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Antimicrobial Potential of Calcium Nanoparticles in Fluoridated Toothpaste Against Lactobacillus and Candida albicans: An In Vitro Analysis

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ABSTRACT

Background: Nanotechnology has emerged as a promising field in dentistry, particularly for improving antimicrobial efficacy in oral healthcare products. Candida albicans and Lactobacillus are key microorganisms associated with early childhood caries and oral infections. While fluoride-based toothpaste is effective against cariogenic bacteria, its efficacy against Candida albicans remains limited. Incorporating calcium nanoparticles into toothpaste formulations may enhance antimicrobial properties and offer broader-spectrum protection.

Aim: To evaluate the antimicrobial efficacy of calcium nanoparticles combined with commercially available fluoride toothpaste against Lactobacillus and Candida albicans through an in vitro analysis.

Materials and Methods: Calcium oxide (CaO) nanoparticles were synthesized using a microwave irradiation method. The antimicrobial efficacy of calcium nanoparticles in combination with fluoride toothpaste (Pediflor) was assessed against Lactobacillus and Candida albicans using the agar well diffusion method. Zones of inhibition were measured, and statistical analysis was performed using one-way ANOVA to determine significant differences across different concentrations.

Results: The combination of calcium nanoparticles and fluoride toothpaste demonstrated a dose-dependent increase in antimicrobial activity, particularly against Candida albicans. However, Pediflor alone exhibited greater inhibition against Lactobacillus. Statistical analysis revealed significant differences at higher concentrations, highlighting the differential susceptibility of the microorganisms.

Conclusion: Calcium nanoparticles enhance the antifungal efficacy of fluoride toothpaste, making them a potential additive in oral care formulations. However, their antibacterial activity against Lactobacillus was lower than that of standard fluoride toothpaste. Further research is needed to optimize formulations and assess long-term effects on oral microbiota.

Keywords: *Calcium Compounds, Fluorides, Candida albicans, Lactobacillus*

1. INTRODUCTION

Nanotechnology has revolutionized various scientific fields, with extensive research focusing on the potential applications of nanoparticle-based materials [1]. In dentistry, the integration of nanoparticles in oral care products has garnered significant interest due to their enhanced antimicrobial properties and biocompatibility. Early Childhood Caries (ECC) remains a prevalent and severe condition affecting children worldwide, despite fluoride being the gold standard in caries prevention. The immature immune system and developing oral microbiota in young children make them highly susceptible to opportunistic microbial colonization in the oral cavity. Among these opportunistic pathogens, Candida albicans is frequently found in the oral microbiota of younger children, indicating an ongoing process of immune system maturation [2]. Studies have shown that Candida albicans can thrive under immunosuppressive conditions, contributing to the rapid progression of caries in children [3].

ECC is often associated with malnutrition and a weakened immune system, making affected children more vulnerable to infections caused by Candida albicans. Several studies have reported the presence of C. albicans in the saliva, dental plaque, and infected dentin of ECC patients. Additionally, Lactobacillus species play a significant role in caries development by fermenting carbohydrates and producing lactic acid, which leads to enamel demineralization and cavitation if left untreated [4]. While fluoride remains effective in caries prevention, commercially available fluoride toothpastes primarily target Streptococcus mutans and may be less effective against Lactobacillus and Candida albicans [5,6].

The acidogenic and aciduric nature of Lactobacillus spp. makes them key contributors to the progression of dental caries [7]. These bacteria thrive in low-pH environments, further exacerbating enamel demineralization and caries formation. On the other hand, Candida albicans is a fungal species that exists as a commensal organism in the oral cavity but can become pathogenic under specific conditions, leading to oral candidiasis and biofilm formation on oral surfaces [8]. The presence of C. albicans in ECC lesions has been linked to its ability to adhere to cariogenic bacteria, enhancing biofilm stability and resistance to antimicrobial agents [9,10].

The incorporation of calcium nanoparticles into fluoride toothpaste offers a promising strategy to enhance its antimicrobial efficacy. Calcium nanoparticles not only strengthen enamel but also possess antimicrobial properties that disrupt microbial biofilms and inhibit both bacterial and fungal pathogens [11,12]. These nanoparticles can potentially provide a broader spectrum of protection by targeting Lactobacillus and Candida albicans, which are commonly associated with ECC progression. In vitro studies play a crucial role in evaluating the antimicrobial efficacy of such formulations, allowing systematic assessment of different nanoparticle concentrations and their interactions with oral microorganisms under controlled conditions [13,14].

This study aims to assess the antimicrobial efficacy of calcium nanoparticles combined with commercially available fluoride toothpaste against Lactobacillus and Candida albicans. By targeting these significant oral pathogens, this research seeks to explore the potential of this novel formulation in improving oral health outcomes. The dual-action mechanism of fluoride and calcium nanoparticles is expected to enhance the antimicrobial spectrum of the toothpaste while promoting enamel remineralization, offering a more comprehensive approach to ECC prevention [15,16].

Thus, the objective of this study is to evaluate whether the incorporation of calcium nanoparticles into fluoride toothpaste provides enhanced antimicrobial effects against Lactobacillus and Candida albicans, ultimately contributing to a more effective strategy for preventing ECC and improving oral health in children.

2. MATERIALS AND METHODS

Materials

1. **Microbial Strains:** Lactobacillus and Candida albicans were obtained from a Department of microbiology from Saveetha dental college .
2. **Synthesis of CaO Nanoparticles** With minimal modifications, the microwave irradiation approach was used to create CaO nanoparticles (CaO-NPs) with a restricted size distribution.In in simple terms, 100 ml of mixture (solutions) were obtained by dissolving 0.5 M Ca(NO324H2O and 0.7 M NaOH in 50 ml of deionized water, respectively. The mixture formed a white gel after being swirled for 10 minutes at room temperature. The mixture was then transferred into a round-bottom flask with a condenser attachment that was stored in a Multi Synth microwave refluxing system. 200 W was the maximum deliverable power output, and a temperature of 160 C was set for 5 minutes. The solution was microwaved and then allowed to naturally cool to room temperature. The resultant precipitate was vacuum-filtered and washed with deionized water and 100% ethanol before being dried in a vacuum at room temperature.(Nasim et al., 2022)
3. **Fluoride Toothpaste:** A commercially available fluoride pedo toothpaste (pediflor toothpaste) was selected for the study.
4. **Culture Media:** De Man, Rogosa, and Sharpe (MRS) agar was used for Lactobacillus cultures, and Sabouraud Dextrose Agar (SDA) was used for Candida albicans.
5. **Equipment:** Sterile petri dishes, micropipettes, an incubator, a vortex mixer, and a well diffusion apparatus were utilized.

Methods

1. **Preparation of Toothpaste-Nanoparticle Mixture:** Calcium nanoparticles were mixed with fluoride toothpaste using a sterile vortex mixer until uniform mixtures were obtained.
2. **Preparation of Microbial Cultures:** Lactobacillus were cultured in MRS broth, and Candida albicans in Sabouraud Dextrose Broth at their optimal growth conditions. Overnight cultures were standardized to match the 0.5 McFarland standard.(Balaganesh et al., 2022)

3. **Agar Well Diffusion Assay:** Laboratory maintained Standard strains of lactobacillus and Candida albicans grown on enriched media and incubated for 12 hours. These fresh cultures were used to make a suspension in saline with turbidity matching 0.5 Mcfarland standard, 50 microliters of the suspension was pipetted and placed on the surface of Mueller Hinton agar and uniformly spread using a sterile swab. Then using a sterile metal tube of 6 mm diameter 3 wells were cut on the media. The wells are filled with CaO nanoparticles mixed with commercially available fluoride toothpaste. After incubation, the zone of inhibition was measured in millimeters and tabulated. Measure the zone of inhibition after 12 hrs. Take care to avoid contamination(Radeva et al., 2024).
4. **Incubation:** Plates inoculated with Lactobacillus were incubated anaerobically at 37°C, while those with Candida albicans were incubated aerobically at 30°C for 24–48 hours.
5. **Measurement of Antimicrobial Activity:** Zones of inhibition around each well were measured using a ruler or caliper. The diameter of the inhibition zones was recorded in millimeters.
6. **Statistical Analysis:** The antimicrobial efficacy of calcium nanoparticles combined with commercially available fluoride toothpaste (Pediflor) against Lactobacillus and Candida albicans was analyzed using one-way analysis of variance (ANOVA). The inhibition zone diameters (mm) were recorded for different concentrations (25 µg, 50 µg, 100 µg, and standard), and ANOVA was performed to assess whether significant differences existed in antimicrobial activity across the tested concentrations. The significance level was set at $p < 0.05$, and results were reported as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS version 27 (IBM Corp., Armonk, NY, USA). All experiments were performed in triplicates to ensure reproducibility and reliability of the findings.

3. RESULTS

The study assessed the antimicrobial efficacy of calcium nanoparticles combined with commercially available toothpaste (Pediflor) against Lactobacillus and Candida albicans at concentrations of 25 µg, 50 µg, and 100 µg, with Pediflor alone as the control. The results demonstrated a dose-dependent increase in the zone of inhibition for Candida albicans, while the effect on Lactobacillus remained relatively constant across different concentrations. At 25 µg, both Lactobacillus and Candida albicans exhibited a 9 mm zone of inhibition. At 50 µg, the inhibition zone remained 9 mm for Lactobacillus but increased to 10 mm for Candida albicans. At 100 µg, the zone of inhibition for Lactobacillus was 9 mm, whereas it increased to 11 mm for Candida albicans. In contrast, Pediflor alone at 100 µg exhibited a higher zone of inhibition (14 mm) for both microorganisms. ANOVA analysis revealed a significant difference in antimicrobial activity at 50 µg ($p = 0.0161$) and 100 µg ($p = 0.0078$), suggesting that calcium nanoparticles with toothpaste exhibit differential inhibition effects at these concentrations. However, no significant differences were observed at 25 µg and the standard concentration ($p = 1.000$). These findings indicate that calcium nanoparticles combined with toothpaste are more effective against Candida albicans, while Pediflor alone showed superior antimicrobial activity against Lactobacillus.

Table 1: Table showing the zone of inhibition of calcium nanoparticles with commercially available toothpaste against lactobacillus and Candida albicans

Column 1	Column 2	Column 3	Column 4
Condition	**F-statistic**	**p-value**	**Significance (p < 0.05)**
25 µg	0	1	Not Significant
50 µg	16	0.0161	Significant
100 µg	24.5	0.0078	Significant
Standard	0	1	Not Significant

The ANOVA analysis revealed a significant difference in antimicrobial activity between Lactobacillus and Candida albicans at 50 µg ($p = 0.0161$) and 100 µg ($p = 0.0078$) concentrations, indicating that these concentrations of CaO nanoparticles with toothpaste exhibit varying inhibition effects on the two microorganisms. However, at 25 µg and the standard concentration, the differences were not statistically significant ($p = 1.000$), suggesting similar inhibitory effects on both microbial strains. These findings highlight the concentration-dependent antimicrobial efficacy of CaO nanoparticles and their potential role in targeted antimicrobial applications.

Figure 1: The image shows the zone of inhibition of calcium nanoparticles with commercially available toothpaste against Candida albican

Figure 2 : The image shows the zone of inhibition of calcium nanoparticles with commercially available toothpaste against lactobacillus

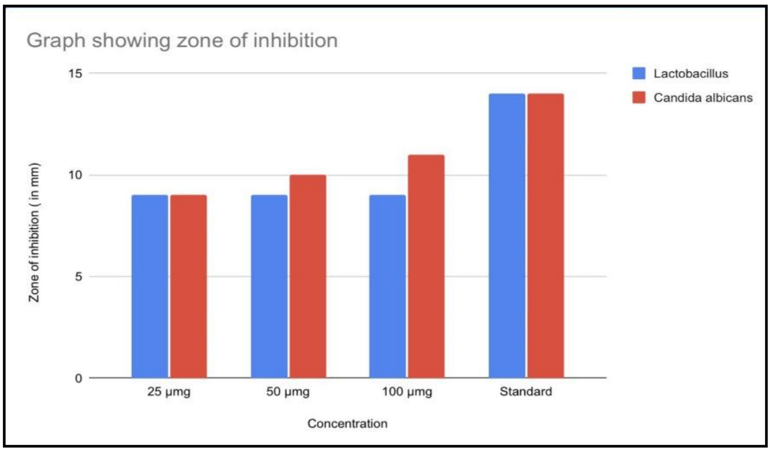

Figure 3: Bar Graph showing the mean zone of inhibition of calcium nanoparticles with commercially available toothpaste against lactobacillus and Candida albicans in different concentrations

4. DISCUSSION

Several studies have demonstrated that nanoparticles, including silver, zinc oxide, and iron oxide, exhibit strong antifungal properties by penetrating biofilms and disrupting fungal cell membranes. Iron oxide nanoparticle-enriched toothpaste, for instance, has shown a dose-dependent reduction in Candida albicans growth, reinforcing the idea that nanoparticles can enhance antifungal efficacy in dental applications [17].

The superior efficacy of Pediflor toothpaste against Lactobacillus is consistent with existing literature on fluoride-based antimicrobial strategies. Fluoride disrupts bacterial metabolism and inhibits the adhesion of cariogenic bacteria such as Lactobacillus and Streptococcus mutans. This suggests that while calcium nanoparticles contribute to antifungal protection, fluoride-based formulations may be more effective against bacterial pathogens commonly associated with dental caries [18].

Comparative studies on different nanoparticles have shown varying degrees of antimicrobial efficacy. Silver nanoparticles, for example, have demonstrated strong antibacterial activity against Lactobacillus, while selenium nanoparticles incorporated into chitosan formulations have exhibited antimicrobial effects against Streptococcus mutans, Lactobacillus acidophilus, and Candida albicans [19,20]. These findings suggest that calcium nanoparticles may be more suitable for antifungal applications, whereas other nanoparticles might be preferable for broader antibacterial action.

Beyond their antimicrobial effects, calcium nanoparticles have been widely studied for their role in enamel remineralization. Calcium phosphate nanoparticles, for example, continuously release calcium and phosphate ions, which strengthen enamel and improve the mechanical properties of dental materials. Incorporating calcium nanoparticles into toothpaste formulations could provide dual benefits—antimicrobial action and enamel repair—offering a promising approach to preventing dental caries while maintaining tooth integrity [21].

The enhanced antifungal efficacy of calcium nanoparticles against Candida albicans may be attributed to their ability to penetrate fungal biofilms and disrupt membrane integrity. Candida albicans is known for its strong biofilm-forming capabilities, which contribute to its resistance against conventional antifungal agents. Nanoparticles, such as zinc oxide and calcium oxide, have been shown to disrupt these biofilms by generating reactive oxygen species (ROS), leading to oxidative stress and membrane damage in fungal cells [22]. The findings of this study support these mechanisms, as calcium nanoparticles demonstrated greater inhibition of Candida albicans compared to Lactobacillus.

In contrast, Lactobacillus appeared more resistant to calcium nanoparticles, possibly due to its thick peptidoglycan layer, which hinders nanoparticle penetration. Similar resistance patterns have been observed in Enterococcus faecalis, another gram-positive bacterium known for its robust cell wall structure. This suggests that the antimicrobial efficacy of calcium nanoparticles may vary depending on the structural characteristics of different microorganisms [23].

One interesting finding was the decreased antimicrobial efficacy of calcium nanoparticles at higher concentrations, which may be due to nanoparticle aggregation. When nanoparticles cluster together, their active surface area is reduced, limiting their interaction with microbial cells. Additionally, excessive nanoparticle concentrations could interfere with bacterial metabolic pathways, further diminishing their effectiveness. This highlights the need for optimizing nanoparticle formulations to maintain antimicrobial efficacy while minimizing potential drawbacks [24].

Calcium nanoparticles show promise as antimicrobial agents in oral healthcare, particularly against Candida albicans, making them suitable for antifungal toothpaste formulations.[25] However, their lower efficacy against Lactobacillus suggests they may need to be combined with other agents for broader protection.[26] Their dual benefits of antimicrobial action and enamel remineralization could enhance preventive dental care, especially for high-risk populations. Further research is needed to assess their long-term safety, impact on the oral microbiome, and potential for combination with other bioactive agents. Advances in nanotechnology may also enable controlled-release formulations for sustained antimicrobial effects.

5. CONCLUSION

This study demonstrates that calcium nanoparticles exhibit antimicrobial activity against Candida albicans, suggesting their potential as antifungal agents in oral healthcare. However, their antibacterial efficacy against Lactobacillus was lower than that of fluoride-based toothpaste, indicating the need for optimized formulations to enhance their broad-spectrum antimicrobial properties. Future research should focus on optimizing nanoparticle concentrations, conducting in vivo studies, and exploring combination therapies to maximize antimicrobial efficacy. The integration of calcium nanoparticles into toothpaste formulations holds promise for improving oral health, but further investigation is necessary to establish their long-term benefits and safety. By advancing research in this field, nanoparticle-based formulations could revolutionize dental care, offering enhanced protection against oral pathogens while promoting overall oral health.

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Author Contribution: Data collection, analysis, and initial drafting of the manuscript were carried out by Lekha Dhanasekaran. Dr. Ramesh R was responsible for structuring the study design, revising the manuscript, and providing critical revisions. The final approval of the manuscript was also granted by Dr. Ramesh R.

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Conflict of interest - The authors declare no potential conflict of interest

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