The Antibacterial Effect of Tea Tree Oil, Clove Oil and 3% Sodium Hypochlorite against Enterococcus faecalis in Endodontics: An in vitro Study

[1]Inas H. Kareem, MSc, [2]Dimah N. Faraj, MSc and [3]Maryam K. Mohammed, MSc
[1]Alshaheed Dr. Waseem Specialized Dental Center, [2, 3]Department of Biology, Collage of Science, Baghdad University, Iraq.
Corresponding author: Inas Hadi Kareem
E.mail: inashadi9@gmail.com

Received October 15, 2021.
Accepted for publication on January 31, 2022.
Published June 22, 2022.

Abstract
Background Irrigation has significant role in endodontic treatment, many types of antimicrobial irrigation solutions have been used, but due to the ineffectiveness, safety concerns and side effects of this irrigation, the herbal alternatives for endodontic irrigants might be beneficial. **Objectives** This study compared the in vitro effectiveness of tea tree oil and clove oil as possible irrigants in endodontics against Enterococcus faecalis in comparison with 3% Sodium hypochlorite. **Materials and Methods** E. faecalis was isolated from patients in need for endodontic treatment; VITEK was employed for E. faecalis isolate conformation. Muller Hinton agar was prepared with 100µl of freshly prepared suspension of E.faecalis. Wells of 6mm diameter and 4mm depth were punched in each petri dish, the well were filled with 50 µl of: Group I: 3% sodium hypochlorite, Group II: 50% clove oil, Group III: 100% clove oil, Group IV: 100% tea tree oil. All petri dishes were incubated at 37°C for 24hr Zone of inhibition of the bacterial growth was calculated **Results** Statistical analysis of the obtained results was calculated. One way ANOVA was performed among four groups which showed as highly statistical difference with a p-value <0.01, LSD test was performed, group I & group IV is significantly was higher than group II, group III (p-value = 0.001), while there were no significant differences between group I & group IV (p-value = 0.165). **Conclusion** Both tea tree oil &clove oil showed antimicrobial activity against Enterococcus faecalis, however, tea tree oil & Sodium hypochlorite had better antimicrobial properties than clove oil.

Key words: Antimicrobials; clove oil; E. faecalis; tea tree oil.

Introduction

Root canal irrigation plays a key role in the success of endodontic treatment because the irrigant eliminate the microorganisms in the root canal, facilitate removal of necrotic tissue, Furthermore, irrigants help in prevent packing infected hard and soft tissue apically in the root canal and into the periapical area. (Kholoud et al, 2020). The most common irrigant used in endodontic is Sodium hypochlorite. It is an effective antimicrobial agent and an excel-

ISSN 6417-2310

lent organic tissue solvent (Ercan et al, 2004), however, NaOCl have numerous disadvantages. Tissue toxicity, unpleasant taste and odor is major drawbacks of NaOCl (Kamath et al, 2013, Jain and Ranjan, 2014). Using herbal extracts such as Aloe Vera in endodontic have many advantages (Babaji et al, 2016) ,like are safety, easy availability, increased shelf life, cost effectiveness, and lack of microbial resistance so far (Pratishta and Ranjan, 2014). Tea tree oil has numerous advantages, it has antibacterial, anti-fungal. The major component, terpenin-4-ol is responsible for its antibacterial and antifungal properties. Also, it has a mild solvent effect (Sinha et al, 2015). Clove oil has antibacterial and antifungal actions. It is used as an antiseptic in oral infections. The high levels of eugenol in clove essential oil are responsible for its strong antimicrobial activity (Borzini et al, 2016). The purpose of this study was to compare the in vitro effectiveness of tea tree oil and clove oil as possible irrigants in endodontics; against Enterococcus faecalis in comparison with 3% Sodium hypochlorite by using agar well diffusion test.

Martials and Methods

Ethical committee approval

This study approved by the ethics committee University of Baghdad\College of Science in January 14, 2021 (Ref.:CSEC/0121/0029).

Sampling procedure

Between April 2021 and May 2021, E. Faecalis bacteria sample were collected& isolated from 10 patients attending to endodontic department in Al-Shaheed Dr. Waseem specialized dental center Baghdad. Two sterile paper points ISO size 20 (Dentsply) were used to collect each sample by inserting them inside the canal to full working length for 60 seconds then each paper point was transferred to plane tube contain 5 ml brain heart infusion broth (Liofilchem-Italy); (AL-Bader and AL-Huwaizi, 2020; AL-Hyali, 2013). Samples were transport to laboratory and incubated an aerobically at 37°C for 24 hr. After incubation each sample was streaked on selective media-Pfizer Selective Enterococcus agar and incubating anaerobically at 37°C for 24hr. The growth of microorganism can be identified based on morphological appearance of colony by size- shape and color. VITEK was employed for E. faecalis isolate conformation; colonies were loaded in VITEK gram positive kite.

Antimicrobial activity

Muller Hinton agar were prepared and inoculated with 100µl of freshly prepared (0.5 McFarland turbidity standard) suspension of E. Faecalis .The inoculum was spread on a petri dish by mean of sterilized cotton swap. Wells of 6 mm diameter and 4 mm depth were punched in each petri dish, the wells were filled with 50 µl of: Group I: 3% sodium Hypochlorite (cerkamed, Poland) Group II: 50% clove oil (essential oil, now), Group III: 100% clove oil (essential oil, now) Group IV: 100% tea tree oil (essential oil, now). All petri dishes were incubated at 37°C for 24hr (AL-Bader and AL-Huwaizi, 2020). Zone of inhibitions which is clear zone of no growth of the bacteria were measured across the diameter of each well by using a digital vernier caliper, no zone indicated a complete resistance of bacteria to the agents (AL-Bader and AL-Huwaizi, 2020).

Results

ANOVA test which showed as highly statistical difference P ≤0.001 (table 1). LSD test was performed to compare among the mean difference between each paired groups in table (2). Group I & group IV were significantly higher than group II,

ISSN 6417-2310

group III, respectively (P-value =0.001), while there were no significant differences between group I & group IV. Also there were no significant differences between group III& group IV

Table (1): ANOVA, Mean, and SD of inhibition zone in millimeter of E. faecalis.

Study Group	Inhibition Zone in millimeter Mean ± SD	*p*-value
Sodium Hypochlorite 3%	28.66 ± 3.4	<0.001
Clove oil 50%	16.0 ± 2.0	
Clove oil 100%	15.5 ± 4.3	
Tea tree 100%	25.83 ± 3.4	

Table (2): LSD test.

	Study groups				*P*-value
	Sodium Hypochlorite 3% Mean ± SD	Clove oil 50% Mean ± SD	Clove oil 100% Mean ± SD	Tea tree 100% Mean ± SD	
Inhibition zone	28.66 ± 3.4	16.0 ± 2.0	-	-	<0.001
	28.66 ± 3.4	-	15.5 ± 4.3	-	<0.001
	28.66 ± 3.4	-	-	25.83 ± 3.4	0.165
	-	16.0 ± 2.0	15.5 ± 4.3	-	0.802
	-	16.0 ± 2.0	-	25.83 ± 3.4	<0.001
	-	-	15.5 ± 4.3	25.83 ± 3.4	<0.001

Discussion

Infections of root canal are multi-bacterial; more than 70% of the bacteria isolated is anaerobic bacteria. E. faecalis had commonly been isolated from root canals of failed endodontic treatment cases (Kumaran et al, 2020). Sodium Hypochlorite is the most widely used irrigation solution in endodontic. It is ideal compared with other irrigation solutions .it has a broad antibacterial spectrum and is sporicidal and viricidal (Topbas et al, 2017). The results of the present study revealed that; 3% Sodium Hypochlorite performed better than all the test groups followed by tea tree oil extract, but there was no significant difference between 3% Sodium Hypochlorite & tea tree oil, this finding presents a great promise to use tea tree oil as an alternative to chemical irrigation. The results of this study showed that tea tree oil have strong antimicrobial potential against E. Faecalis (inhibition zone ≥ 20 mm) as stated by Rusenova and Parvanov, 2009. This result is in agreement with several studies, such as (Kamath et al, 2013; Sinha et al, 2015). Tea tree oil is the volatile essential oil derived mainly from the Australian native plant, Melaleuca alternifolia. (Thosar et al, 2014). The antibacterial activity of tea tree oil may be due to its active component terpinen-4-ol, and /or other constituents like alpha terpinene, (Lee et al, 2013) Mechanisms of action of tea tree oil is also attributed to its hydrocarbon structure and inherent lipophilicity (Mickienė et al, 2011). It is assumed tea tree oil can cause inhibition of respiration of bacterial cell, loss of intracellular material and /or loss of membrane integrity and function (Lee et al, 2013). Furthermore ,this study revealed that, there was no significant difference between clove oil 50% vs. clove oil 100%, furthermore, there was highly significant difference between clove oil 50%, clove oil 100% vs 3% Sodium Hypochlo-

ISSN 6417-2310

rite &tea tree oil. Clove oil has antibacterial and antifungal actions (Kouidhi et al, 2010), (Thosar et al, 2014). Its strong antimicrobial activity due to the presence of several constituents, mainly phenyl-propanoides such as carvacrol, thymol, eugenol and cinnamaldehyde (Chaieb et al, 2007). The high levels of eugenol &phenolic compound being able to denature protein and react with cell membranes' phospholipids changing their permeability and inhibiting Gram-negative and Gram-positive bacteria (Chaieb et al, 2007).In this study were investigated the antibacterial of these irrigants in vitro. Further ex vivo studies are required to investigate the antibacterial effect on biofilm.

Conclusion

Within the limitation of this study:

1.Both tea tree oil & clove oil showed antimicrobial activity against Enterococcus faecalis. In vitro observations of these irrigants appears promising as intra canal irrigation, however, further clinical studies are required to investigate biocompatibility and safety.

2.Sodium hypochlorite and tea tree oil had better antimicrobial properties than clove oil.

3.Clove oil, both at 50% & 100% concentrations showed antimicrobial activity against Enterococcus faecalis.

Reference

AL-Bader R and AL-Huwaizi H.2020.Antimicrobial Evaluation for novel solution of Iron Oxide Nanoparticles Functionalized with Glycin and coated by Chitosan Root Canal Final Irrigation. Sys.Rev Pharm; 11(6):633-642.

AL-Hyali N .2013.Inhibition of Bacterial Growth around Gutta Percha Cones by Different Antimicrobial Solutions Using Antibiotic Sensitivity test (An in vitro study) .JBCD ;(25):26-32.

Babaji P, Jagtap K, Lau H, Bansal N, Thajuraj S, Sondhi P. 2016 Comparative evaluation of antimicrobial effect of herbal root canal irrigants (Morinda citrifolia, Azadirachta indica, Aloe vera) with sodium hypochlorite: An in vitro study. J Int Soc Prevent Communit Dent; 6:196-9.

Borzini L, Condò R, De Dominicis P, Casaglia A and Cerroni L.(2016).Root Canal Irrigation: Chemical Agents and Plant Extracts Against Enterococcus faecalis. The Open Dentistry Journal; 10, 692-703 .https://doi: 10.2174/1874210601610010692

Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A. (2007). The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review. Phytother Res; 21(6):501-6.

Ercan E, Ozekinci T, Atakul F, Gül K. (2004). Antibacterial activity of 2 % chlorhexidine and 5.25 % sodium hypochlorite in infected root canal: in vivo study. J Endod; 30: 84-7.

Jain P, Ranjan M. (2014). Role of herbs in root canal irrigation-A review .Journal of Pharmacy and Biological Sciences; 9, 2: 06-10.

Kamath U, Sheth H, Ramesh S, Singla K (2013). Comparison of the antibacterial efficacy of tea tree oil with 3% sodium hypochlorite and 2% Chlorhexidine against E.faecalis: An in vitro study. Journal of Contemporary Dentistry; 3(3): 117-120.

Kholoud M. Esmail , Wael H. Kamel, Mohsen Nour El-dein,Mohamed M. El Sherif (2020). Comparative Evaluation of Natu-

ISSN 6417-2310

ral Herbal Extracts as Root Canal Irrigation versus Routine Chemical Root Canal Irrigation AL-AZHAR Dental Journal For G i rls; 7, (1): 125:134.

Kouidhi B, Zmantar T, Bakhrouf A. (2010). Anticariogenic and cytotoxic activity of clove essential oil (Eugenia caryophyllata) against a large number of oral pathogens. Ann Microbiol; 60:599–604 .https://doi:10.1007/s13213-010-0092-6

Kumaran G, Antony S, Muralidharan N .(2020).Comparison of antimicrobial activity of tea tree oil extract and hydrogen peroxide with 3% sodium hypochlorite against Enterococcus faecalis, Candida albicans – An in vitro study. Drug Invention Today; 13, 5.

Lee C-J, Chen L-W, Chen L-G, Chang T-L, Huang C-W, Huang M-C. (2013).Correlations of the components of tea tree oil with its antibacterial effects and skin irritation. J of food and drug Ana; 21:169-76.

Mickienė R, Bakutis B, Baliukonienė V. (2011).Antimicrobial activity of two essential oils. Ann Agric Environ Med; 18: 139–144.

Pratishta J, Ranjan M. (2014). Role of herbs in root canal irrigation A review. IOSR J Pharm Biol Sci; 9: 6 10.

Rusenova N, Parvanov P. (2009) Antimicrobial Activities of Twelve Essential Oils against Microorganisms of Veterinary Importance. Trakia Journal of Sciences; 7(1):37-43.

Sinha DJ, Vasudeva A, Jaiswal N, Garg P, Tyagi SP, Singh J. Antibacterial efficacy of Melaleuca alternifolia (Tea tree oil), Curcuma longa (Turmeric), 2% chlorhexidine, and 5% sodium hypochlorite against Enterococcus faecalis: An in vitro study. Saudi Endod J 2015; 5:182-6. https://doi:10.4103/1658-5984.163623

Thosar N, Basak S, Bahadure RN, Rajurkar M. Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study. Eur J Dent. 2013 Sep;7(Suppl 1):S071-S077. https:/doi: 10.4103/1305-7456.119078.

Topbas C, Adiguzel O. (2017) Endodontic Irrigation Solutions: A Review. Int Dent Res; 7, 3:54-61. https://doi.org/10.5577/intdentres.2017.vol7.no3.2

ISSN 6417-2310