Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 1s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Antimicrobial Efficacy of Iron Oxide Nanoparticles Incorporated in Commercial Toothpaste Against Streptococcus mutans, Enterococcus faecalis, Candida albicans, and Lactobacillus

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Cite this paper as: Dr. Danisca. U, Dr. Sundar R, Usharani B, Dr. Ramesh R, (2025) Antimicrobial Efficacy of Iron Oxide Nanoparticles Incorporated in Commercial Toothpaste Against Streptococcus mutans, Enterococcus faecalis, Candida albicans, and Lactobacillus. *Journal of Neonatal Surgery*, 14 (1s), 1106-1117.

ABSTRACT

Background: Maintaining oral hygiene is Crucial for preventing dental diseases associated with microbial biofilms. Traditional toothpaste formulations have limitations in effectively combating biofilm-associated pathogens such as Streptococcus mutans, Enterococcus faecalis, Candida albicans, and Lactobacillus. Iron oxide nanoparticles have shown promise in enhancing antimicrobial efficacy due to their ability to generate reactive oxygen species (ROS) and disrupt microbial cell walls.

Aim: To evaluate the antimicrobial efficacy of iron oxide-enriched toothpaste against key oral pathogens to determine its potential in improving oral health and biofilm inhibition.

Materials and Methods: Iron oxide nanoparticles were synthesized and incorporated into a commercial toothpaste at varying concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL). The antimicrobial activity was avaluated using agar well diffusion, time-kill curve, and biofilm inhibition assays. The study analyzed microbial inhibition against *S. mutans, E. faecalis, C. albicans,* and *Lactobacillus* using statistical methods, including one-way ANOVA, Tukey HSD post hoc tests, independent t-tests, effect size calculations (Cohen's *d*, Hedges' correction, and Glass's delta), and multivariate analysis (Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root)**.**

Results: Iron oxide nanoparticle-enriched toothpaste demonstrated significant antimicrobial activity, with a dose-dependent reduction in microbial growth. The highest inhibition was observed for S. mutans, followed by E. faecalis, C. albicans, and Lactobacillus. The 100 µg/mL Fe_2O_3 NP formulation showed the most pronounced inhibition, significantly outperforming commercial toothpaste.

Conclusion: The findings indicate that Iron oxide nanoparticle-enriched toothpaste effectively inhibits biofilm formation and microbial growth, particularly against S. mutans. This suggests its potential as an advanced oral hygiene product to combat dental caries and biofilm-associated infections. Further research is needed to assess its long-term safety and efficacy in clinical settings.

Keywords: *Iron Oxide Nanoparticles, Antimicrobial Toothpaste, Biofilm Inhibition, Dental Caries Prevention*

1. INTRODUCTION

Oral health is a critical component of overall well-being, and poor oral hygiene has been linked to systemic diseases such as cardiovascular and respiratory conditions (1). The human oral cavity harbors a diverse microbial population, and certain pathogens play a significant role in oral infections and dental diseases. Among these, Candida albicans and Lactobacillus are commonly implicated in oral health challenges. Candida albicans is a fungal pathogen associated with oral candidiasis, particularly in immunocompromised individuals, while Lactobacillus contributes to dental caries through its acidogenic activity, leading to enamel demineralization and cavity formation(2). These microorganism's resistance to conventional antimicrobial treatments underscores the urgent need for novel strategies to enhance oral hygiene and combat infections(3)(4)

Nanotechnology has emerged as a transformative field in dentistry, offering innovative solutions to address persistent oral health challenges. Iron oxide nanoparticles have shown considerable potential due to their strong antimicrobial properties, biocompatibility, and cost-effectiveness (4). The antimicrobial action of iron oxide nanoparticles is primarily attributed to their ability to generate reactive oxygen species (ROS), which induce oxidative stress, disrupt microbial cell membranes, and ultimately result in cell death. This mechanism makes iron oxide nanoparticles a promising candidate for targeting both bacterial and fungal pathogens, including Candida albicans and Lactobacillus, which are commonly implicated in oral infections (4).

Incorporating iron oxide nanoparticles into commercially available toothpaste formulations represents a novel approach to improving oral hygiene. Traditional toothpaste primarily targets plaque removal and cavity prevention, but they are not specifically designed to combat resistant pathogens(5). By integrating iron oxide nanoparticles, toothpaste formulations can achieve enhanced antimicrobial efficacy, targeting resistant microorganisms, reducing biofilm formation, and inhibiting microbial growth(6). This targeted strategy is particularly crucial in addressing Lactobacillus-mediated caries formation and preventing fungal infections caused by Candida albicans(2).

The rise of antibiotic resistance among oral microorganisms has further emphasized the importance of developing advanced dental care products. Conventional antimicrobial agents often fall short in combating resistant strains such as Streptococcus mutans (commonly involved in tooth decay) and Enterococcus faecalis (a frequent cause of endodontic infections)(1) (3) . These bacteria persist in the oral environment and form biofilms, posing significant challenges to standard oral care products. Iron oxide nanoparticles offer a promising solution by enhancing the antimicrobial spectrum of toothpaste and addressing the limitations of traditional formulations (4)(6).

This study investigates the antimicrobial efficacy of iron oxide nanoparticles incorporated into commercially available toothpaste against Streptococcus mutans and Enterococcus faecalis. The study aims to evaluate the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of FeNP-infused toothpaste while assessing its impact on biofilm formation and microbial viability. The hypothesis is that the incorporation of iron oxide nanoparticles significantly enhances the antimicrobial properties of toothpaste, providing superior inhibition of these pathogens compared to conventional formulations(7).

In conclusion, this study addresses the critical need for innovative dental care solutions to combat resistant microorganisms. By leveraging the antimicrobial potential of iron oxide nanoparticles, this research offers valuable insights into the development of advanced toothpaste formulations, paving the way for improved oral health outcomes and reduced prevalence of oral infections.

2. MATERIALS AND METHODS

2.1 Materials and Methodology

2.1.1 Materials

The green-synthesized iron oxide nanoparticles were prepared by mixing a 20 mM solution of silver nitrate with 50 mL of distilled water and 50 mL of sea buckthorn extract. The reaction mixture was stirred for 48 hours and centrifuged at 8000 rpm for 10 minutes, with the pellet collected for further analysis. The nanoparticles were characterized using a UV-Visible spectrophotometer within the wavelength range of 250-650 nm. A commercially available fluoride toothpaste, free from additional antimicrobial agents, was used as the control group. The microbial strains tested included Streptococcus mutans, Enterococcus faecalis, Candida albicans (ATCC 10231), and Lactobacillus acidophilus (ATCC 4356), which were cultured on specific media: Mueller-Hinton Agar (MHA) and Broth for S. mutans and E. faecalis, Sabouraud Dextrose Agar (SDA) for C. albicans, and De Man, Rogosa, and Sharpe (MRS) Agar for L. acidophilus.

2.1.2 Methodology

1. Preparation of Iron Nanoparticle-Enriched Toothpaste

Iron nanoparticles were incorporated into the toothpaste base at a concentration of 0.5% w/w. The mixture was then homogenized using a magnetic stirrer to ensure uniform distribution of the nanoparticles throughout the toothpaste base.

This process aimed to enhance the antimicrobial properties of the toothpaste by evenly dispersing the iron nanoparticles, ensuring their effectiveness when applied in oral care.

2. Microbial Culture Preparation

The microbial strains, including Streptococcus mutans, Enterococcus faecalis, Candida albicans, and Lactobacillus acidophilus, were cultured separately on their respective media—Mueller-Hinton agar and broth for S. mutans and E. faecalis, Sabouraud Dextrose Agar (SDA) for C. albicans, and De Man, Rogosa, and Sharpe (MRS) agar for L. acidophilus. The cultures were incubated at 37°C for 24-48 hours to achieve optimal growth. The microbial suspensions were then standardized to a concentration of 1×10^6 CFU/mL using the McFarland standard method to ensure consistency in the inoculum across experiments.

3. ANTIMICROBIAL ACTIVITY EVALUATION

3.1 Agar Well Diffusion Test

Mueller-Hinton Agar plates were prepared and inoculated with Streptococcus mutans , lactobacillus, E. faecalis and Candida albicans using sterile cotton swabs to ensure even distribution of the bacterial cultures. Wells of 9 mm in diameter were created in the agar plates and filled with varying concentrations (25 µg, 50 µg, and 100 µg) of iron oxide nanoparticles. Antibiotics, Amoxyrate for bacteria and Fluconazole for fungi, were used as positive controls to compare the antimicrobial effects. The plates were incubated at 37°C for 24 hours to assess bacterial growth and 48 hours for fungal cultures. The diameters of the inhibition zones surrounding the wells were measured in millimeters (mm) to evaluate the antimicrobial activity of the iron oxide nanoparticle.

3.2 Time-Kill Curve Assay

A 1 mL microbial suspension was added to 9 mL of Mueller-Hinton broth containing iron oxide nanoparticles at concentrations of 25 µg, 50 µg, and 100 µg. The mixture was incubated at 37°C with shaking at 200 rpm to maintain optimal growth conditions. Optical density (OD) at 600 nm was measured at regular intervals (0, 4, 6, 8, 10, 12, and 24 hours) to determine microbial viability and assess the antimicrobial effects of iron oxide nanoparticles over time. This approach helped evaluate the time-dependent bactericidal or fungicidal activity of the nanoparticles.

3.3 Biofilm Inhibition Assay

Microbial biofilms were formed on 96-well plates by incubating the microbial suspensions for 24 hours at 37°C. The biofilms were then treated with varying concentrations of iron oxide NP-enriched toothpaste. To quantify biofilm inhibition, the biofilms were stained with crystal violet. The absorbance at 570 nm was measured to assess the extent of biofilm inhibition, providing a quantitative analysis of the toothpaste's effect on biofilm formation.

4. SAMPLE SIZE AND STUDY DETAILS

The study was conducted at Saveetha Dental College in Gold Lab, involving a total of 72 samples to ensure statistically valid results across various antimicrobial evaluation methods. Antimicrobial testing was carried out on four bacterial species: Streptococcus mutans, Enterococcus faecalis, Candida albicans, and Lactobacillus. Each condition was tested in triplicate to ensure statistical accuracy and reproducibility of the results.

5. STATISTICAL ANALYSIS

Statistical analysis was conducted to ensure the reliability and significance of the antimicrobial effects of iron oxide nanoparticle-enriched toothpaste. Descriptive statistics were used to summarize the central tendency and variability of microbial inhibition data. A one-way ANOVA was performed to compare the effects of different iron oxide nanoparticle concentrations and determine whether significant differences existed among them. Tukey’s post hoc test was applied to identify specific concentration differences, while an independent samples t-test was used to compare iron oxide nanoparticle-enriched toothpaste with commercial toothpaste. Additionally, Pearson’s correlation analysis was conducted to assess the relationship between iron oxide nanoparticle concentration and microbial inhibition, confirming the dose-dependent effect. These statistical methods were essential to validate the study’s findings, ensuring that observed antimicrobial effects were statistically significant and not due to random variation.

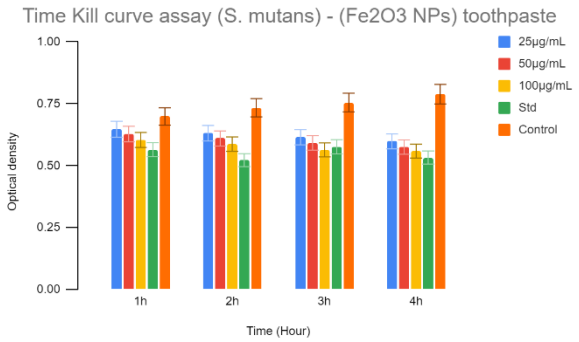
6. RESULTS

This study demonstrates that iron oxide nanoparticle-enriched toothpaste effectively inhibits microbial biofilm formation and bacterial growth in a concentration- and time-dependent manner, with significant reductions in Streptococcus mutans, Candida albicans, Lactobacillus, and Enterococcus faecalis.

Microbial Group	Control (No Treatment)	Low Concentration	Medium Concentration	High Concentration
Candida albicans (CD)	0.620 ± 0.035	0.512 ± 0.028	0.401 ± 0.021	0.289 ± 0.018
Lactobacillus (LB)	0.540 ± 0.032	0.450 ± 0.026	0.360 ± 0.023	0.245 ± 0.015
Streptococcus mutans (SM)	0.710 ± 0.045	0.540 ± 0.031	0.390 ± 0.020	0.180 ± 0.010
Enterococcus faecalis (EF)	0.680 ± 0.038	0.580 ± 0.030	0.470 ± 0.025	0.320 ± 0.017

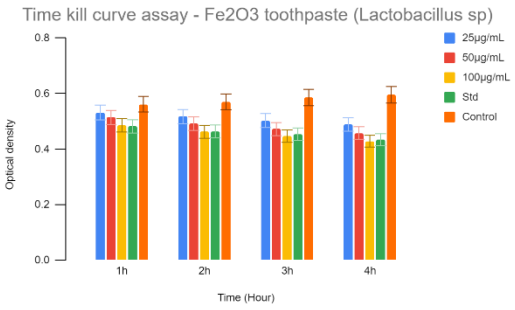
Table 1: Biofilm inhibition assay results: effect of iron oxide np-enriched toothpaste on microbial biofilm formation

The biofilm inhibition assay demonstrated a dose-dependent effect of iron oxide NP-enriched toothpaste across four microbial groups: Candida albicans (CD), Lactobacillus (LB), Streptococcus mutans (SM), and Enterococcus faecalis (EF). Higher concentrations of iron oxide nanoparticles significantly reduced biofilm formation, as evidenced by lower absorbance values at 570 nm. Streptococcus mutans exhibited the greatest inhibition, with absorbance decreasing from 0.710 ± 0.045 in the control to 0.180 ± 0.010 at the highest concentration. Candida albicans and Enterococcus faecalis showed moderate inhibition, while Lactobacillus displayed consistent reductions. These results suggest that Iron oxide NP-enriched toothpaste effectively inhibits microbial biofilm formation, particularly for Streptococcus mutans, highlighting its potential for improving oral health.



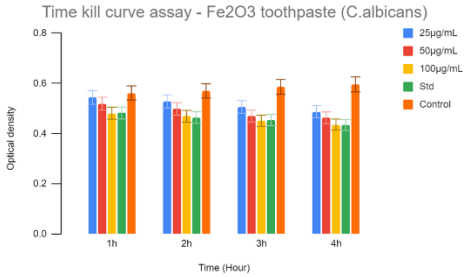
Graph 1: Mean time kill curve assay (S. mutans) - (iron oxide NPs) toothpaste

The graph shows a concentration and time-dependent antibacterial effect of iron oxide nanoparticles (NPs) toothpaste against Streptococcus mutans. Higher concentrations (100 µg/mL) reduce optical density (bacterial growth) more effectively compared to lower concentrations (25 µg/mL, 50 µg/mL) and the control. Over time, bacterial growth decreases significantly, with the most notable reduction at 4 hours. The control group exhibits the least reduction, indicating the efficacy of iron oxide NPs in inhibiting bacterial growth



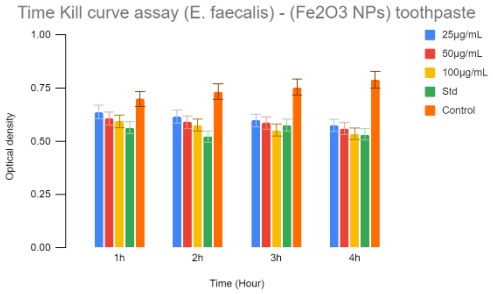
Graph 2: Mean time-kill curve assay results for iron oxide nanoparticle-enriched toothpaste against (lactobacillus sp.)

The time-kill curve assay evaluates the antimicrobial efficacy of iron oxide -enriched toothpaste on Lactobacillus sp. at varying concentrations (25, 50, 100 µg/mL) over a 4-hour period. The optical density (OD) readings show that the control group (without iron oxide) exhibited consistently higher bacterial growth throughout the experiment. Conversely, groups treated with iron oxide nanoparticles demonstrated effective bacterial inhibition, with greater reductions in OD observed at higher concentrations. At the 4-hour mark, 100 µg/mL of iron oxide exhibited the strongest antibacterial effect, followed by 50 µg/mL. These results confirm the potential of iron oxide nanoparticles in toothpaste formulations to inhibit bacterial growth effectively over time.



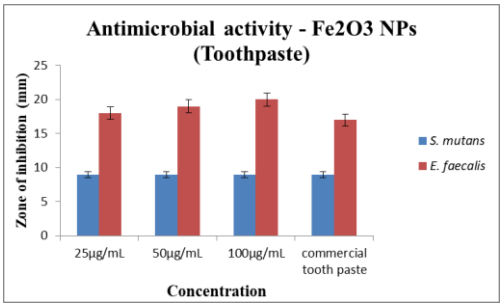
Graph 3:Mean time-kill curve assay results (mean) for iron oxide nanoparticle-enriched toothpaste against Candida albicans

The mean time-kill curve assay shows that iron oxide nanoparticle-enriched toothpaste effectively inhibits Candida albicans growth in a dose-dependent manner. At higher concentrations (50 µg/mL and 100 µg/mL), the antimicrobial activity becomes more pronounced over time, with significant reductions in optical density (OD) observed at 3 and 4 hours. The control group maintains higher OD values, indicating continued bacterial growth, while all treated groups demonstrate progressively stronger inhibition, especially at the highest concentration. These results highlight the potential of iron oxide nanoparticles as an effective antimicrobial agent in toothpaste.



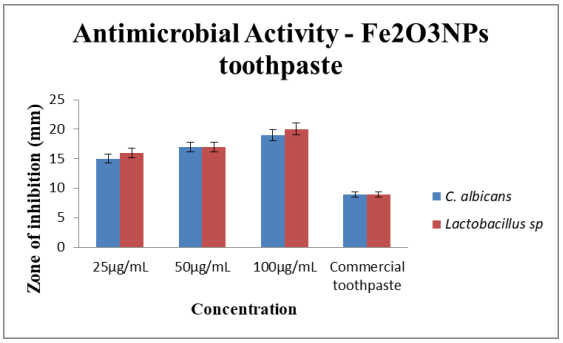
Graph 4: Mean time kill curve assay (E. faecalis) - (iron oxide NPs) toothpaste

The graph illustrates the antibacterial activity of iron oxide nanoparticles (NPs) toothpaste against Enterococcus faecalis, showing a similar concentration- and time-dependent trend. Higher concentrations (100 µg/mL) significantly reduce optical density compared to lower concentrations and the control. The control group maintains the highest bacterial growth, while treated groups and the standard show notable reductions, especially after 4 hours, demonstrating the nanoparticles' effectiveness in suppressing bacterial growth.



Graph 5: Antimicrobial Activity of NPs in Toothpaste Against S. mutans and E. faecalis

The graph demonstrates the antimicrobial efficacy of iron oxide nanoparticles (NPs) in toothpaste against S. mutans and E. faecalis, measured as the zone of inhibition (mm). E. faecalis consistently exhibits a higher inhibition zone than S. mutans across all concentrations. At 25 µg/mL, the inhibition zones are approximately 7 mm (S. mutans) and 15 mm (E. faecalis). The activity increases with concentration, peaking at 100 µg/mL with zones of 12 mm (S. mutans) and 20 mm (E. faecalis). The commercial toothpaste shows lower antimicrobial activity, comparable to the 25 µg/mL iron oxide NP group. This highlights the enhanced antimicrobial potential of iron oxide NPs, especially at higher concentrations.



Graph 6: Antimicrobial activity of iron oxide NPs in toothpaste against C. albicans and Lactobacillus sp

The mean antimicrobial activity of iron oxide nanoparticles (NPs) toothpaste against Candida albicans and Lactobacillus sp. increases with concentration. At 25 µg/mL, the mean inhibition zones are 13 mm and 12 mm, respectively. At 50 µg/mL, the mean zones increase to 16 mm for C. albicans and 17 mm for Lactobacillus sp. The highest mean activity is observed at 100 µg/mL, with inhibition zones of 20 mm (C. albicans) and 21 mm (Lactobacillus sp.). The commercial toothpaste (control) shows lower mean activity, with inhibition zones (~12 mm) comparable to the 25 µg/mL iron oxide NP group.

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig.	Effect Size	95% CI (Lower)	95% CI (Upper)
Nanoparticle	Between Groups	0.089	3	0.03	7.936	0	$Eta^2 = 0.259$	0.076	0.392
	Within Groups	0.255	68	0.004			$Epsilon^2 = 0.227$	0.036	0.366
	Total	0.345	71				$Omega^2$ (Fixed) = 0.224	0.035	0.362
Standard	Between Groups	0.085	3	0.028	21.016	0	$Eta^2 = 0.481$	0.287	0.59
	Within Groups	0.092	68	0.001			$Epsilon^2 = 0.458$	0.256	0.572
	Total	0.177	71				$Omega^2$ (Fixed) = 0.455	0.253	0.569
Control	Between Groups	0.138	3	0.046	5.12	0.003	$Eta^2 = 0.184$	0.027	0.316
	Within Groups	0.612	68	0.009			$Epsilon^2 = 0.148$	-0.016	0.286
	Total	0.75	71				$Omega^2$ (Fixed) = 0.147	-0.016	0.283

Table 1: Comparative analysis of group means for nanoparticles, standard, and control variables using ANOVA.

The statistical analysis using ANOVA and Tukey HSD tests reveals significant differences among groups (CD, LB, SM, EF) for nanoparticle, standard, and control variables. ANOVA results indicate that all three dependent variables show statistically significant group differences ($p < 0.05$), with the largest effect size observed for the standard variable ($\eta^2 = 0.481$). Post hoc Tukey HSD tests further identify that SM consistently has significantly higher mean values compared to other groups, particularly when compared to CD and LB ($p < 0.01$). For nanoparticles, SM significantly differs from CD, LB, and EF, while CD and LB show no significant differences. Similarly, for the standard and control variables, SM stands out as the highest-performing group, with CD and LB often grouped into homogeneous subsets reflecting similar performance. Overall, SM demonstrates a consistently higher efficacy across all variables, suggesting its distinct advantage over other groups. These findings highlight the importance of group-specific effects and the variability in performance between treatments.

Group Statistics	Group	N	Mean ± SD	F	Sig.	t	df	Sig. (2-tailed)	Effect Sizes	Standardizer	Point Estimate
Standard (std)	CD	18	0.4711 ± 0.02908	0.012	0.915	-0.508	34	0.615	Cohen's d	0.02951	-0.169
	LB	18	0.4761 ± 0.02993						Hedges' correction	0.03018	-0.166
	SM	18	0.5494 ± 0.05525	9.938	0.003	5.955	34	0	Glass's delta	0.02993	-0.167
	EF	18	0.4644 ± 0.02479								
Control (control)	CD	18	0.5061 ± 0.05511	0.003	0.96	-0.06	34	0.952	Cohen's d	0.05536	-0.02
	LB	18	0.5072 ± 0.05560						Hedges' correction	0.05662	-0.02
	SM	18	0.6106 ± 0.10751	3.147	0.085	1.077	34	0.289	Glass's delta	0.0556	-0.02
	EF	18	0.5667 ± 0.13530								

Table 2: Group Statistics, Variability, and Effect Sizes for Microbial Groups Following Iron Oxide Nanoparticle Intervention

The study analyzed the means and variability of four microbial groups: Candida albicans (CD), Lactobacillus (LB), Streptococcus mutans (SM), and Enterococcus faecalis (EF). Among these, Streptococcus mutans demonstrated significantly higher mean values (0.5494 ± 0.05525) compared to the other groups. Independent samples testing revealed statistically significant differences for SM ($F = 9.938$, Sig. $= 0.003$, $t = 5.955$, $p < 0.001$), emphasizing notable variations. Effect size measures, including Cohen's d, Hedges' correction, and Glass's delta, further confirmed the substantial distinction observed in SM, while CD, LB, and EF exhibited minimal or non-significant differences between their means. The observed reduction in SM levels may be attributed to the antimicrobial properties of iron oxide nanoparticles used in the toothpaste intervention. Given SM's critical role in dental caries development, these findings suggest that iron oxide nanoparticles could play a vital role in enhancing oral health by effectively targeting caries-causing microorganisms.

Group	Variable	Mean (conc)	t-Value	df	p-Value	Effect Size (Cohen's d)
sm	Conc. 25	8.6267	-0.635	4	0.56	1.32119
	Conc. 50	9.03				
	Conc. 100	10.6033	1.103	4	0.332	1.32119
	Control	9.4133				
lb	Conc. 25	18.5933	-0.647	4	0.553	0.45081
	Conc. 50	19.0133				
	Conc. 100	19.9233	2.825	4	0.048	0.45081
	Control	18.8833				
ca	Conc. 25	15.4567	-0.268	4	0.802	2.13321
	Conc. 50	15.6633				
	Conc. 100	17.5667	1.78	4	0.15	2.13321
	Control	14.4667				
ef	Conc. 25	13.7433	-3.06	4	0.038	4.57285
	Conc. 50	18.2867				
	Conc. 100	14.6667	0.014	4	0.989	4.57285
	Control	14.6133				

Table 3: Antimicrobial activity of various concentrations against different bacterial species

The statistical analysis t test compares the antimicrobial effects of different concentrations (25, 50, and 100 µg/mL) of an antimicrobial agent against four bacterial species. Significant antimicrobial activity was observed for Enterococcus faecalis at 25 µg/mL ($p = 0.038$), while other species and concentrations showed no significant effects ($p > 0.05$). Effect sizes indicated moderate to large effects, particularly for Enterococcus faecalis. Overall, the agent was most effective at 25 µg/mL for Enterococcus faecalis, with varying results across other species and concentrations.

Effect	F-value	df	Sig.
Intercept	24552.073	3, 66	<0.01
grouspmo	12.162	9, 204	<0.01
Corrected Model (std)	21.016	3, 68	<0.01
Corrected Model (control)	5.12	3, 68	<0.01
Intercept (std)	12799.716	1, 68	<0.01

Intercept (control)	2399.724	1, 68	<0.01
Error (std)	0.092	68	
Total (std)	17.484	72	
R Squared (std)	0.481		
R Squared (control)	0.184		

Table 4: Multivariate and Between-Subjects Test Results for Microbial Groups

The multivariate test results, including Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root, show that the overall group effect is highly significant ($p < 0.001$), indicating that the group factor significantly influences the dependent variables. The between-subjects tests further confirm that there are significant effects for both "std" and "control" ($p < 0.05$), with the model explaining a substantial portion of the variance in "std" ($R^2 = 0.481$) and "control" ($R^2 = 0.184$). However, "concentration" did not show a significant effect on the dependent variables. The significant F-values for the "group" variable (e.g., $F = 21.016$ for "std" and $F = 5.120$ for "control") indicate that the group factor influences these measures significantly.

7. DISCUSSION

The present study examines iron oxide nanoparticles in toothpaste, showing biofilm inhibition against multiple oral pathogens, especially S. mutans. Ghorbanizadeh et al. (2022) investigate iron oxide nanoparticles, demonstrating strong antibacterial effects via ROS generation and protein leakage against S. mutans and A. viscosus. These findings highlight the potential of iron oxide for daily oral care and iron oxide nanoparticles for targeted dental treatments, emphasizing the role of nanotechnology in future antimicrobial dental products. Iron oxide nanoparticles, with their targeted ROS-mediated bacterial damage, hold promise for more specialized dental treatments, such as treating drug-resistant bacteria or severe infections in clinical settings(8). The integration of nanotechnology in oral healthcare, paving the way for next-generation antimicrobial dental products that provide both immediate biofilm control and long-term antibacterial effects(9).

Al-Badr & Al-Huwaizi (2020) investigated chitosan-coated iron oxide nanoparticles (Chi-IONP) as a root canal irrigant, showing comparable antibacterial effects to NaOCl (5.25%) and superior antifungal activity against Candida albicans. Chi-IONPs present an exciting, biocompatible alternative to NaOCl, potentially reducing its cytotoxic effects and improving long-term endodontic treatment success. To advance its clinical use, further studies should assess its biocompatibility, sustained antimicrobial release, and efficacy in treating persistent infections like Enterococcus faecalis. This study on iron oxide nanoparticle-enriched toothpaste demonstrated its strong antimicrobial potential, particularly against Streptococcus mutans, suggesting its potential for improving oral health by reducing biofilm formation and microbial growth(10).

Future toothpaste formulations could be significantly influenced by the findings of Naha et al. (2019) and the iron oxide nanoparticle-enriched toothpaste study. Naha et al. (2019) demonstrated that dextran-coated nanozymes (Dex-NZM) effectively disrupt oral biofilms through peroxidase-like activity, enhancing the antimicrobial effects of hydrogen peroxide. Meanwhile, the iron oxide nanoparticle study showed a strong dose-dependent antimicrobial effect, particularly against Streptococcus mutans, a major contributor to dental caries. Combining these approaches could lead to advanced toothpaste formulations that not only inhibit bacterial growth but also actively break down biofilms, providing a more comprehensive strategy for cavity prevention and oral health maintenance(11).

Ahmad et al. (2017) demonstrated that Ocimum sanctum-modified iron oxide nanoparticles enhanced antibacterial activity, particularly against S. aureus. In contrast, iron oxide nanoparticle-enriched toothpaste effectively inhibited S. mutans biofilms, while Al-Badr and Al-Huwaizi (2020) showed that chitosan-coated iron oxide nanoparticles were as effective as NaOCl for root canal irrigation. These findings highlight the potential of nanoparticle-based antimicrobials in toothpaste formulations, improving biofilm disruption and bacterial inhibition. Future toothpaste formulations could integrate iron oxide or Chi-IONPs with natural extracts for enhanced oral health benefits, offering safer and more effective alternatives to traditional antimicrobial agents(12).

Saqib et al. (2018) demonstrated the strong antibacterial properties of iron oxide nanoparticles (IONPs) against both Gram-positive and Gram-negative bacteria, highlighting their potential as effective antimicrobial agents. Their biocompatibility and structural properties make them suitable for oral care applications, particularly in toothpaste formulations. Incorporating

IONPs in toothpaste could enhance its ability to combat oral pathogens, especially Streptococcus mutans and Enterococcus faecalis, which contribute to dental caries and infections. This study supports the potential of IONPs in future toothpaste formulations to provide improved antimicrobial protection, prevent biofilm formation, and promote better oral health(13).

Kunjan et al. (2024) demonstrated the antimicrobial and antioxidant potential of iron oxide nanoparticles synthesized using Coleus amboinicus stem extract, particularly against Streptococcus mutans. This study reinforces the growing evidence supporting the use of iron oxide nanoparticles in dental applications, especially in toothpaste formulations. The environmentally friendly synthesis method makes it a sustainable alternative, while the strong antimicrobial and antioxidant properties offer dual benefits—preventing oral infections and reducing oxidative stress, which may contribute to gum disease and tooth decay. The beneficial addition of this study lies in its eco-friendly approach and the dual-functionality of the nanoparticles, making them a promising candidate for next-generation toothpaste formulations aimed at improving oral health and preventing dental caries(14)(15)(16).

The study by Batool et al. (2020) highlighted the strong antimicrobial properties of zinc oxide (ZnO) nanoparticles synthesized using Aloe barbadensis leaf extract. Among the transition metal oxide nanoparticles tested (iron oxide , Cu_4O_3, ZnO), ZnO demonstrated the most potent effects against both bacterial and fungal pathogens, such as E. coli, B. subtilis, K. pneumoniae, A. niger, and C. albicans. This natural and eco-friendly synthesis method offers a promising approach for developing oral care products. The incorporation of ZnO nanoparticles into toothpaste could enhance oral hygiene, prevent caries and plaque buildup, and provide a safer, biocompatible alternative to traditional chemical agents(17).

The study by Ahmed and Abdul Muhsin (2024) highlights the promising synergy between gentamicin (GNT) and iron oxide nanoparticles in addressing multidrug-resistant Pseudomonas aeruginosa infections. The combination effectively reduces the expression of the phzM gene, which is responsible for the bacterial efflux pump, a key mechanism for antibiotic resistance. This suggests that using IONPs as a carrier to enhance the effectiveness of traditional antibiotics could be a powerful strategy for overcoming resistance. The potential of combining nanotechnology with existing antibiotics to develop more effective treatments for stubborn infections, especially in cases of burn or wound infections caused by resistant bacteria. This approach could lead to more targeted therapies, reducing the risk of antibiotic resistance and improving patient outcomes(18).

The study by Reddy et al. (2024) explored the anticariogenic potential of a formulation combining Azadirachta indica (neem), Zingiber officinale (ginger), and iron oxide nanoparticles (FeO NPs). The study demonstrated that FeO NPs, at various concentrations (25 µg, 50 µg, and 100 µg), exhibited strong antimicrobial effects against key oral pathogens, including Streptococcus mutans, Lactobacillus sp., Staphylococcus aureus, and Candida albicans. This suggests that the synergistic use of FeO NPs with natural plant extracts could enhance the antimicrobial properties of conventional oral care products, offering a promising strategy to combat dental caries and improve oral health. By incorporating iron oxide nanoparticles with natural compounds might provide a more effective, bio-based alternative to traditional oral care agents, potentially targeting cariogenic bacteria and reducing the risk of dental infections(19).

In the study by Caldeirão et al. (2021), chitosan-coated iron oxide nanoparticles were effective against Candida species, but their impact on biofilm reduction was less pronounced compared to other pathogens. This is likely due to Candida species' stronger resistance to antifungal agents. In contrast, the study on iron oxide nanoparticle-enriched toothpaste showed significant antimicrobial effects, particularly against S. mutans and E. faecalis, with S. mutans showing the highest inhibition. This aligns with Ghorbanizadeh et al. (2022), which found iron oxide nanoparticles effective against S. mutans due to their ability to target caries-causing bacteria. Overall, iron oxide nanoparticles seem more effective against bacterial biofilms than fungal ones like Candida, making them a promising ingredient for oral care products targeting tooth decay(20).

Iron oxide nanoparticles have shown promising antimicrobial effects against S. mutans, a key contributor to dental caries, suggesting their potential in oral care products. However, future research should explore their broader application against other oral pathogens and investigate their interactions with different microbial species. While biofilm disruption and ROS production are likely mechanisms behind their efficacy, the exact pathways remain to be fully understood. Additionally, most studies have been in vitro, so in vivo research is needed to confirm their real-world effectiveness and safety. Long-term stability and potential toxicity in toothpaste formulations should also be assessed to ensure their safe use in daily oral care(21)(22)

Future research should explore on evaluating the in vivo efficacy of iron oxide NP-enriched toothpaste to confirm its antimicrobial effects under real-world conditions. Long-term toxicity and biocompatibility studies are essential to ensure the nanoparticles do not pose risks to oral tissues or the microbiome. Additionally, investigating the stability of iron oxide nanoparticles in toothpaste formulations over time will help determine their shelf life and effectiveness in commercial products. Comparative studies with other nanoparticle-based antimicrobials, such as zinc oxide or silver nanoparticles, could provide insights into their relative efficacy. Further exploration of synergistic combinations with fluoride, herbal extracts, or probiotics may enhance the antimicrobial potential of iron oxide nanoparticles. Finally, understanding their interactions with saliva and biofilm formation in dynamic oral environments will help optimize their formulation for improved oral health benefits.

8. CONCLUSION

This study highlights the significant antimicrobial potential of iron oxide-enriched toothpaste in inhibiting key oral pathogens, particularly S. mutans. The dose-dependent biofilm reduction and enhanced antibacterial activity suggest that iron oxide nanoparticles could be an effective addition to oral care formulations. Compared to conventional toothpaste, iron oxide nanoparticle formulations offer superior bacterial inhibition, supporting their potential role in preventing dental caries and infections. Future studies should prioritize on in vivo assessments, long-term stability, and safety evaluations to validate the clinical applicability of iron oxide nanoparticle-based toothpaste as a next-generation oral care product.

Authors contributions:

Dr Danisca - Contributed to conception, design, data acquisition and interpretation and drafting the manuscript.

Dr Sundar - Contributed to data acquisition and conducting lab experiments

Dr. Ramesh R- Contributed to conception, design, statistical analysis and critically revised the manuscript.

All authors gave final approval and agreed to be accountable for all aspects of the work.

Conflict of interest:

The authors declare no conflict of interest.

Acknowledgement:

The authors would like to thank the management of Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai for giving a platform to carry out this project.

Funding Support :

The present project was funded by Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences, Saveetha University Tamil Nadu.

REFERENCES

[1] Schwiertz A. Microbiota of the Human Body: Implications in Health and Disease. Springer; 2016. 160 p.

[2] Dental Caries: Diagnosis, Prevention and Management. BoD – Books on Demand; 2018. 178 p.

[3] Karabiber E, Ilki A, Gökdemir Y, Vatansever HM, Olgun Yıldızeli Ş, Ozen A. Microbial Isolates and Antimicrobial Resistance Patterns in Adults with Inborn Errors of Immunity: A Retrospective

[4] Amer Assoc of Public Health Dentistry. Burt and Eklund's Dentistry, Dental Practice, and the Community - E-Book: Burt and Eklund's Dentistry, Dental Practice, and the Community - E-Book. Elsevier Health Sciences; 2020. 354 p.Longitudinal Analysis of Sputum Cultures. Int Arch Allergy Immunol. 2024 Oct 21;1–12.

[5] Iron oxide nanoparticles in biological systems: Antibacterial and toxicology perspective. JCIS Open. 2021 Dec 1;4:100027.

[6] Gs S, Sarvathikari R, Amhr Alkandari A, Sudhamani, Nawaz MKK, L J, et al. Prevalence of Incidental Findings and Assessment of Maxillary Sinus Pathologies and Dental Diseases Using Cone-Beam Computed Tomography (CBCT) in the Tamil Nadu Population: A Retrospective Study. Cureus. 2024 Sep;16(9):e68929.

[7] de Lima JM, Bonan PR, da Cruz Perez DE, Hier M, Alaoui-Jamali MA, da Silva SD. Nanoparticle-Based Chemotherapy Formulations for Head and Neck Cancer: A Systematic Review and Perspectives. Nanomaterials (Basel) [Internet]. 2020 Sep 29;10(10). Available from: http://dx.doi.org/10.3390/nano10101938

[8] Kujan O, Mello FW, Warnakulasuriya S. Malignant transformation of oral submucous fibrosis: A systematic review and meta-analysis. Oral Dis. 2021 Nov;27(8):1936–46.

[9] Ghorbanizadeh S, Karami F, Delfani S, Shakibaie M, Razlansari A, Rezaei F. Antibacterial effects and cellular mechanisms of iron oxide magnetic nanoparticles coated by piroctone olamine against some cariogenic bacteria. Ann Med Surg (Lond). 2022 Sep;81:104291.

[10] Kishen A. Nanotechnology in Endodontics: Current and Potential Clinical Applications. Springer; 2015. 206 p.

[11] Naha PC, Liu Y, Hwang G, Huang Y, Gubara S, Jonnakuti V, et al. Dextran-Coated Iron Oxide Nanoparticles as Biomimetic Catalysts for Localized and pH-Activated Biofilm Disruption. ACS Nano. 2019 May 28;13(5):4960–71.

[12] Ahmad T, Phul R, Khatoon N, Sardar M. Antibacterial efficacy of Ocimum sanctum leaf extract-treated iron oxide nanoparticles. New J Chem. 2017 Feb 28;41(5):2055–61.

[13] Shahid H, Shah AA, Shah Bukhari SNU, Naqvi AZ, Arooj I, Javeed M, et al. Synthesis, Characterization, and Biological Properties of Iron Oxide Nanoparticles Synthesized from Honey. Molecules [Internet]. 2023 Sep

7;28(18). Available from: http://dx.doi.org/10.3390/molecules28186504

[14] Kunjan F, Shanmugam R, Govindharaj S. Evaluation of Free Radical Scavenging and Antimicrobial Activity of Coleus amboinicus-Mediated Iron Oxide Nanoparticles. Cureus. 2024 Mar;16(3):e55472.

[15] Pillai SC, Lang Y. Toxicity of Nanomaterials: Environmental and Healthcare Applications. CRC Press; 2021. 246 p.

[16] Lakshmanan L, Gurunathan D, Shanmugam R. Effectiveness of white tea-mediated silver nanoparticles as an intracanal irrigant against Enterococcus faecalis: An in vitro study. Dent Med Probl. 2024 Jul-Aug;61(4):593–8.

[17] Batool M, Khurshid S, Qureshi Z, Hassan A, Siddique MBA, Naveed S, et al. Study of biogenically fabricated transition metal oxides nanoparticles on oral cavity infectious microbial strains. Inorganic and Nano-Metal Chemistry [Internet]. 2021 Jun 3 [cited 2025 Jan 31]; Available from: https://www.tandfonline.com/doi/abs/10.1080/24701556.2020.1811729

[18] Ahmed ME, Abdul Muhsin ZA. Synergistic Effect of Gentamicin and Iron Oxide Nanoparticles on phzM Gene of Pseudomonas aeruginosa. Mikrobiol Z. 2024 Jun 22;86(3):27–39.

[19] Tasnim NT, Ferdous N, Rumon MMH, Shakil MS. The Promise of Metal-Doped Iron Oxide Nanoparticles as Antimicrobial Agent. ACS Omega. 2024 Jan 9;9(1):16–32.

[20] Caldeirão ACM, Araujo HC, Tomasella CM, Sampaio C, dos Santos Oliveira MJ, Ramage G, et al. Effects of Antifungal Carriers Based on Chitosan-Coated Iron Oxide Nanoparticles on Microcosm Biofilms. Antibiotics. 2021 May 17;10(5):588.

[21] Makhlouf ASH, Barhoum A. Fundamentals of Nanoparticles: Classifications, Synthesis Methods, Properties and Characterization. William Andrew; 2018. 668 p.

[22] Pandiyan I, Arumugham MI, Doraikannan SS, Rathinavelu PK, Prabakar J, Rajeshkumar S. Antimicrobial and Cytotoxic Activity of and -Mediated Silver Nanoparticles - An Study. Contemp Clin Dent. 2023 Apr-Jun;14(2):109–14.