

In Vitro Antifungal Efficacy of Different Intracanal Irrigants against *Candida Albicans*

Mansoreh Abbasi ¹, Anahita Norouzifard ²✉, Masoud Sharifi ³

¹ Assistant Professor, Department of Endodontics, School of Dentistry, Islamic Azad University, Tehran, Iran

² Postgraduate Student, Department of Periodontology, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran

³ Assistant Professor, Department of Microbiology, School of Medical, Qazvin University of Medical Sciences, Qazvin, Iran

Abstract

Background and Aim: An intracanal irrigant is essential for a successful root canal therapy. The aim of this study was to compare the antifungal efficacy of 5.25% sodium hypochlorite, 2% chlorhexidine gluconate (CHX) and 94% carvacrol against *Candida albicans* (*C. albicans*) in vitro.

Materials and Methods: In this experimental study, after crown removal and canal preparation of 48 extracted human maxillary central incisors, they were randomly divided into 3 experimental groups of 14 teeth, plus two groups of positive and negative controls (n=3). A suspension of *C. albicans* (ATCC=10261) was injected into the experimental and positive control group root canals. The teeth were then incubated for 72 hours. Then, root canals in each group were rinsed with one of the irrigants for 1 minute and samples were taken from the canals and inoculated on plates. After 48 hours of incubation, the colony growth was assessed and colony forming units (CFUs) served as a measure of antifungal activity. Data were analyzed using the Kruskal Wallis test.

Results: In carvacrol, sodium hypochlorite and CHX groups, 6, 10 and 1 specimen did not show bacterial growth and the mean CFU was 86.3, 53.3 and 271.2 in the mentioned groups, respectively.

Conclusion: Carvacrol and 5.25% sodium hypochlorite had similar antifungal efficacy against *C. albicans* and this effect was significantly greater than that of 2% CHX.

Key Words: *Candida albicans*, Carvacrol, Chlorhexidine gluconate, Root canal, Sodium hypochlorite

✉ Corresponding author:

Anahita Norouzifard,
Postgraduate Student,
Department of Periodontology,
School of Dentistry, Kerman
University of Medical Sciences,
Kerman, Iran
anahita_norouzifard@yahoo.com

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Introduction

The main goal of root canal therapy is to eliminate the microorganisms from the root canal system and prevent infection [1, 2]. Different microorganisms have been isolated from the infected root canals. The role of fungi has been well documented in infections of the pulp, root canals and dentinal tubules [3]. Fungi have been reported to be present in 7-55% of infected root canals. *Candida albicans* is the most common fungus involved in endodontic infections [4]. Disinfection of the root canal system is done via mechanical preparation, chemical

irrigation and placement of intro-canal medicaments in-between treatment sessions [5]. Use of irrigating solutions is necessary for complete elimination of debris and microorganisms from the irregularities of the root canal system [6]. Intra-canal irrigating solutions must have strong antimicrobial properties. They must be able to dissolve organic residues, disinfect the intra-canal space, and remove debris from the prepared canals. Moreover, they should have a lubricating effect with no toxicity for peri-radicular tissues [6].

At present, sodium hypochlorite (NaOCl) is the most commonly used root canal irrigating solution. However, it does not have all the properties of an ideal solution [7]. Chlorhexidine gluconate is another suitable irrigating solution for root canal disinfection. It is capable of bonding to hydroxy apatite and has a subsequently slow release [8]. However, in contrast to sodium hypochlorite, CHX cannot dissolve necrotic tissues [7]. Use of herbal extracts is another suitable technique for treatment of some conditions due to minimal side effects and low toxicity [9].

Carvacrol is a phenolic compound found in essence of plants such as *Thymus vulgaris* and *Satureja Khuzestanica*. It has well-recognized efficacy for alleviation of inflammation, pain relief and elimination of microorganisms. Carvacrol has been recommended for root canal irrigation.

However, its antifungal activity in the root canal system has yet to be confirmed [9]. This in vitro study aimed to compare the antifungal effect of sodium hypochlorite, CHX and carvacrol on *C. albicans* in the root canal system.

Materials and Methods

This experimental study was conducted on 48 human maxillary central incisors extracted for different purposes. After collection of the teeth, the crowns were cut using a high speed handpiece and a diamond disc (Jota, Switzerland) in order to match all the specimens and eliminate the confounding effect of the variable anatomy of the crown, in such way that the remaining root segment was 15 ± 1 mm long in all specimens. The working length was determined 0.5 mm short of the anatomic apex. The canals were prepared with stainless steel K files (Mani, Japan) using the step back technique with the master apical file (MAF) of #40. In-between filing, the root canals were rinsed with 5 mL of sterile saline solution. After completion of cleaning and shaping, in order to eliminate the smear layer, the canals were irrigated with 10mL of 17% EDTA for one minute followed by rinsing with 5 mL of 2.5% sodium hypochlorite for one minute. Next, the teeth were randomly divided into three experimental groups of 14 teeth and two positive and negative control groups of three specimens each. In order to seal the root dentinal tubules, the root surface was covered with

two layers of nail varnish. Then, the teeth were autoclave-sterilized at 121°C and 15 pounds per square inch pressure for 15 minutes. A suspension of *C. albicans* (ATCC 10261) was prepared in Tryptic soy broth (TSB) liquid medium and its turbidity was adjusted to 0.5 McFarland standard. All specimens were inoculated with the suspension of *C. albicans* using an insulin syringe under aseptic conditions (flames) and a biological hood except for the negative control group. Sterile saline solution was used in the negative control group instead of the *C. albicans* suspension. The plates were incubated at 35.4°C and 100% humidity for 72 hours (Reyhan Teb, Iran). To ensure active presence of *C. albicans* in the root canal system, a sample was obtained from the root canal system using #35 sterile paper points (VDW, Germany) every 24 hours and cultured on Tryptic soy agar (TSA). After 72 hours, The root canals in group 1 were rinsed with one milliliter of 94% carvacrol solution (10, 11)(Khoraman Pharmaceuticals, Iran) using an insulin syringe for one minute followed by rinsing with 5 mL of sterile saline solution for one minute in order to eliminate the carvacrol residues. Samples were obtained from inside the root canal system using a 35 sterile paper point and cultured on TSA medium. Each of the teeth in groups two and three were disinfected with 1mL of 5.25% sodium hypochlorite (Golrang, Iran) and 2% CHX (FGM, Brazil), respectively for one minute, followed by rinsing with five milliliters of sterile saline solution. Specimens were obtained as in group one using paper points and cultured. No disinfecting solution was used in the positive control group. The three experimental groups and the two positive and negative control groups were incubated for 48 hours. The CFUs were counted. Data were analyzed using SPSS version 16 software (Microsoft, IL, USA). The groups were compared using the Kruskal Wallis test. Level of significance was set at 0.05.

Results

Growth of *C. albicans* was evident in the positive control group. No growth was detected in the negative control group. None of the understudy solutions were capable of totally eliminating *C. albicans* (Diagram 1). In group one (carvacrol), no fungal growth was detected in 6 out of 14

specimens (43%). The mean CFU in the remaining eight specimens with fungal growth was found to be 86.3. In group two (sodium hypochlorite), fungal growth was not detected in 9 out of 14 specimens (64.2%) and the mean CFU in the remaining five specimens with fungal growth was 53.3. In group 3 (CHX), no fungal growth was detected in 1 out of 14 specimens (7%) and the mean CFU in the remaining 13 specimens with fungal growth was found to be 271.2.

Root canal irrigation with sodium hypochlorite and carvacrol had similar antifungal efficacy against *C. albicans* and no significant difference was detected in this regard between the two ($p=0.923$).

However, the antifungal effects of sodium hypochlorite and carvacrol were significantly higher than that of CHX ($p=0.018$ and $p=0.047$, respectively) (Diagram 1).

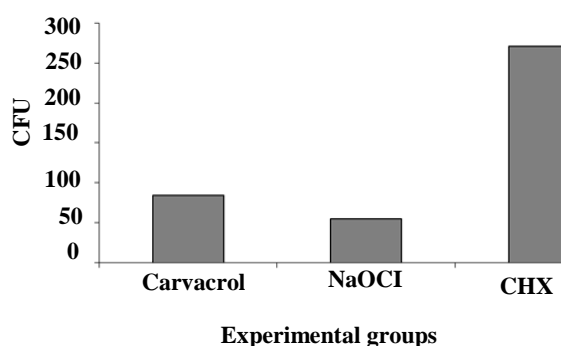


Diagram 1. The mean CFUs in the experimental groups

Discussion

Common microorganisms present in the root canal system may be very well eliminated after exposure to different disinfecting solutions. However, studies have demonstrated that fungi are highly resistant to many disinfecting solutions [1]. In the current study, complete antifungal affect (no growth at all in the specimens) was not observed in any group. In a study by Ruff et al, [12] application of 2% CHX for one minute exerted complete antifungal effect. This difference seems to be due to the time of sampling. Ruff et al. obtained samples from the root canal system 24 hours after inoculation and incubation. It must be noted that CHX has long-lasting antimicrobial efficacy due to its unique capability in bonding to dentin hydroxyapatite for 72 hours. Gradual release of the bonded CHX can maintain a constant concentration of this molecule and a sustained

antifungal activity [5]. Thus, it appears that even after irrigation with sterile saline solution, the antifungal effect of CHX may remain far beyond 24 hours (the time of sampling) in the study by Ruff et al. In the current study, complete antifungal effect of CHX was not observed because sampling was done immediately after irrigation.

In a study by Vianna et al, [7] application of 5.25% sodium hypochlorite and 0.2% CHX for 15 seconds resulted in complete elimination of fungal cells. The technique used in their study was broth dilution, which may explain different results from those of the current study. In a study by Sen et al. the complete anti-fungal effect of 1 and 5% sodium hypochlorite and 0.12% CHX was only observed after the application of solution for one hour in absence of the smear layer in extracted teeth [4].

In the direct contact and broth dilution techniques, the disinfecting agents are directly exposed to fungal cells. However, in the current study, *C. albicans* was grown on root canal walls in order to form a biofilm, simulating intraoral conditions. The microorganisms present in the biofilm are resistant to host defense mechanisms and antimicrobial drugs [4]. When irrigating the root canal system, the superficial layer of microorganisms is in direct contact with relatively high concentrations of irrigating solution.

However, the extracellular matrix of the biofilm prevents complete penetration of the solution into deeper layers. Therefore, the microbial effect of irrigating solutions is often overrated when using methods such as direct contact technique. Moreover, the buffering capacity of dentin can decrease the anti-microbial effect of disinfecting solutions in the root canal system [4]. In the current study, the antifungal effect of solutions was found to be lower than that in studies, which used the direct contact technique. This finding is in accord with the results of Sen et al.

Considering the growing resistance to the conventional antimicrobial agents, scientists have focused on the application of new natural or synthetic drugs. The anti-microbial effect of herbal extracts on cariogenic microorganisms, *Enterococcus faecalis* and fungi has been demonstrated in many previous studies [9, 13]. Studies have demonstrated that essential oils can be a suitable alternative to chemical agents in order to overcome microbial resistance [11, 13]. In the current study, the antifungal effect of carvacrol

was similar to that of sodium hypochlorite and greater than that of CHX. In a study by Botelho et al, carvacrol showed strong antimicrobial efficacy and *C. albicans* and *Streptococcus mutans* were found to be the most susceptible microorganisms to carvacrol [14]. According to a study by Chami et al, carvacrol had significant antifungal effect on oral candidiasis [15]. In a study by Samadi et al, the antibacterial activity of *Satureja Khuzestanica* extract containing 94% carvacrol against *Enterococcus Faecalis* was similar to that of 2.5% sodium hypochlorite and 0.2% CHX [11]. In the current study, 94% carvacrol was used, which is the form supplied by the manufacturer. Studies on the suitable concentration of carvacrol for elimination of *C. albicans* are scarce, Although 94% carvacrol did not show complete antifungal efficacy, future studies with lower concentrations of carvacrol seem necessary in order to obtain maximum antifungal effect with minimum concentration of the solution.

Conclusion

None of the understudy solutions (2% CHX, 94% carvacrol or 5.25% sodium hypochlorite) were capable of complete elimination of *C. albicans*. The anti-fungal effects of sodium hypochlorite and carvacrol were significantly greater than that of CHX.

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