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Effect of Herbal solutions on antimicrobial efficacy of Triple Antibiotics when used as delivery vehicle: An in-vitro study.

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Abstract

Introduction: Microbes elimination in the root canal system is endodontic therapy's prime goal. It is advised to utilise intracanal medicaments between the appointments to diminish bacterial counts. This study was conducted to investigate the effect of herbal solutions on antimicrobial efficacy of triple Antibiotics when used as delivery vehicle for intracanal medicament.

Methodology: 160 extracted single-rooted teeth were manually instrumented. After contaminating the canals with E. Faecalis and C. albicans, the specimens were divided randomly into 4 equal groups (n \20) for each C. albicans and Faecalis according to the intra-canal medications used: Group Ⅰ: Clove Oil, Group Ⅱ: Neem oil, Group Ⅲ: Cinnamon oil, Group ⅠV: Saline, All the three Groups were mixed with triple Antibiotics Ciprofloxacin, Metronidazole and Doxycycline. Microbial samples were obtained from the root canals after 24 hours, and cultures were determined after 24 hours of incubation. The Colony Forming unit was analysed with Kruskal- Wallis, Dunn's, and Chi-Square tests to find significant differences among different groups.

Results: The number of colony-forming units was significantly lower in all experimental groups compared to the control group in both agars, the highest mean rank for colony count in E. Faecalis was 34.1 and in Candida Albicans was 29.30. Neem oil had better antimicrobial efficacy compared to other medications when it was mixed with Triple antibiotics.

Conclusions: The study suggests that Neem oil increased the efficacy of triple antibiotics to eliminate the two different tested microorganisms at the root canal lumen. C.albicans was the most sensitive tested microorganism to the whole tested medications.

Keywords: Clove Oil; Cinnamon oil; Candida albicans; Enterococcus Faecalis; Neem oil; Triple Antibiotics.

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Introduction

The main causative factors of pulpal and periapical inflammation are microorganisms. Various studies have shown that the disposal or reduction of microorganisms from the root canal system results in higher healing rates of periapical lesions. The main aim of root canal treatment is to eliminate or reduce the number of microorganisms residing within the infected root canal[1]. Chemo mechanical cleaning and shaping of the root canal can significantly reduce the number of microorganisms but not eliminate them because of anatomical complexity and the limitation in accessing the canal system by instruments and irrigants [2,3]. In this regard, the present study aims to compare neem extract, cinnamon extract, and clove as intracanal medicament. The comparison is based on the effectiveness of these medications on the antimicrobial efficacy of triple Antibiotics against E. Faecalis and C. Albicans in extracted human permanent teeth.

Methods

This study was an in vitro study conducted in a Microbiological laboratory at Qassim University.

Preparation of Sample

One hundred sixty extracted intact single-rooted human teeth with single root canals were selected. Teeth were autoclaved and stored in a sterile solution.

Inclusion criteria: fully formed, single-rooted, intact permanent human teeth

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Exclusion criteria: carious teeth, damaged teeth, unformed roots, multi-rooted teeth.

The teeth were accessed and flared of the coronal part using the Gates Glidden (GG) drill number 3. After that, the working length was measured 1 mm shorter than the tip of the file that was visible at the apical foramen. All canals were sequentially prepared using the step-back technique up to the size #35 master apical file. Flaring of the canal was performed up to size #60 under irrigation with 2.5% sodium hypochlorite solution (NaOCl). The apical foramen was then sealed with a varnish to prevent leakage. Finally, the canals were flushed with ethylene diamine tetra acetic acid 17% EDTA for 2 minutes, followed by 5 ml of 5.25% NaOCl for 2 minutes to remove the smear layer. Then each tooth was flushed with 5 ml of normal physiologic saline solution, and each tooth was placed in a closed Eppendorf test tube containing 4 ml of nutrient broth.

Sample inoculation with suspension

Two types of microorganisms isolated from clinical trials, including E. Faecalis (ATCC 29212) and C. Albicans (ATCC 27853), were used. 2ml of the sterile nutrient broth from each tube were replaced by 2ml of the prepared bacterial suspension, and then the test tubes were closed and incubated at 37C for 24 hours. After the contamination period, each specimen was removed from its test tube under aseptic conditions in the laminar airflow chamber, rinsed with 5 ml of sterile saline, and dried with sterile paper points #40.

Triple Antibiotic Powder Preparation

Standard preparation:
To prepare 1 g/mL TAP, 1 g of USP-grade antibiotic powders comprising Ciprofloxacin 14%, Metronidazole 43%, and Doxycycline 43% (King Saud Hospital, Unaizah, Qassim) were mixed with 1 mL of sterile water. To prepare a 1 mg/mL TAP solution, 100 mg of each compounded powder mentioned above was dissolved in 100 mL of sterile water.
Triple Antibiotic Preparation:

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A fine powder of antibiotic powder was mixed with herbal and homoeopathic agents until a workable smooth paste was achieved.

Placement of Intracanal Medicaments

The specimens were divided randomly into four equal groups (n1\20) according to the intra-canal medications used; Group Ⅰ: was medicated with Clove Oil, Group Ⅱ: Neem oil, Group Ⅲ: Cinnamon Oil and all the Three Groups were mixed with Triple Antibiotics (ciprofloxacin, metronidazole, and Doxycycline) and Group ⅠV: sterile physiologic saline has been used as a negative control group. The orifices of the canals were sealed with Temporary filling and placed in humid sterile gauze in closed sterile Petri dishes, then incubated for 24 hours at 37C.

Assessment of Antimicrobial Efficacy

After the incubation period, the temporary filling and the intra-canal medications were removed. The root canals were irrigated using sterile saline solution and then dried with sterile paper points #40 left in the root canal for 1min to absorb the canal fluid and placed in a sterile Eppendorf test tube containing 0.5ml of sterile saline, vortexed for 30s. This suspension represents the specimen taken from the main canal lumen.

Sterile disposable plastic inoculating loops were standardised to carry 1uL of the microbial suspension to be seeded on the two media specific for the growth of the tested microorganisms. Blood agar for counting E. faecalis colonies and Sabouraud Dextrose agar with chloramphenicol for counting C. Albicans colonies. The plates were incubated at 37 C for 24 h. Growing colonies were counted and recorded as colony-forming units CFU.

Statistical Analysis

Statistical analysis was done using the Statistical Package of Social Science (SPSS Version 19.0; Chicago Inc, USA). Data comparison was made by applying statistical tests to determine the statistical significance of the comparisons. P value of <0.05 is considered significant, and p value of <0.0001 is highly significant. Kruskal- Wallis test, Dunn's Test and Chi-Square test were applied to find significant differences among different groups.

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Results

Counting colonies is traditionally performed manually using a click-counter (figure 1) (Figure 2). The current study showed that all three medicaments studied exerted antibacterial and antifungal activity on the effect of Tripe antibiotics and demonstrated a significantly higher percentage of bacterial and fungal reduction (P<0.05). The number of colony-forming units in all the experimental groups was remarkably lower in comparison with the control group (Saline) after 24 hours ($p < 0.05$), as shown in Tables (1), (2). The present study shows better antibacterial and antifungal effects of neem extract compared to other herbal agents. Further studies are required to evaluate the effectiveness of the new medicament at the molecular level and realise the exact mechanism against E. faecalis. and C. Albicans.

Figure 1: Colony forming unit for E. faecalis.

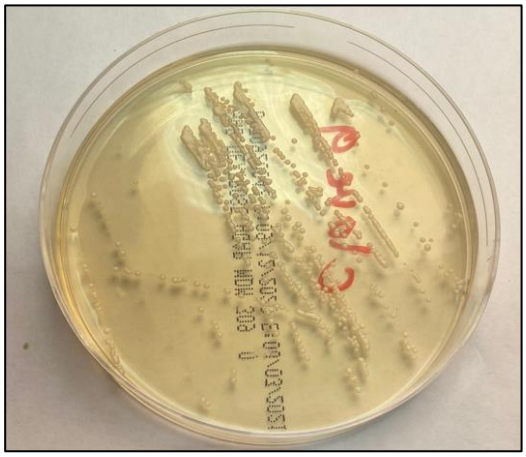

Figure 2: Colony forming unit for C. albicans.

S.N	Study Groups	N	Mean Rank	Chi-Square Value	p value
1	Group A (Clove)	20	34.1	51.35	0.0000**(P<0.0001) Very very Highly Significant**
2	Group B (Neem)	20	21.3		
3	Group C (Cinnamon)	20	40.5		
4	Group D (Saline)	20	66.1		

Table 1: Comparison of Mean rank of Colony count in E. Faecalis among all the groups with different medicaments after applying the Kruskal Wallis test.

S.N	Study Groups	N	Mean Rank	Chi-Square Value	p value
1	Group A (Clove)	20	29.30	38.6	0.0000**(P<0.0001) Very very Highly Significant**
2	Group B (Neem)	20	31.70		
3	Group C (Cinnamon)	20	34.50		
4	Group D (Saline)	20	66.5		

Table 2: Comparison of Mean Rank of Colony count in Candida Albicans among all the groups with different medicaments after applying the Kruskal Wallis test.

Discussion

The basis of root canal treatment is the eradication of intra-radicular microorganisms. Using a biocompatible intracanal medicament with antimicrobial properties between appointments may eliminate the microorganisms from the root canal system. It could significantly increase the success of root canal treatment [4]. Although several studies have demonstrated the importance of interappointment intracanal medication in killing the microorganisms, E. faecalis is chosen for this study as it is one of the most resistant found in case of failed root canal treatment. Its virulence factors and survival mechanisms make it an essential pathogen in post-treatment diseases. It is the most common microorganism found in secondary root canal infection and the primary cause of treatment failure [5]. The development of antibiotic-resistant strains and side effects of chemical irritants and intracanal medicaments has led to exploring alternative herbal medicaments. Herbal alternatives have gained attention due to their antioxidant, antimicrobial, sedative, anxiolytic, and anti-inflammatory properties, making them ideal for root canal disinfection [6,7,8].

In this research, the assembly for instrumentation was the same for all the experimental groups. To assure sufficient purification of the apical third, the canals were uniformly instrumented 1 mm shorter than the tip of the apex. The smear layer was removed using EDTA and Sodium hypochlorite NaOCl [9]. Sodium hypochlorite (NaOCl) is the most widely used irrigant during endodontic treatment [10] because it has effective antimicrobial activity [11]. Several concentrations of NaOCl ranging from 0.5%–5.25% were found in the endodontic literature, and the most widely used concentration is 2.5% [12]. The present study used 5.25% sodium hypochlorite to make the root canals sterile before inoculation with E. faecalis and C. Albicans.

The antimicrobial effect of steam-distillation C. zeylanicum bark EO against E. faecalis ATCC 29212 was reported by Abbaszadegan et al., with a MIC at 0.01 mg/mL and MBC at 0.1 mg/mL. Cinnamon EO and the triple antibiotic paste used in the study could eliminate planktonic E. faecalis after 24 h, where calcium hydroxide paste failed. Cinnamon EO showed better biocompatibility with experimental fibroblast cells compared to the other two substances[13]. In this study, the cinnamon oil mixed with TAP was the least effective compared to neem and clove oils. Literature suggested that the Neem (Azadirachta indica) leaf extract has a powerful antimicrobial effect against E. faecalis from infected root canal samples. Neem oil mixed with the TAP was the best canal medicament in the present study, as Ethanolic and aqueous extract of Neem leaf showed significant anticandidal effect against C. Albicans and antibacterial activity against E. faecalis [14,15]. The section was found to be more effective compared with 2% sodium hypochlorite. Supplemental research of these alternative herbal oils is necessary before the clinical application is considered.

Conclusion

In light of this study, it can be concluded that Neem, Clove, and Cinnamon extracts were demonstrated to contain a significant level of antimicrobial, antiproliferative and antioxidant agents and increased Triple's antimicrobial efficacy Antibiotics when it was mixed with them on all the microorganisms tested. The results of our study also revealed that Neem Oil had the greatest antibacterial and antifungal activity while the most sensitive tested microorganism is candida Albicans. Herbal oil represents a promising area of future research that is likely to include in vivo testing and determination of the mechanism of action.

Ethical approval

Study approval was acquired from the Dental Research Institutional Review Committee on 19 April 2020 (EA/F-2020-3013) at the dental school of Qassim University, KSA.

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Conflict of interest

The authors declare that they have no competing interests.

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Authors contributions

The authors are involved in writing and finalising the draft of this article. MS conducted the research, provided research materials, and collected and organised the data to draft the manuscript. SD conceived and designed the study, analysed, and interpreted the data. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Consent

Not required

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