exposure. This contrasts with the activity observed for these acids against *E. faecalis* suspensions in our study. Previous studies have shown citric acid to be effective against planktonic bacteria in concentrations of 10% or over and at times of 10 and 15 minutes.[32]

CONCLUSION

8% concentration of citric acid can be used as an irrigant and gave an inhibition zone almost as much as that of 10% citric acid. However, in vivo studies have to be done to substantiate the effect of 8% solution of citric acid when used as an irrigating solution in effective elimination of debris and microorganisms.

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