ISSN 2515-8260 Volume 10, Issue 01, 2023

Antimicrobial Efficacy of Non-Antibiotic Drugs Against E. Faecalis: An In-Vitro Study

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Abstract

Aim: The present in-vitro experimental study was undertaken to evaluate and compare the antimicrobial activity of cetirizine, ibuprofen, and tramadol against the micro-organism Enterococcus faecalis.

Methods and Material: Three medicaments were selected for the study, cetirizine, ibuprofen, and tramadol. The experimental groups for the test were as follows: Group 1: cetirizine, Group 2: ibuprofen, Group 3: tramadol, and Group 4: Saline. An agar well diffusion test was used to determine the efficacy of the experimental medicaments against E. faecalis (ATCC 29212). The diameter of inhibition zones was measured in millimeters using an inhibition zone measuring scale and the results were recorded.

Statistical Analysis: The statistical analysis was done using ANOVA and post hoc analysis. The p-value was set at <0.05.

Results: There was a significant difference in the diameter of growth inhibition zones, with the greatest diameter noted for cetirizine followed by ibuprofen, and tramadol.

Conclusions: The antimicrobial effectiveness of cetirizine was found to be superior to the other two medicaments (ibuprofen and tramadol).

Funding sources: None

Introduction: The key role of microorganisms is well established in initiating, progressing, and establishing pulpal and periradicular conditions [1]. Mostly connected to unsuccessful endodontic treatment, Enterococcus faecalis is a non-spore producing, fermentative, facultative anaerobic, Gram-positive cocci [2]. Elimination of microorganisms is crucial for the success of root canal treatment. However, chemomechanical preparation alone is not effective in eliminating the microorganism [3]. Previously calcium hydroxide was considered an effective intracanal medicament but recent studies have reported the development of resistance to it [4]. Recent studies have focused on researching medications whose main therapeutic function is not antibacterial action due to rising bacterial resistance to various routinely used antimicrobial medicines [5]. Non-steroidal anti-inflammatory medicines, neuroleptics, antihistamines, antidepressants, and antiplatelet drugs are examples of non-antibiotic medications that can function in a variety of ways, either directly against microbes or by boosting the effectiveness of antibiotic treatment (NSAIDs) [6].

An anti-histaminic of the second generation known as cetirizine is frequently prescribed as an outpatient treatment for Upper respiratory tract infection. [7]. Both gram-positive and gram-negative bacteria are found to be significantly sensitive to cetirizine. [8]. Ibuprofen, the commonly prescribed NSAID has the potential to act as an antimicrobial agent along with its analgesic activity [9]. A few microorganisms are inhibited by the synthetic opioid tramadol, which is used to treat individuals with mild to fairly severe pain. [10]. To our knowledge, the antimicrobial efficacy of these three non-antibiotic drugs against Enterococcus faecalis remains unexplored. To assess and compare the antibacterial effectiveness of cetirizine, ibuprofen, and tramadol against Enterococcus faecalis, the current in vitro investigation was carried out.

Materials and Methods: This study evaluated the antibacterial efficacy of Cetirizine, ibuprofen, and tramadol. The drugs in pure form were obtained from Sigma-Aldrich, India.

Bacterial strains and media

The American-type culture collection 29212 of E. faecalis was obtained and nourished in brain-heart infusion broth (BHI). To adjust the turbidity, inoculum density was set at 0.5 McFarland (1.58 × 108 bacteria/ml) [11]. Agar diffusion test methodology was used to evaluate the antibacterial activity of various materials.

Preparation of medicament: Cetirizine, ibuprofen, and tramadol were procured in powder form (Sigma-Aldrich; India).

Groups (n = 72)

Group 1: Cetirizine (1:1 w/v) - cetirizine was mixed with distilled water at 1 mg/ ml and diluted at 2000 µg/ml.
Group 2: Ibuprofen (1:1 w/v) - 400 mg of the test materials' powder was gradually added to a vial containing one milliliter of distilled water while being mixed with a spatula. In this manner, mixtures with test material concentrations of 400 mg/ml were created.
Group 3: Tramadol (1:1 w/v) – Tramadol was mixed with distilled water at a concentration of 1 mg/ ml and dilutions made at 25 µg/ml.
Group 4: Normal saline (negative control)
Agar well diffusion assay: The study required a total of 72 Mueller- Hinton agar plates were required, 18 for each group, to have a power of 95% confidence intervals. In this investigation, Mueller-Hinton agar plates with wells that were 5 mm in diameter and 2 mm deep were employed. Cotton swabs were used to ensure an even distribution of bacterial suspension on agar plates. 30 µl of the test material was filled into each well. Incubation of the plate was done aerobically at 37º C for 24 hrs.

After incubation, a blinded examiner measured the zone of bacterial inhibition around each well as the shortest distance (mm) from the initial point of bacterial growth to the outer margin of the well with an inhibition zone measuring scale.
Statistical analysis: IBM SPSS version 25 was used to analyze the data. Using ANOVA and post hoc analysis, the diameter of inhibition zones in the four groups was compared.For each analysis, a p-value of 0.05 or less was considered significant.
Results: On comparing the medicaments, a significant difference in the diameter of growth inhibition zones was observed between the three medicaments, with the greatest diameter seen for cetirizine followed by ibuprofen and tramadol ($p< 0.001$) [Table 1], [Figure 1].

On inter-group comparison, there was a significant difference in the diameter of the growth inhibition zones between each group, with the greatest diameter observed for cetirizine followed by ibuprofen and tramadol [Table 2].

Table 1: Comparison of the diameter of the zone of inhibition among the four groups using ANOVA

Group	Minimum	Maximum	Mean	SD	F statistic (p-value)
Cetirizine	20.00	22.00	21.16	.78	2897.63 (<.001)
Ibuprofen	15.00	17.00	16.00	.84	
Tramadol	10.00	12.00	11.00	.84	
Saline	0	0	0	0	

Figure 1: Comparison of the diameter of zone of inhibition among the four groups

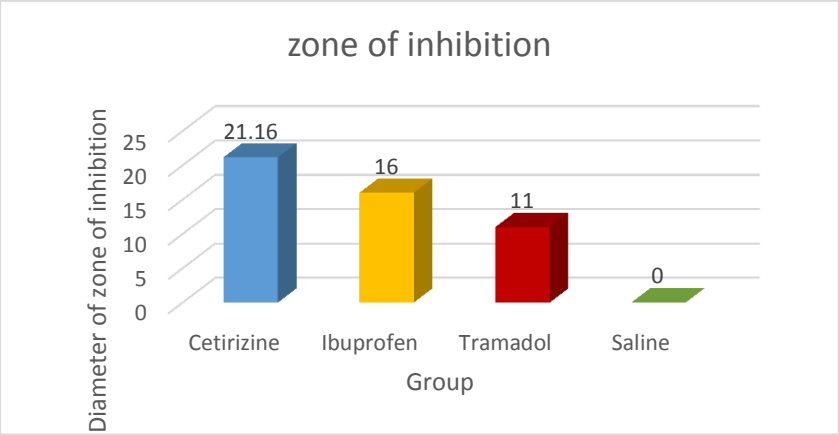

Table 2: Post-hoc analysis to check for individual group differences

Group 1	Group 2	Mean difference	P value
Cetirizine	Ibuprofen	5.16	<.001
	Tramadol	10.16	<.001
	Saline	21.16	<.001
Ibuprofen	Tramadol	5.00	<.001
	Saline	16.00	<.001
Tramadol	Saline	11.00	<.001

Discussion: Many non-antibiotic medications, including antihistamines, NSAIDs, and opioids, are now widely utilized as a result of the substantial rise in bacterial resistance to several widely used antibacterial agents and the global transmission of that resistance. Bearing in mind, in this study cetirizine (antihistamine), ibuprofen (NSAIDs), and tramadol (opioid) were compared to analyze their effectiveness as an intracanal medicament against E. Faecalis in this study.

In this investigation, E. faecalis, which Haapasalo and Orstavik et al. deemed to be the most resilient intracanal bacterium, was employed. Without food, these bacteria can endure for at least 10 days inside dentinal tubules. They are the organisms that cause persistent apical periodontitis and have the capacity to attach, collect, and develop into a biofilm, which increases their chances of survival and decreases their susceptibility to antibiotics [12].

The agar diffusion method has been used for many years by dentists and pharmaceutical firms to assess the antibacterial activity of dental materials and drugs. This method enables a direct comparison of the effectiveness of several medications against the intended pathogens, enabling us to determine which medication would be most successful in getting rid of germs in the pulp space [13].

For many years, antihistamines have been a trailblazer in the treatment of allergic illnesses. Recent research suggests that antihistamines may have a different role in medicine

by acting as an antibacterial agent against microbes [14]. All antihistamines feature a bulky lipophilic aromatic moiety and a tertiary amino group, which give them an amphipathic structure and some surfactant-like properties [15,16]. Numerous amphipathic substances are found to change biological membranes mostly due to their surface activity [17]. The minimum inhibitory concentration of cetirizine against the majority of the studied bacteria was 200–2000 g/ml, as measured by both the agar dilution and broth dilution methods [18].

Ibuprofen is one of the most well-known and often prescribed nonsteroidal anti-inflammatory medicines. Ibuprofen's antibacterial action was demonstrated by Hersh et al. against six prevalent periodontal infections [19]. It is uncertain how exactly NSAIDs like diclofenac and ibuprofen work to fight germs. However, investigations have suggested that bacterial DNA synthesis is inhibited [20] or that membrane function is impaired [21].

Tramadol was examined at two different concentrations by Tamanai-Shacoori et al. in cultures of Escherichia coli and Staphylococcus aureus. Tramadol inhibited both Staphylococcus aureus and Escherichia coli growth at 12.5 mg/mL; however, at 25 mg/mL, Staphylococcus aureus had an increased inhibitory impact while Escherichia coli experienced a bactericidal effect. [10]. Tramadol works by stimulating inflammatory responses, potentially increasing TNF-alpha and other inflammatory cytokines production, promoting phagocyte activity, and removing S. aureus [22]. However, tramadol appears to grow P. aeruginosa rather than aid in its eradication. Pseudomonas aeruginosa is a Gram-negative bacterium that operates as an opportunistic pathogen, causing hospital infections. The perfect habitat for P. aeruginosa to hide in the tissue matrix and neutrophil cortex appears to be created by tramadol, which appears to stimulate inflammatory reactions and draw inflammatory cells like neutrophils to the infection site. [23].

The comparison of the diameter of growth inhibition zones among the medicaments showed the highest diameter for cetirizine, followed by ibuprofen and tramadol.

However, further studies have to be conducted on the evaluation of these non-antibiotics against other intracanal pathogens. Since there was a substantial variation in the diameter of the growth inhibition zones between each of the four groups, the null hypothesis was rejected.

Conclusion: Within the confines of the current investigation, cetirizine was determined to have superior antibacterial efficacy, followed by ibuprofen and tramadol. Thus to effectively combat endodontic infections more, non-antibiotic medication use can therefore be advocated in clinical settings.

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