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Exploring non-antimicrobial agents as antibiotic alternatives: an *in vitro* study

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Abstract

Background: Microorganisms cause periapical and pulpal disorders, making root canal treatment (RCT) essential. Due to complex canal anatomy, bacteria may persist despite cleaning. Intracanal medicaments, such as calcium hydroxide and chlorhexidine, help eliminate residual bacteria, reduce inflammation, and enhance treatment outcomes by improving disinfection and compatibility.
Aim: To assess the antimicrobial effectiveness of Triple Antibiotic Paste (TAP), Modified TAP, Sertraline (SSRI), Atorvastatin and the combination of Atorvastatin and Sertraline against *Enterococcus faecalis*.
Materials and methods: A total of 30 samples were made, with 6 samples from each group: TAP, Sertraline, Atorvastatin, Modified TAP, and Sertraline + Atorvastatin. The antimicrobial efficacy of the medicaments was evaluated using an agar well diffusion test by measuring inhibition zones around the medicaments. Statistical analysis included one-way ANOVA, with significance set at $p < 0.05$.
Results: A significant difference (p=0.001) in the diameter of growth inhibition zones was observed. The maximum inhibitory zone was found in Group 5: Sertraline+Atorvastatin.
Conclusion: The combination of sertraline and atorvastatin demonstrated superior antimicrobial efficacy compared to modified TAP, TAP, sertraline, and atorvastatin used alone.
Keywords: Neo Atorvastatin, *Enterococcus faecalis*, Selective Serotonin Reuptake Inhibitor, Triple antibiotic paste.

1. Introduction

Microorganisms are integral contributors to the onset of periapical and pulpal disorders [1]. The root canal treatment procedure eradicates bacteria residing within the infected root canal. The intricate anatomy of root canals often poses challenges in achieving thorough disinfection of the entire canal system. Consequently, employing antibacterial agents in the form of intracanal medicaments becomes necessary to effectively eradicate endodontic microflora [2].

Enterococcus faecalis, a Gram-positive facultative anaerobe, is a vital constituent of the microbial flora found in unsuccessfully treated endodontic teeth. Demonstrating resilience in harsh environments, it possesses the capability to develop biofilms [3]. In recent studies, calcium hydroxide (Ca(OH)2) has been disregarded as an efficient intracanal medicament due to its inability to eradicate the biofilm of E. *faecalis*. Consequently, newer antibacterial compounds are being suggested as alternatives [4].

Triple antibiotic paste (TAP), comprising metronidazole, ciprofloxacin, and minocycline, stands out as a widely employed intracanal medicament in endodontic therapies. Research findings indicate that TAP effectively eliminates bacteria up to a depth of 400 µm within the dentin, surpassing the reach of Ca(OH)2, which extends only up to 200 µm [5,6]. The overutilization of TAP may foster bacterial resistance, diminishing its efficacy as a medicament. Consequently, there has been a quest for non-antibiotic compounds possessing potent antibacterial attributes that operate via alternative mechanisms [7]. Some non-antibiotic drugs, such as antihistamines, antipsychotics, tranquillizers, and statins, have demonstrated antibacterial properties. Research has revealed that certain selective serotonin reuptake inhibitors (SSRIs) also possess antibacterial characteristics. Notably, Sertraline, which is classified as an SSRI, has shown significant bactericidal effects against both Gram-positive and Gram-negative bacteria by inhibiting bacterial DNA synthesis [7,8].

To our knowledge, the antimicrobial efficacy of modified TAP and Sertraline in combination with Atorvastatin remains unexplored. Therefore, the present in vitro study was conducted to evaluate the antibacterial efficacy of Sertraline (SSRI), TAP, Modified TAP, Atorvastatin and the combination of Sertraline with Atorvastatin against E. *faecalis*.

2. Materials and methods

This protocol was approved by the Institutional Ethics Committee (IEC number O.R.: GSLDC/IEC/2024/024), GSL Dental College and Hospital, Rajahmundry, Andhra Pradesh, India. A total of 30 samples were made and divided into 5 groups with 6 (n=6) in each. The study assessed the antibacterial effectiveness of TAP, Modified TAP, Atorvastatin, and Sertraline. These medications were sourced in their pure forms from Sigma-Aldrich, India.

2.1 Bacterial strains and media

The E. *faecalis* strain ATCC 29212 from the American Type Culture Collection (ATCC) was acquired. Their growth was confirmed on Bile esculi medium (Figure 1) and cultivated in blood agar. Inoculum density was standardized at 0.5 McFarland units (equivalent to 1.58 X 108 bacteria/ml) to account for turbidity [9]. The strains were isolated from a blood agar plate inoculated in peptone nutrient broth and were again incubated for 24 hours at 37 degrees centigrade in the incubator. The antibacterial activity of materials was assessed using the agar diffusion test method.

Figure 1. Bile esculin medium with *Enterococcus faecalis* growth

2.2 Preparation of medicament

Ciprofloxacin, metronidazole, and minocycline were used for TAP, while ciprofloxacin, metronidazole, and clindamycin were used for Modified TAP. Sertraline and atorvastatin were obtained in powder form from Sigma-Aldrich, India (Figure 2). A total of 30 specimens were prepared and divided into five groups, each containing six samples (n=6) from each medication. The samples were grouped as described below.

Group 1: TAP (1:1:1 w/v) – Equal amounts of metronidazole, ciprofloxacin, and minocycline were mixed with distilled water (1 mg/mL).

Group 2: Sertraline (1:1 w/v) – One millilitre of distilled water was mixed with 60 µg powder to attain a concentration of 60 µg/ml.

Group 3: Atorvastatin (1:1 w/v) –One millilitre of distilled water was mixed with 80 µg powder to attain a concentration of 80 µg/ml.

Group 4: Modified TAP (1:1:1 w/v) – Equal amounts of Metronidazole, Ciprofloxacin, and Clindamycin were mixed with distilled water (1 mg/mL).

Group 5: Sertraline+Atorvastatin (1:1 w/v) – Equal amounts of Sertraline and Atorvastatin were mixed with distilled water.

2.3 Agar well diffusion assay

A total of 5 Mueller-Hinton agar plates (Figure 7) were taken for the experiment, with 1 plate allocated to each group, ensuring 80% power and 95% confidence intervals. A total of six wells, each measuring 5 mm in diameter and 2 mm in depth, were made on the Mueller-Hinton agar plates (Figure 3a). Approximately 30 µl of the test material was dispensed into each well (Figure 3b). In order to achieve a uniform distribution of the cultured bacteria on the agar plates, cotton swabs were utilized (Figure 3c). The plates were then aerobically incubated at 37°C for 24 hours. Following incubation, a blinded examiner utilized an inhibition zone measuring scale to gauge the zone of bacterial inhibition surrounding each well, measuring from the initial point of bacterial growth to the outer margin of the well (Figure 3d).

2.4 Statistical analysis

The obtained data were analysed using Statistical Package for Social Sciences, SPSS version 25.0, Chicago, IL, USA. One-way ANOVA was employed to compare the diameter of growth inhibition zones across different groups. A significance level of p-value less than 0.05 was considered statistically significant.

Figure 2. Powdered form of drugs

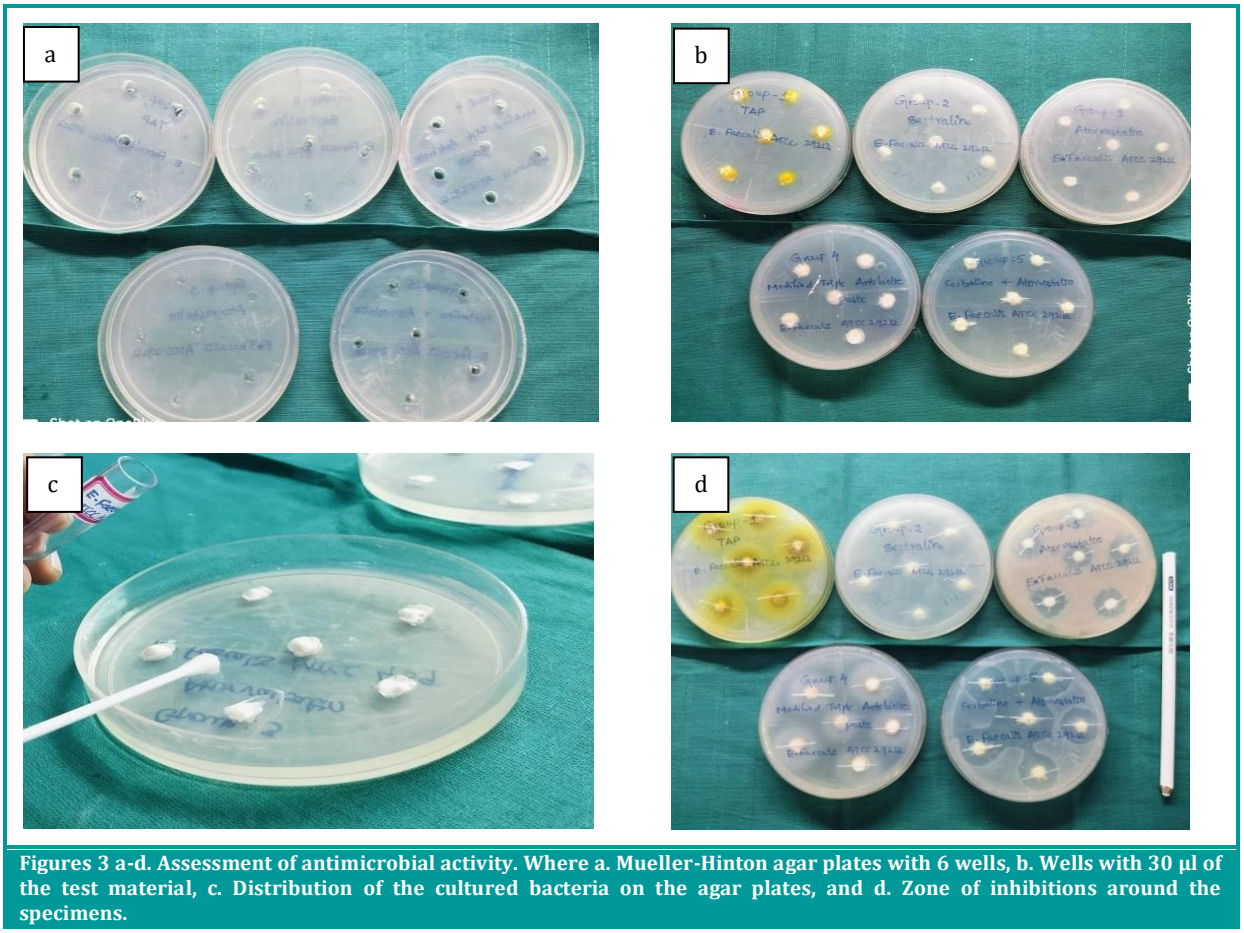
3. Results

Table 1 provides a comparative analysis of the inhibitory zones formed against E. *faecalis* in the 5 groups after incubating for 24hrs. The highest inhibitory zone was observed in Group 5, Sertraline+Atorvastatin, (23.500±1.516 mm) followed by Group 4, Modified TAP, (23.333±1.966 mm), Group 1, TAP (22.666±3.077 mm),

and Group 2, Sertraline (19.166±2.787 mm). The least zone of inhibition was observed in Group 3, Atorvastatin (18.333±1.366 mm). One-way ANOVA displayed a significant difference among the groups for all five categories of the bacterial inhibition zones against E. *faecalis* (Table 1).

4. Discussion

It's fascinating how research is exploring alternative methods for disinfection in situations where antibiotic resistance poses challenges. Evaluating the antimicrobial effectiveness of statins and SSRIs alongside conventional methods such as Triple Antibiotic Paste (TAP) and Modified TAP against E. *faecalis* in root canal treatments appears to be a novel approach. Statins and SSRIs have primarily been associated with cardiovascular health and mood disorders respectively, so seeing them being investigated for their antimicrobial properties in dentistry is intriguing. Root canal infections, often caused by bacteria like E. *faecalis*, require effective treatment to prevent complications, so finding new approaches is crucial [7,8].



Figures 3 a-d. Assessment of antimicrobial activity. Where a. Mueller-Hinton agar plates with 6 wells, b. Wells with 30 µl of the test material, c. Distribution of the cultured bacteria on the agar plates, and d. Zone of inhibitions around the specimens.

Table 1. Mean zone of inhibitions (mm) around the specimens against E. *faecalis* (One-way ANOVA)

Groups	N	Mean	Standard Deviation	Significance (p-Value)
1	6	22.666	3.077	
2	6	19.166	2.787	
3	6	18.333	1.366	0.001
4	6	23.333	1.966	
5	6	23.500	1.516	

This study investigated the effectiveness of various alternative methods for combating *Enterococcus faecalis*. It is crucial to identify which of these alternative approaches have shown particular efficacy against E. *faecalis*. Additionally, a comparative analysis of these methods against traditional treatments regarding efficacy and safety is needed.

The resilience of E. *faecalis*, as highlighted by Haapasalo and Orstavik, underscores its designation as one of the most resistant intracanal bacteria, thereby emphasizing the critical need to evaluate and compare these alternative treatment strategies. It possesses the remarkable capability to endure within dentinal tubules for up to 10 days without external nourishment. These bacteria are responsible for persistent apical periodontitis due to their adeptness in adhering, aggregating, and forming biofilms, attributes that enhance their survival and amplify their resistance to antibiotic treatments [10].

The antimicrobial efficacy of dental materials and medications has long been assessed by dentists and pharmaceutical companies through the agar diffusion method. This approach enables a direct comparison of the effectiveness of various medicaments against specific

pathogens, aiding in the identification of the most potent medicament for eradicating bacteria within the pulp space [11]. TAP has garnered widespread usage as an intracanal medicament against E. *faecalis* [12]. Comprising metronidazole, minocycline, and ciprofloxacin, TAP harnesses the non-cytotoxic properties of the tetracycline drug group to inhibit collagenases and matrix metalloproteinases. Notably, these antibiotics have demonstrated the capacity to elevate interleukin-10 levels, an anti-inflammatory cytokine. Additionally, metronidazole and ciprofloxacin have been shown to stimulate fibroblast production [13]. Various researchers substantiated the efficacy of MTAP through in vitro studies [14]. In clinical practice, the inclusion of minocycline has been observed to correlate with specific complications, such as tooth structure discoloration, antiangiogenic effects, and the chelation of radicular dentin, which can result in demineralization and compromised root integrity [15]. Karczewski *et al.* conducted a recent in vitro study examining the antimicrobial effectiveness, dentin discolouration potential, and cytotoxicity of Clindamycin MTAP. Their findings suggested that clindamycin has the potential to be a suitable substitute for minocycline within TAP formulations [16].

In this study, a blend of ciprofloxacin, metronidazole, and clindamycin was utilized. This particular drug combination was selected due to its broad spectrum of activity against endodontic pathogens. Considerable evidence substantiates the notion that serotonin reuptake inhibitors, the primary class of antidepressant medications, exhibit notable antimicrobial efficacy [7]. In 2015, Ayaz *et al.* [8] investigated the potential activity of sertraline against ATCC strains and clinical isolates of S. aureus, E. coli, and P. aeruginosa. They examined its effects both independently and in conjunction with seven different antibiotics. To assess the inherent antibacterial properties of sertraline against S. aureus, agar dilution and well assay methods were employed. The diameter of inhibitory zones (DIZ) increased proportionally with higher concentrations of sertraline. To enhance the understanding of sertraline's antimicrobial potential, the researchers investigated 28 bacterial strains, comprising 3 ATCC strains and 25 clinical isolates, along with 13 fungal strains. Sertraline demonstrated a 50% inhibition rate against clinical isolates of E. coli at a concentration of 60 µg/mL [7]. Statins, a group of drugs used to lower lipid levels and decrease the risk of heart-related issues, have shown additional benefits in reducing inflammation and modulating the immune system over time. Moreover, researchers have proposed that statins may offer protection against various infectious diseases. Atorvastatin and simvastatin, in particular, have been studied for their potential antimicrobial properties among the different types of statins [7]. The results of the current study aligned with the above study findings, demonstrating that the antimicrobial efficacy of sertraline is enhanced when combined with other drugs rather than used alone.

In this study, sertraline was administered both alone and in combination with atorvastatin, showing a larger inhibitory zone in the group that received the combination, which illustrates the concept of drug synergism. The comparison of growth inhibition zone diameters revealed that the values for Modified TAP were significantly greater than those for TAP. These findings are consistent with the study by Cunha *et al.* (2021) [18], which assessed the antibacterial efficacy of Triple Antibiotic Medication with Macrogol (3Mix-MP), traditional Triple Antibiotic Paste, calcium hydroxide, and ethanol extract of propolis, concluding in the above study that MTAP was more effective than TAP. The growth inhibition zones' diameters were notably larger when sertraline was combined with atorvastatin compared to when each medicament was used alone. This suggests a potential synergistic effect of combining these two substances. However, additional research is needed to explore various combinations of antibiotics and non-antibiotics against E. *faecalis*. Thus, the null hypothesis was rejected, indicating a significant variance in the growth inhibition zones' diameters across the five groups.

The main limitations of this study include it's *in vitro* design, which may not fully replicate the complex root canal environment, and its focus solely on E. *faecalis*, limiting the assessment of efficacy against other endodontic pathogens. The sample size, while statistically adequate, could be expanded for greater generalizability. Additionally, the antimicrobial efficacy was evaluated only after 24 hours, leaving questions about long-term effects and resistance development. Future research should focus on in vivo studies, testing a broader range of bacterial strains, and exploring synergistic combinations of antibiotics and non-antibiotic agents to enhance endodontic treatment outcomes.

5. Conclusion

This study found that the combination of sertraline and atorvastatin was more effective against E. *faecalis* than modified TAP, TAP, sertraline, or atorvastatin used alone. Hence, in clinical scenarios, employing medication combinations instead of single agents should be advocated for more effective control of endodontic biofilms. Additionally, utilizing non-antibiotic intracanal medications mitigates the potential for systemic complications and curbs the development of antibiotic resistance.

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