May 30, 2025 Version 1

Evaluation of Antibacterial Efficacy of Zinc Oxide Nanoparticles, Garlic Extract, and Their Combination versus Sodium Hypochlorite as Endodontic Irrigants Against Enterococcus faecalis in Single Rooted Teeth (A Comparative In Vitro Study) V.1

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqq8q5gk5/v1

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Protocol Citation: Mohamed Hatem Mohamed Ahmed, Prof. Dr. Lubna Abbas Shafie, Dr. Radwa Sameeh Emara, Dr. Reham Mohammad Raafat Hamed 2025. Evaluation of Antibacterial Efficacy of Zinc Oxide Nanoparticles, Garlic Extract, and Their Combination versus Sodium Hypochlorite as Endodontic Irrigants Against Enterococcus faecalis in Single Rooted Teeth (A Comparative In Vitro Study). **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqq8q5gk5/v1

Protocol status: In development
We are still developing and optimizing this protocol

Created: May 30, 2025

Last Modified: May 30, 2025

Protocol Integer ID: 219242

Keywords: endodontic irrigants against enterococcus, disinfection of the root canal system, root canal preparation, endodontic infection, root canal irrigant, mechanical process of root canal preparation, root canal treatment failure, root canal system, endodontic irrigant, major cause of root canal treatment failure, effective irrigation protocols in endodontic, gold standard irrigant in endodontic, endodontic treatment, persistent apical periodontitis, periodontitis, enterococcus faecali, endodontic, enterococcus, predominant enterococcus species, single rooted teeth, zinc oxide nanoparticle, internal root anatomy, positive facultative anaerobic coccus, structural complexities of the internal root anatomy, zno nanoparticle, enhanced antimicrobial efficacy, dentinal tubule, significant antibacterial activity, potent antibacterial property, antibacterial efficacy, synergistic combination of zno nanoparticle, evaluation of antibacterial efficacy, disinfection, complex microbiology, advanced antimicrobial agent, infectio

Abstract

Endodontic infections pose significant challenges in clinical practice due to their complex microbiology and the resilient nature of the causative organisms. These infections often lead to persistent apical periodontitis, which remains a major cause of root canal treatment failure. Effective cleaning and disinfection of the root canal system are critical for the success of endodontic treatment. Despite the structural complexities of the internal root anatomy, root canal irrigants are vital in enhancing the chemo-mechanical process of root canal preparation. Enterococcus faecalis, a Gram-positive facultative anaerobic coccus, is the predominant Enterococcus species identified in refractory lesions and is associated with up to 77% of treatment failures. E. faecalis possesses a unique ability to invade dentinal tubules, demonstrates strong adhesion to collagen, and often persists within the root canal system even after instrumentation and irrigation. Sodium hypochlorite (NaOCl) has long been considered the gold standard irrigant in endodontics due to its potent antibacterial properties and tissue-dissolving capabilities. However, its cytotoxic effects on periapical tissues and potential to cause chemical burns and unpleasant taste have prompted the search for safer alternatives. Nanotechnology offers a promising avenue in the development of advanced antimicrobial agents. Zinc oxide nanoparticles (ZnO Nps), characterized by their high surface area and ability to generate reactive oxygen species, exhibit significant antibacterial activity against a wide range of microorganisms, including E. faecalis. Natural products, such as garlic (Allium sativum), have also garnered attention as potential alternatives to conventional irrigants. The synergistic combination of ZnO nanoparticles and garlic extract could provide enhanced antimicrobial efficacy while mitigating the drawbacks associated with traditional irrigants. By leveraging the complementary mechanisms of these agents, this study aims to evaluate their effectiveness against E. faecalis in comparison to NaOCl. The findings could pave the way for safer, more effective irrigation protocols in endodontics.

Guidelines

The aim of this study is to evaluate and compare the antibacterial efficacy of Zinc oxide nanoparticles, garlic extract, and their combination to 2.5% sodium hypochlorite as endodontic irrigants against Enterococcus faecalis in single rooted teeth with single canal. The study employs a PICO approach: Population - Single rooted teeth with single canal; Intervention - Irrigation with garlic extract (250 mg/ml), ZnO nanoparticles (1000 µg/ml), and a combination of both; Control - Irrigation with Sodium hypochlorite 2.5%; Outcome - Antimicrobial efficacy assessed by counting the number of colony forming units per milliliter of each dilution (CFUs) on the agar medium.

Materials

Human permanent single rooted teeth extracted due to orthodontic, periodontal, or prosthodontic reasons will be collected. All the teeth will be examined to exclude those with previous root canal treatment, root caries, root resorption, and root fracture or cracks. Pre-operative radiograph will be taken to ensure the presence of straight single root canals (less than 5° angle of curvature according to Schneider's method). Collected teeth will be cleaned from calculus and any soft tissues by using ultrasonic scaler, then disinfected with 5% sodium hypochlorite at 37°C for 1 hour and after that immediately immersed and stored in distilled water at ambient temperature (25°C) for a period of a maximum of three months.

Introduction

1 Statement of the problem

1.1 Endodontic infections pose significant challenges in clinical practice due to their complex microbiology and the resilient nature of the causative organisms. These infections often lead to persistent apical periodontitis, which remains a major cause of root canal treatment failure.

1.2 Effective cleaning and disinfection of the root canal system are critical for the success of endodontic treatment. Despite the structural complexities of the internal root anatomy, root canal irrigants are vital in enhancing the chemo-mechanical process of root canal preparation.

1.3 Enterococcus faecalis, a Gram-positive facultative anaerobic coccus, is the predominant Enterococcus species identified in refractory lesions and is associated with up to 77% of treatment failures.

1.4 E. faecalis possesses a unique ability to invade dentinal tubules, demonstrates strong adhesion to collagen, and often persists within the root canal system even after instrumentation and irrigation.

1.5 Sodium hypochlorite (NaOCl) has long been considered the gold standard irrigant in endodontics due to its potent antibacterial properties and tissue-dissolving capabilities. However, its cytotoxic effects on periapical tissues and potential to cause chemical burns and unpleasant taste have prompted the search for safer alternatives.

2 Rationale for conducting the research

2.1 Nanotechnology offers a promising avenue in the development of advanced antimicrobial agents. Zinc oxide nanoparticles (ZnO Nps), characterized by their high surface area and ability to generate reactive oxygen species, exhibit significant antibacterial activity against a wide range of microorganisms, including E. faecalis.

2.2 Natural products, such as garlic (Allium sativum), have also garnered attention as potential alternatives to conventional irrigants. Garlic contains allicin, a sulfur-containing compound with broad-spectrum antimicrobial properties.

2.3 The synergistic combination of ZnO nanoparticles and garlic extract could provide enhanced antimicrobial efficacy while mitigating the drawbacks associated with traditional irrigants.

2.4 By leveraging the complementary mechanisms of these agents, this study aims to evaluate their effectiveness against E. faecalis in comparison to NaOCl. The findings

could pave the way for safer, more effective irrigation protocols in endodontics.

3 Research question

3.1 Is there any difference between anti-bacterial efficacy of ZnO nanoparticles, garlic extract, combination of both and sodium hypochlorite as an endodontic irrigant against Enterococcus faecalis in single rooted teeth with single canal?

4 Review of literature

4.1 In 2010, Shrestha et al. evaluated the efficacy of chitosan (CS-np) and zinc oxide (ZnO-np) nanoparticles in disinfecting Enterococcus faecalis biofilms and their long-term stability post-aging.

4.2 In 2013, Guerreiro-Tanomaru et al. evaluated the pH stability and antimicrobial activity of micro- and nanoparticulate zinc oxide (ZnO) pastes, with or without calcium hydroxide (CH), against pathogens including Enterococcus faecalis.

4.3 In 2014, Gopalakrishnan & Ravi assessed the antimicrobial efficacy of cinnamon and garlic as endodontic irrigants against Enterococcus faecalis, compared to 5.25% sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX).

4.4 In 2015, Karkare et al. compared the antimicrobial efficacy of plant-derived irrigants [saturated and diluted (1:1) hydroalcoholic extracts of Aloe vera and garlic] against Enterococcus faecalis, with 5% sodium hypochlorite (NaOCl) as the gold standard.

4.5 In 2015, Birring et al. evaluated garlic extract (GE) as a biocompatible alternative to sodium hypochlorite (NaOCl), a cytotoxic yet widely used root canal irrigant.

4.6 In 2017, Daga et al. compared the antimicrobial efficacy of herbal irrigants (neem, garlic extract, and Tridax procumbens) with sodium hypochlorite (NaOCl) against Enterococcus faecalis.

4.7 In 2018, De Almeida et al. assessed the antimicrobial efficacy of silver (Ag) and zinc oxide (ZnO) nanoparticle (Np) solutions compared to conventional irrigants (2% chlorhexidine [CHX], 5% and 1% sodium hypochlorite [NaOCl]) against Enterococcus faecalis biofilm in root canals.

4.8 In 2019, Hadi & Al-Mizraqchi evaluated the antimicrobial efficacy of zinc oxide nanoparticles (ZnO NPs) against Enterococcus faecalis, Candida, and total root canal microbiota in 30 patients with necrotic or previously failed root canal treatments.

4.9 In 2019, Octavia et al. investigated the efficacy of garlic extract against Enterococcus faecalis, a resilient Gram-positive bacterium commonly found in nonvital primary root canals.

4.10 In 2020, Mehta et al. compared the antibacterial efficacy of Allium sativum (garlic) extract, aqueous ozone, diode laser, and 3% sodium hypochlorite (NaOCl) as root canal irrigants against aerobic and anaerobic bacteria.

4.11 In 2022, Mahfouz Omer et al. compared the antibacterial efficacy of Allium sativum (garlic extract), calcium hydroxide $Ca(OH)_2$, and their combination as intracanal medicaments in infected mature anterior teeth with asymptomatic apical periodontitis.

4.12 In 2023, Navayath et al. evaluated the antimicrobial efficacy of a novel endodontic irrigant combining garlic extract and magnesium oxide (MgO) nanoparticles against Enterococcus faecalis, comparing it to sodium hypochlorite (NaOCl) and saline.

5 Choice of Comparators

5.1 The selection of appropriate comparators is essential for a comprehensive evaluation of the antibacterial efficacy and potential clinical applications of new endodontic irrigants. This study employs four comparators: zinc oxide nanoparticles, garlic extract, combination of both and sodium hypochlorite (control).

5.2 Sodium Hypochlorite (NaOCl) - Control Group: Sodium hypochlorite (NaOCl) is considered the gold standard endodontic irrigant due to its potent antimicrobial properties and tissue-dissolving capabilities.

5.3 Zinc Oxide Nanoparticles (ZnO NPs): Zinc oxide nanoparticles (ZnO NPs) have gained attention in endodontics due to their unique physicochemical properties, including a high surface area, ability to generate reactive oxygen species, and strong antibacterial action.

5.4 Garlic Extract (Allium sativum): Garlic extract contains allicin, a bioactive sulfur-containing compound with broad-spectrum antimicrobial properties.

5.5 Combination of Zinc Oxide Nanoparticles and Garlic Extract: The synergistic combination of ZnO NPs and garlic extract is hypothesized to enhance antibacterial efficacy by leveraging their complementary antimicrobial mechanisms.

5.6 Rationale for Comparison: Sodium hypochlorite serves as the benchmark comparator due to its established role in clinical practice. Zinc oxide nanoparticles and garlic extract are selected as innovative alternatives based on their documented antimicrobial properties and potential for improved biocompatibility.

Methods

6 Samples, Interventions & Outcomes

6.1 Calculated sample size: A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference between different tested groups regarding antibacterial efficacy.

6.2 Description of study sample: Human permanent single rooted teeth extracted due to orthodontic, periodontal or prosthodontic reasons will be collected.

6.3 Intervention for each group: Teeth will be randomly divided into four groups according to the type of irrigation.

6.4 Outcomes: Root canal sampling (outcome assessment) will be determined using culture technique in the microbiological department, Cairo University.

7 Assignment to intervention

7.1 Sequence generation: Random allocation and sequence generation will be performed using computer random sequence generator program.

7.2 Allocation concealment: To prevent the selection bias in the interventions, the allocated sequence will be protected and concealed until assignment using sequentially numbered opaque sealed envelopes.

7.3 Implementation: Random allocation, sequence generation and the allocation concealment will be performed by the Co-supervisor.

8 Blinding

8.1 The outcome assessor will be blinded to the intervention.

9 Statistical methods

9.1 Data will be coded and entered using the statistical package SPSS version 22.

9.2 Numerical data will be summarized using mean and standard deviation. Comparisons between groups for normally distributed data will be done using analysis of variance

(ANOVA), while for non-normally distributed numeric variable will be done by Kruskal Wallis test.