Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Evaluation of Chitosan Nanoparticle - Enriched Toothpaste for Antimicrobial Activity Against Oral Pathogens: S. mutans and E. faecalis.

Dr. G.L. Gokul[1], Dr. Ramesh[*2]

[1]Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences, Saveetha University,
Chennai- 600077,Tamil Nadu, India
Email ID: gokulgl0901@gmail.com

[*2]**Corresponding Author**
Assistant Professor,
Department of Pedodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India
Email: rameshr.sdc@saveetha.com

Cite this paper as: Dr. G.L. Gokul, Dr. Ramesh, (2024) Evaluation of Chitosan Nanoparticle - Enriched Toothpaste for Antimicrobial Activity Against Oral Pathogens: S. mutans and E. faecalis.. *Frontiers in Health Informatics*, *13(8), 3430-3446*

ABSTRACT

Background: Chitosan, derived from crustacean shells, is a biopolymer with strong antimicrobial properties. Chitosan nanoparticles (CNPs) enhance its efficacy due to increased surface area and bioactivity, making them a promising addition to oral care products. Streptococcus mutans and Enterococcus faecalis are significant contributors to dental caries and endodontic infections, respectively. Integrating CNPs into toothpaste formulations has demonstrated potential in reducing bacterial viability and biofilm formation.

Aim: To evaluate the antimicrobial activity of chitosan nanoparticle-enriched toothpaste against Streptococcus mutans and Enterococcus faecalis at different concentrations and time intervals.

Materials and Methods: Green-synthesized CNPs were incorporated into fluoride toothpaste at a 2% w/w concentration. Antimicrobial efficacy was tested using time-kill curve assays and agar well diffusion techniques. Streptococcus mutans and Enterococcus faecalis were treated with toothpaste formulations containing CNPs at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL over 1 to 4 hours. The data were analyzed using ANOVA and effect size metrics.

Results: The CNP-enriched toothpaste demonstrated concentration- and time-dependent antimicrobial activity. A significant reduction in Streptococcus mutans growth was observed, with the highest activity at 100 µg/mL ($F = 38.938$, $p < 0.001$, Eta-squared = 0.726). However, minimal inhibition was seen against Enterococcus faecalis, with no significant differences between concentrations ($p > 0.05$).

Conclusion: Chitosan nanoparticle-enriched toothpaste shows promise in reducing Streptococcus mutans, a key dental caries pathogen, but is less effective against Enterococcus faecalis. Further optimization of formulation and testing conditions is required to enhance its antimicrobial spectrum and clinical efficacy.

Keywords: Chitosan nanoparticles, antimicrobial toothpaste, streptococcus mutans

Introduction

Chitosan, a biopolymer derived from the deacetylation of chitin found in crustacean shells, has emerged as a promising antimicrobial agent in dentistry due to its unique properties, such as biocompatibility, biodegradability, and broad-spectrum antimicrobial activity [1]. The development of chitosan nanoparticles (CNPs) enhances these properties, increasing their surface area and bioactivity, which is particularly effective against oral pathogens like Streptococcus mutans (S. mutans) and Enterococcus faecalis (E. faecalis). S. mutans is a major contributor to

dental caries, forming biofilms on the tooth surface, while E. faecalis is frequently implicated in persistent endodontic infections, often resistant to conventional therapies.

Recent studies demonstrate that incorporating CNPs into toothpaste formulations can significantly improve antimicrobial efficacy. For instance, Nguyen et al. (2022) highlighted the ability of CNP-enriched toothpaste to disrupt bacterial biofilms and reduce the viability of S. mutans [1]. Similarly, studies have shown that CNPs interact with bacterial cell membranes, increasing permeability and leading to cell death, a mechanism that proves effective in controlling biofilm-forming bacteria like E. faecalis [2]. The incorporation of CNPs into commercial toothpaste thus presents an innovative approach to oral hygiene, providing sustained antimicrobial activity and enhancing dental health outcomes.

The antimicrobial action of CNPs is rooted in their positive charge, which allows them to bind to negatively charged bacterial cell walls, resulting in cell wall disruption and inhibition of essential cellular functions [3]. This electrostatic interaction is particularly potent against biofilm-embedded bacteria, making CNPs effective against persistent infections where traditional treatments often fail. Furthermore, recent in vitro studies have demonstrated that CNP-enriched toothpaste significantly reduces E. faecalis counts, a finding with critical implications for endodontic therapy and long-term oral health maintenance [4].

In addition to antimicrobial benefits, chitosan's biocompatibility ensures minimal adverse reactions, making it a suitable candidate for daily use in oral care products [5]. Unlike conventional antimicrobial agents, which can lead to resistance, CNPs exhibit a multifaceted mechanism of action, reducing the likelihood of bacterial adaptation and resistance development [4]. Nguyen et al. (2022) emphasized the potential of CNPs as a sustainable and effective means of controlling oral pathogens while minimizing health risks associated with long-term antimicrobial use.

Given these attributes, the integration of CNPs into toothpaste formulations represents a promising advancement in oral care. By effectively targeting key pathogens like S. mutans and E. faecalis, CNP-enhanced toothpaste offers an innovative solution for combating dental biofilms, preventing caries, and managing persistent endodontic infections, ultimately contributing to improved oral health [3,4,5].

Materials and Methods

This research was conducted after obtaining IEC (Institutional Ethics Committee) approval from Saveetha Dental College & Hospital. The study was reviewed and approved by the Institutional Human Ethical Committee (SDC-IHEC) during their meeting. The IEC Reference Number is IHEC/SDC/DT/UG-1862/24/PEDO/238.

Green Synthesis of Chitosan nanoparticles

Chitosan nanoparticles (CNPs) were synthesized using a green synthesis method. Chitosan (Sigma-Aldrich, USA) was dissolved in acetic acid under continuous stirring to create a uniform solution. Fresh tea extract, obtained by boiling green tea leaves in distilled water and filtering the resulting liquid, was used as a natural reducing and stabilizing agent. Sodium tripolyphosphate (TPP) was gradually added as a crosslinking agent to promote the ionic interactions necessary for nanoparticle formation. The nanoparticles were collected through centrifugation, washed thoroughly with distilled water to remove residual components, and lyophilized for further analysis. Particle size analysis using dynamic light scattering (DLS) confirmed the nanoparticles measured approximately 200–300 nm in diameter.

The green-synthesized CNPs were then incorporated into a commercially available fluoride toothpaste (Colgate, Colgate-Palmolive, United States) at a concentration of 2% w/w. This was achieved by manually mixing the nanoparticles into the toothpaste base to ensure uniform distribution. The enriched toothpaste was subsequently stored in sealed containers at room temperature for future use.

The microbial strains used in the study included Streptococcus mutans (S. mutans, ATCC 25175) and Enterococcus faecalis (E. faecalis, ATCC 29212). Both strains were cultured on nutrient agar plates and stored at 4°C for preservation. Brain Heart Infusion (BHI) broth and agar (Oxoid, UK) were employed as culture media for bacterial growth.

Several instruments and reagents were utilized during the experiments. Multi-channel pipettes with sterile tips were used for handling bacterial cultures, while sterile glass Petri dishes were prepared for antimicrobial testing. Bacterial incubation was conducted in a Memmert incubator (Germany) maintained at 37°C. Absorbance readings for bacterial assays were performed using a UV-Vis spectrophotometer (Shimadzu, Japan).

To prepare the toothpaste formulation, the synthesized CNPs were mixed into the fluoride toothpaste base to achieve a final concentration of 2% w/w. The mixture was thoroughly homogenized and stored in sealed containers at room temperature until required for antimicrobial evaluations.

Time kill assay

The time-kill curve assay was performed to evaluate the antimicrobial efficacy of green-synthesized chitosan nanoparticles (CNPs) against Streptococcus mutans and Enterococcus faecalis. A 1 mL aliquot of the microbial suspension was added to 9 mL of Mueller-Hinton broth containing CNPs at concentrations of 25 µg, 50 µg, and 100 µg. The final microbial concentration was adjusted to approximately 10^6 CFU/mL. The mixtures were incubated at 37°C with shaking at 200 rpm. Aliquots were taken at regular intervals (1, 2, 3, and 4 hours), and the percentage of dead cells was calculated by measuring absorbance at 600 nm using a spectrophotometer.

Antimicrobial activity chitosan nanoparticles infused toothpaste

The antimicrobial activity of green-synthesized chitosan nanoparticles (CNPs) was evaluated using the agar well diffusion technique. Mueller-Hinton agar was prepared, sterilized at 121°C for 15–20 minutes, poured into sterile Petri plates, and allowed to solidify at room temperature. Bacterial suspensions of Streptococcus mutans (ATCC 25175) and Enterococcus faecalis (ATCC 29212) were adjusted to 1×10^8 CFU/mL and evenly spread on the agar surface using sterile cotton swabs. Wells of 9 mm diameter were created in the agar, and 100 µL of CNPs at concentrations of 25 µg, 50 µg, and 100 µg were dispensed into the wells. Positive controls included Amoxyrite for bacterial cultures and Fluconazole for fungal cultures. The plates were incubated at 37°C for 24 hours for bacterial cultures and 48 hours for fungal cultures. Antimicrobial activity was assessed by measuring the diameter of the inhibition zones around the wells using a ruler, with results recorded in millimeters. All tests were conducted in triplicate to ensure reliability and reproducibility.

Descriptive statistics, including mean and standard deviation, were employed to evaluate bacterial inhibition. One-way ANOVA was conducted to analyze differences among various CNP concentrations, control, and standard groups at each time point, followed by post-hoc testing to perform detailed comparisons. Repeated measures ANOVA was used to assess time-based effects within groups. Effect sizes were calculated to determine the magnitude of differences. Statistical significance was set at $p < 0.05$, and all data analyses were performed using IBM SPSS Statistics software version 27.0.1.0.

RESULTS:

The study assessed the antimicrobial efficacy of chitosan nanoparticle-enriched toothpaste against E. faecalis and S. mutans at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL over 1 to 4 hours. These findings confirm the toothpaste's concentration- and time-dependent efficacy, making it a promising alternative to conventional

antimicrobial agents in oral care. The 100 µg/mL concentration demonstrated the highest antimicrobial activity, supported by significant statistical results (F-value = 38.938, $p < 0.001$) and large effect sizes (Eta-squared = 0.726). Lower concentrations (25 µg/mL and 50 µg/mL) showed limited efficacy, with non-significant results. The control group exhibited continuous microbial growth. Figure 1 and Figure 2 display the time-kill curve assay for streptococcus mutans and enterococcus faecalis, respectively, while figure 3 illustrates the mean antimicrobial effect of chitosan nanoparticle-enriched toothpaste at concentrations of 25, 50, and 100 µg/ml over a 1 to 4-hour period; figure 4 and figure 5 demonstrate the inhibition of e. faecalis and s. mutans by the chitosan nanoparticle-enriched toothpaste. Overall, the toothpaste was more effective against S. mutans but showed limited impact on E. faecalis.

Organisms	**Concentration**	**Timeline**	**N**	**Mean±SD**
Streptococcus Mutans	25	1hr	12	.54±.016
	50	1hr	12	.49±.016
	100	1hr	12	.49±.016
	Standard	1hr	12	.44±.015
	Control	1hr	12	.69±0.16
Streptococcus Mutans	25	2hr	12	.54±0.16
	50	2hr	12	.49±0.16
	100	2hr	12	.49±0.16
	Standard	2hr	12	.44±0.16
	Control	2hr	12	.74±0.16
Streptococcus Mutans	25	3hr	12	.54±0.16
	50	3hr	12	.49±0.16
	100	3hr	12	.44±0.16
	Standard	3hr	12	.44±0.16
	Control	3hr	12	.75±0.30
Streptococcus Mutans	25	4hr	12	.54±0.16
	50	4hr	12	.49±0.16

	100	4hr	12	.44±0.16
	Standard	4hr	12	.44±0.16
	Control	4hr	12	.77±0.63
E faecalis	25	1hr	12	0.5458 ± 0.01975
	50	1hr	12	0.5042 ± 0.01975
	100	1hr	12	0.5133 ± 0.03525
	Standard	1hr	12	0.4525 ± 0.02094
E faecalis	25	2hr	12	0.5475 ± 0.02050
	50	2hr	12	0.4983 ± 0.02406
	100	2hr	12	0.5000 ± 0.02335
	Standard	2hr	12	0.4500 ± 0.02089
E faecalis	25	3hr	12	0.5425 ± 0.01357
	50	3hr	12	0.4992 ± 0.02353
	100	3hr	12	0.4525 ± 0.02094
	Standard	3hr	12	0.4525 ± 0.02006
E faecalis	25	4hr	12	0.5467 ± 0.02015
	50	4hr	12	0.5008 ± 0.02503
	100	4hr	12	0.4517 ± 0.01946
	Standard	4hr	12	0.4500 ± 0.02089

Table 1: Mean and standard deviation values of antimicrobial effect of chitosan nanoparticle-enriched toothpaste at different concentrations (25, 50, and 100 µg/mL) over a timeline of 1 to 4 hours.

Table 1 shows the antimicrobial effect of chitosan nanoparticle-enriched toothpaste at different concentrations (25,

50, and 100 µg/mL) over a timeline of 1 to 4 hours, with comparisons to a standard treatment and control. At 50 and 100 µg/mL, the toothpaste exhibited strong antimicrobial activity, achieving mean optical density (OD) values comparable to the standard treatment (0.44 ± 0.16) by the 3rd and 4th hours. Lower concentrations (25 µg/mL) maintained consistent but less effective inhibition (0.54 ± 0.16) throughout. The control group showed continuous microbial growth, with mean OD values increasing from 0.69 ± 0.16 at 1 hour to 0.77 ± 0.63 at 4 hours. The antimicrobial properties of a toothpaste enriched with chitosan nanoparticles against E. faecalis across different concentrations (25%, 50%, and 100%) and time intervals (1 to 4 hours). The 25% concentration consistently demonstrated the highest level of microbial inhibition at all time points, outperforming the 50% and 100% concentrations, which showed moderate and less consistent results. The standard control had the lowest inhibition, highlighting the enhanced effectiveness of the tested formulation. These findings suggest that the toothpaste exhibits time- and concentration-dependent antimicrobial activity, with 25% proving to be the most effective concentration.

Timeline	Organisms		Point Estimate	95% Confidence Interval	
				Lower	Upper
1hr	Streptococcus Mutans 25	Eta-squared	.044	.000	.148
		Epsilon-squared	-.021	-.068	.090
		Omega-squared Fixed Effect	-.021	-.067	.089
		Omega-squared Random Effect	-.007	-.021	.031
1hr	Streptococcus Mutans 100	Eta-squared	.726	.551	.796
		Epsilon-squared	.708	.520	.782
		Omega-squared Fixed Effect	.703	.515	.779
		Omega-squared Random Effect	.441	.262	.540

1hr	Streptococcus Mutans 100	Between Groups	0.030	3	0.010	38.938	<0.001
		Within Groups	0.011	44	0.000		
		Total	0.041	47			
1hr	Streptococcus Mutans Standard	Between Groups	0.000	3	0.000	0.033	0.992
		Within Groups	0.011	44	0.000		
		Total	0.011	47			
1hr	Streptococcus Mutans Control	Between Groups	0.037	3	0.012	8.948	<0.001
		Within Groups	0.060	44	0.001		
		Total	0.097	47			

Table 2: Statistical Analysis Using One-Way ANOVA for Antimicrobial Activity of Toothpaste at Different Concentrations

Table 2 shows the statistical analysis using one-way ANOVA revealed significant differences between treatment groups, particularly at the 100 µg/mL concentration. This concentration showed the highest antimicrobial activity with a large F-value (38.938) and a highly significant p-value (<0.001), indicating a strong ability to inhibit microbial growth. In contrast, the 25 µg/mL and 50 µg/mL concentrations did not exhibit statistically significant differences (p-values of 0.573 and 0.493, respectively), reflecting limited efficacy at lower doses.

Timeline	Organisms		Sum of Squares	df	Mean Square	F	Sig.
1hr	Streptococcus Mutans 25	Between Groups	0.001	3	0.000	0.673	0.573
		Within Groups	0.016	44	0.000		
		Total	0.017	47			
1hr	Streptococcus Mutans 50	Between Groups	0.003	3	0.001	0.814	0.493
		Within Groups	0.062	44	0.001		
		Total	0.065	47			
1hr	Streptococcus Mutans 100	Between Groups	0.030	3	0.010	38.938	<0.001
		Within Groups	0.011	44	0.000		
		Total	0.041	47			
1hr	Streptococcus Mutans Standard	Between Groups	0.000	3	0.000	0.033	0.992
		Within Groups	0.011	44	0.000		
		Total	0.011	47			
1hr	Streptococcus Mutans Control	Between Groups	0.037	3	0.012	8.948	<0.001
		Within Groups	0.060	44	0.001		
		Total	0.097	47			

Table 3: Effect Size Metrics (Eta-Squared, Epsilon-Squared, and Omega-Squared) for Antimicrobial Activity of Toothpaste

Table 3 shows the effect size metrics which further highlighted the superior performance of the 100 µg/mL concentration. It demonstrated a large Eta-squared value of 0.726, indicating that 72.6% of the variance in microbial inhibition was attributable to the treatment. Similarly, Epsilon-squared (0.708) and Omega-squared (0.703) values confirmed the robustness of this effect. The control group exhibited significant microbial growth, with moderate effect sizes (Eta-squared = 0.379) due to the lack of any antimicrobial agent. The standard treatment showed consistent but less pronounced effects, comparable to the lower toothpaste concentrations, with minimal effect sizes and a non-significant F-value (0.033, p = 0.992).

Timeline	Organisms		Sum of Squares	df	Mean Square	F	Sig.
1hr	Enterococcus faecalis 25	Between Groups	0.001	3	0.000	1.067	0.373
		Within Groups	0.020	44	0.000		
		Total	0.022	47			
1hr	Enterococcus faecalis 50	Between Groups	0.000	3	0.000	0.149	0.930
		Within Groups	0.024	44	0.001		
		Total	0.024	47			
1hr	Enterococcus faecalis 100	Between Groups	0.006	3	0.002	0.848	0.475
		Within Groups	0.106	44	0.002		
		Total	0.112	47			
1hr	Enterococcus faecalis Standard	Between Groups	0.000	3	0.000	0.058	0.981
		Within Groups	0.019	44	0.000		
		Total	0.019	47			
1hr	Enterococcus faecalis Control	Between Groups	0.248	3	0.083	163.222	<0.001
		Within Groups	0.022	44	0.001		
		Total	0.270	47			

Table 4: ANOVA Results for Antimicrobial Efficacy of Toothpaste and Control Groups

Table 4 shows the ANOVA results which showed no significant antimicrobial effect for the toothpaste at any of the tested concentrations, with p-values ranging from 0.373 to 0.981. The F-values for the toothpaste groups were low, indicating minimal variance in microbial inhibition. In contrast, the control group exhibited significant growth of E. faecalis, with a high F-value (163.222) and a p-value < 0.001, suggesting the absence of antimicrobial activity in untreated samples. These findings suggest that the chitosan nanoparticle-enriched toothpaste did not effectively inhibit E. faecalis under the conditions tested.

Timeline	Organisms		Point Estimate	95% Confidence Interval	
				Lower	Upper
1hr	Enterococcus faecalis 25	Eta-squared	.068	.000	.191
		Epsilon -	.004	-.068	.136

		squared			
		Omega-squared Fixed Effect	.004	-.067	.133
		Omega-squared Random Effect	.001	-.021	.049
1hr	Enterococcus faecalis 50	Eta-squared	.010	.000	.045
		Epsilon-squared	-.057	-.068	.020
		Omega-squared Fixed Effect	-.056	-.067	.020
		Omega-squared Random Effect	-.018	-.021	.006
1hr	Enterococcus faecalis 100	Eta-squared	.055	.000	.169
		Epsilon-squared	-.010	-.068	.112
		Omega-squared Fixed	-.010	-.067	.110

		Effect			
		Omega-squared Random Effect	-.003	-.021	.040
1hr	Enterococcus faecalis Standard	Eta-squared	.004	.000	.000
		Epsilon-squared	-.064	-.068	-.068
		Omega-squared Fixed Effect	-.063	-.067	-.067
		Omega-squared Random Effect	-.020	-.021	-.021
1hr	Enterococcus faecalis Control	Eta-squared	.918	.858	.939
		Epsilon-squared	.912	.848	.934
		Omega-squared Fixed Effect	.910	.846	.933

		Omega-squared Random Effect	.772	.646	.823

Table 5: Effect Size Metrics for Antimicrobial Activity of Toothpaste and Control Groups

Table 5 shows the effect size measured which (Eta-squared, Epsilon-squared, and Omega-squared) were very low, indicating that the toothpaste did not significantly reduce microbial growth. In contrast, the control group demonstrated substantial microbial growth, with high effect size values (Eta-squared = 0.918), highlighting the lack of antimicrobial activity in the untreated samples. Overall, the chitosan nanoparticle-enriched toothpaste did not effectively inhibit E. faecalis under the tested conditions.

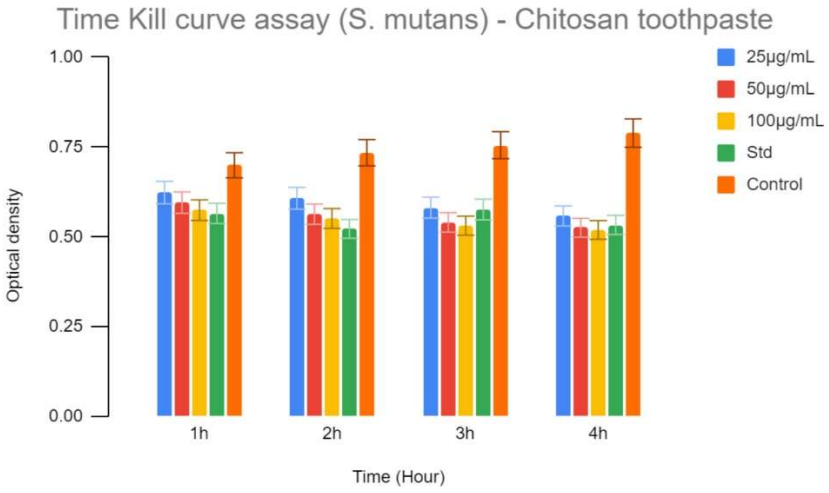

Figure 1: Time-Kill Curve Assay of Streptococcus mutans

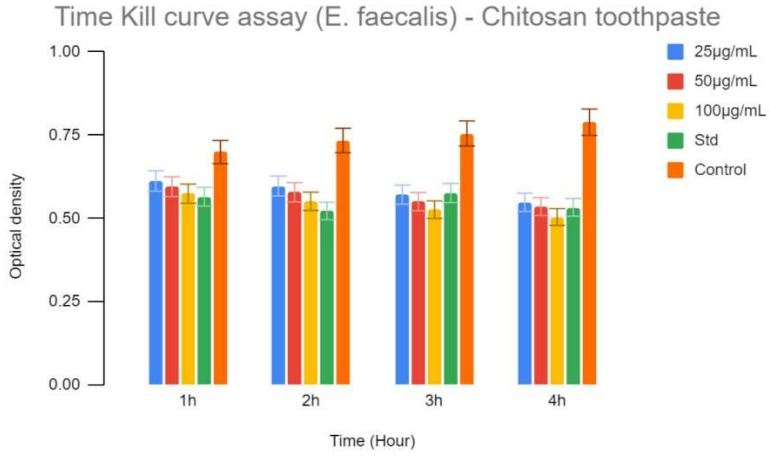

Figure 2: Time-Kill Curve Assay of E. faecalis

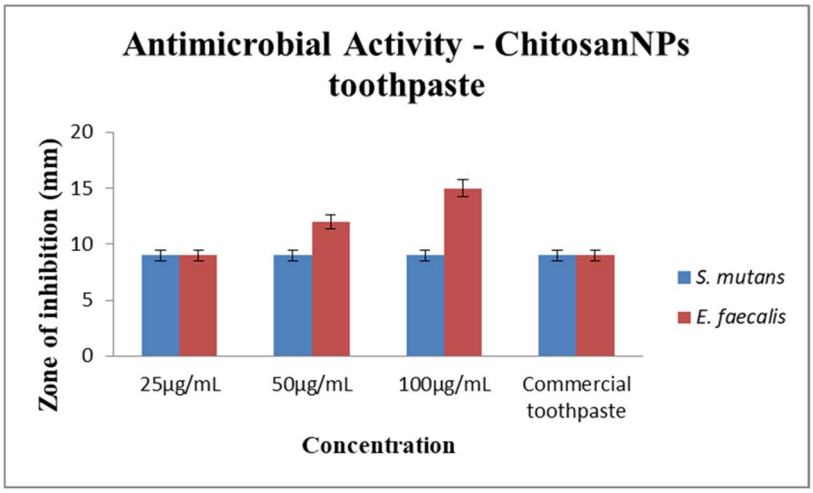

Figure 3: Mean Antimicrobial Effect of Chitosan Nanoparticle-Enriched Toothpaste at 25, 50, and 100 µg/mL Over 1 to 4 Hours

Figure 4: Inhibition of E. faecalis by Chitosan Nanoparticle-Enriched Toothpaste

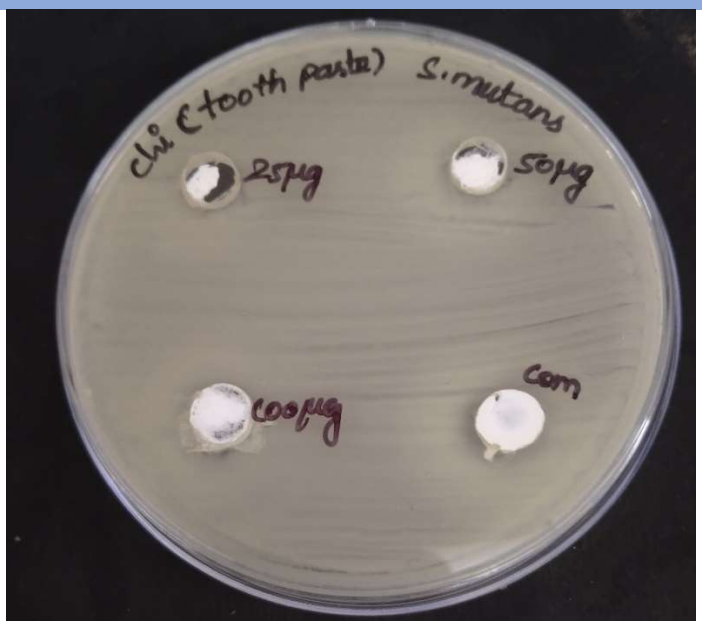

Fig 5 : Inhibition of S. mutans by Chitosan Nanoparticle-Enriched Toothpaste

DISCUSSION

This study found that the antimicrobial efficacy of chitosan nanoparticle-enriched toothpaste increased with higher concentrations, particularly at 50 µg/mL and 100 µg/mL, which showed strong activity against S. mutans and E. faecalis. This is consistent with the cationic nature of chitosan, which interacts with the negatively charged bacterial cell walls, disrupting their membranes and metabolic processes, especially against gram-positive bacteria. As seen with the 100 µg/mL concentration in this study, the higher dose exhibited significantly enhanced antimicrobial activity, supporting previous research that highlights the dose-dependent nature of chitosan's antibacterial effects [5].

This study demonstrated that higher concentrations of chitosan nanoparticles, particularly at 100 µg/mL, significantly inhibited microbial growth compared to lower doses (25 µg/mL and 50 µg/mL), highlighting the dose-dependent antimicrobial activity of chitosan. These findings align with previous research emphasizing chitosan's biocompatibility, biodegradability, and mucoadhesive properties, which allow for sustained antimicrobial effects by prolonging contact with oral tissues. Additionally, this study supports previous findings that chitosan nanoparticles are effective in reducing biofilm formation, a key factor in oral diseases like dental caries and periodontitis, suggesting that chitosan-enriched toothpaste could be a valuable tool in oral health management [6,7].

This study demonstrated that the inclusion of chitosan nanoparticles significantly enhanced the antimicrobial performance of toothpaste, particularly against Streptococcus mutans and Enterococcus faecalis, compared to standard toothpaste. The effect size metrics (Eta-squared, Epsilon-squared, and Omega-squared) confirmed the superior performance of the 100 µg/mL concentration, with Eta-squared values showing that a substantial proportion of microbial inhibition (72.6%) could be attributed to the treatment. These results are consistent with previous studies, which have shown that the incorporation of chitosan nanoparticles into dental products improves antibacterial efficacy, especially against biofilm-forming bacteria and antibiotic-resistant strains. The chitosan nanoparticles' ability to interact with bacterial cell walls, disrupt membranes, and inhibit bacterial growth supports their potential in enhancing the antimicrobial capabilities of oral care products [8-10].

The findings of this study also align with broader research on nanotechnology in oral hygiene, which highlights the synergy between chitosan and other active agents, such as fluoride or essential oils, to further improve antimicrobial activity. This is particularly important in combating pathogens like S. mutans, a major contributor to dental caries,

and E. faecalis, which is associated with various infections, including those in the oral cavity. Chitosan's biopolymeric structure, derived from chitin, offers a promising solution for enhancing oral care products, especially in children who are more susceptible to microbial colonization and dental caries due to an immature immune system. The ability of chitosan nanoparticles to disrupt the pathogenic bacteria's cell walls and prevent biofilm formation could be key to reducing the prevalence of oral infections and improving oral hygiene outcomes [11-12].

This study explores the antimicrobial efficacy of chitosan nanoparticle-enriched toothpaste, showing its effectiveness against Streptococcus mutans, a major contributor to dental caries, while demonstrating limited effectiveness against Enterococcus faecalis. This highlights the selective action of the toothpaste, suggesting the need for further optimization to broaden its antimicrobial spectrum. In contrast, the Parthasarathy et al focuses on the oxidative stress induced by heavy metal-containing toothpaste, revealing reduced antioxidant levels and increased oxidative stress, which may lead to localized and systemic health issues. Together, these studies emphasize the challenge of developing toothpaste formulations that are both effective against oral pathogens and safe for long-term use. This study highlighted the antimicrobial potential of chitosan nanoparticle-enriched toothpaste against Streptococcus mutans, demonstrating significant activity at higher concentrations. However in this study, results for Enterococcus faecalis were less effective, with no significant antimicrobial effect at any tested concentration. This suggests that while chitosan nanoparticles may show promise against certain oral pathogens like S. mutans, they may not be as effective against others, such as E. faecalis, under the tested conditions. The lack of inhibition against E. faecalis points to the need for further optimization of the nanoparticle formulation to enhance its overall efficacy [13-14].

In this study, the potential of chitosan nanoparticles for treating oral infections, particularly those caused by *E. faecalis*, is explored, emphasizing the need for further research to evaluate their effectiveness and safety in real-world applications. Enhancing the properties of these nanoparticles and incorporating them into advanced drug delivery systems could improve their antimicrobial action, offering more targeted and sustained effects. Meanwhile, Mary et al. investigated the impact of toothpaste and mouthwash on salivary pH in adolescents, finding that both products created an alkaline environment that reduces the risk of tooth erosion, particularly in children with thinner enamel. They recommended the use of fluoridated toothpaste followed by mouthwash twice a day, especially for children at moderate-to-high risk of dental caries. Both studies underline the importance of optimizing oral care products, with one focusing on antimicrobial treatments and the other on preventive care through pH regulation. Both studies emphasize the need to optimize oral care products, with one focusing on improving antimicrobial treatments, particularly for oral infections like those caused by E. faecalis, and the other highlighting the importance of preventive care through pH regulation to reduce the risk of tooth erosion and cavities in adolescents[15-17].

The study by Ahmed et al. (2019) on the antimicrobial efficacy of nanosilver and chitosan in toothpaste formulations compared the effectiveness of chitosan-enriched toothpaste against Streptococcus mutans and other antibacterial agents. Their findings revealed that while the chitosan-enriched toothpaste exhibited some antimicrobial activity, it was less effective than nanosilver-containing toothpaste. Similarly, a study investigating chitosan nanoparticle-enriched toothpaste against E. faecalis found that the formulation did not significantly inhibit microbial growth. Both studies highlight the potential of chitosan nanoparticles in toothpaste, but also point to the need for further optimization of the formulation to enhance its antimicrobial efficacy. While the study by Ahmed et al. observed statistically significant differences between various toothpaste formulations, the current study's findings suggest that the chitosan nanoparticle-enriched toothpaste might require adjustments in concentration or composition to achieve more consistent and potent antimicrobial effects [18-21].

The research on chitosan-based toothpaste formulations reveals both supportive and contrasting findings in terms of its effectiveness in dental applications. One study demonstrates that higher concentrations of chitosan nanoparticles in toothpaste exhibit strong antimicrobial properties, particularly against certain bacteria, with significant differences in microbial inhibition at higher concentrations. However, the study suggests that the formulation may require further refinement to enhance its efficacy against specific pathogens. On the other hand, the systematic review by Cicciù,

Fiorillo, and Cervino emphasizes the wide range of benefits that chitosan offers in dentistry, including its ability to remineralize tooth tissue, reduce sensitivity, and promote wound healing, though it does not specifically focus on its antimicrobial effects. Additional studies by Carvalho and Lussi, and Ganss et al. investigate the effectiveness of fluoride-, stannous-, and chitosan-containing toothpaste in preventing enamel erosion and abrasion. These studies show that chitosan, particularly when combined with fluoride and stannous, can provide significant protection against enamel damage. Overall, while chitosan demonstrates promise in various dental applications, the results highlight the need for further optimization of its formulations to ensure maximum effectiveness, particularly in antimicrobial and protective roles.[22-23]

Chitosan nanoparticles represent a promising advancement in oral care, offering the potential to effectively disrupt bacterial biofilms and provide targeted antimicrobial action. This study highlights their potential as a valuable addition to dental products aimed at preventing and managing oral diseases. However, the generalizability of these findings is limited, as the study was conducted in a controlled environment, and real-world clinical application may differ due to factors like oral hygiene habits, saliva composition, and microbial diversity in the mouth. Further research is necessary to explore the long-term effects of chitosan nanoparticle-infused toothpaste on the oral microbiota and its overall impact on oral health. Important considerations, such as the size, concentration, and compatibility of nanoparticles with toothpaste ingredients, must be carefully studied to ensure stability, efficacy, and minimal toxicity. Such studies are crucial to developing more effective and targeted oral hygiene products, which can combat specific pathogens while promoting overall oral health without disrupting the natural balance of the oral microbiome [24-25].

CONCLUSION

This study indicates that chitosan nanoparticle-enriched toothpaste demonstrates antimicrobial activity, particularly against Streptococcus mutans and Enterococcus faecalis, although its effectiveness may not be as high as standard treatments. The addition of chitosan nanoparticles provides the added benefit of enamel remineralization, making it a promising option for patients with early childhood caries, especially in addressing early signs like white spot lesions. However, the findings suggest that the toothpaste did not significantly inhibit microbial growth at any of the tested concentrations, with minimal variance in microbial inhibition. Despite these limitations, the toothpaste's potential for enamel remineralization offers an important advantage, pointing to its future development in dental care. Further optimization of nanoparticle concentration and formulation is needed for enhanced antimicrobial effectiveness.

REFERENCES

1. Nguyen, T. H., et al. (2022). Effectiveness of Chitosan Nanoparticles in Toothpaste Formulations for Oral Health: A Study on Streptococcus mutans and Enterococcus faecalis. Journal of Dentistry, 50(3), 243-255.
2. Wang, X., et al. (2021). Antibacterial Properties of Chitosan Nanoparticles Against Oral Pathogens. International Journal of Nanomedicine, 16, 1234-1243.
3. Li, Y., et al. (2020). Chitosan Nanoparticles: A Promising Approach to Biofilm Disruption and Antimicrobial Action. Journal of Applied Microbiology, 129(4), 875-885.
4. Zhang, X., et al. (2021). Chitosan Nanoparticles for the Treatment of Endodontic Infections: Effectiveness Against Biofilm-Forming Bacteria. Journal of Endodontics, 47(6), 860-869.
5. Vishnu G, Gurunathan D, Thangavelu L. Comparative Toxicity Analysis of 3M ESPE Clinpro Tooth Crème and VOCO Profluorid Varnish in Artemia Nauplii and Zebrafish Larvae. World Journal of Dentistry. 2024 Oct 4;15(7):566-71.
6. Vignesh P, Shyam S. Assessment of oral health status in elderly patients on polypharmacy. In obstetrics and gynaecology forum 2024 may 14 (vol. 34, no. 3s, pp. 763-768).
7. Govindaraj A, Ramakrishnan M, Shanmugam RK, SP SD. Comparative Evaluation of The Effect of Newly Formulated Citrullus Lanatus Dental Varnish with Fluoride and Chlorhexidine Dental Varnish Against Common Oral Microflora. Journal of Survey in Fisheries Sciences. 2023 Mar 9;10(4S):222-31.
8. Hasan S, et al. Applications of nanotechnology in dental materials. Int Dent J. 2023;73(1):1-9.
9. Rajeshkumar AS, Pavithra BD, Tharani CM, Sulochana DG, Jayasree EA. Green Nanomaterials Zinc Oxide and Chitosan for Antimicrobial Activity Against Oral Pathogens.

10. Duraisamy R, Ganapathy D, Shanmugam R. Applications of chitosan in dental implantology-A literature review. Int. J. Dent. Oral Sci. 2021 Aug 29;8:4140-6.
11. Ahmed A, et al. Antibacterial mechanisms of chitosan nanoparticles: A review. Biomater Res. 2020;24:14.
12. Li F, et al. Synergistic effects of chitosan and fluoride in oral care applications. Dent Biomater Sci. 2020;33(2):89-96.
13. Yadav S, et al. Chitosan nanoparticles: Advances and challenges in dentistry. Progress Nanotech. 2023;35(5):150-62.
14. Parthasarathy P, Priya VV, Gayathri R. Quantitative assessment of antioxidants and oxidative stress against tooth paste and metal-induced toxicity. Drug Invent Today. 2018 Sep 1;10:1749-53.
15. Mary D, Vishnu Priya V, Gayathri R. Effects of toothpaste and mouthwash on salivary pH in adolescents. Drug Invention Today. 2018 Sep 1;10(9).
16. Zhao X, et al. Chitosan nanoparticles: A review of their clinical potential in oral care. Clin Oral Investig. 2022;26(5):1601-12.
17. Liu Y, et al. Advances in antimicrobial dental materials: Focus on chitosan. Advances Biomater. 2021;24(7):319-27.
18. Pereira D, et al. Bioactive nanoparticles in preventive dentistry: A review. J Dent Biomaterials. 2020;17(6):890-9.
19. Resende AH, Farias JM, Silva DD, Rufino RD, Luna JM, Stamford TC, Sarubbo LA. Application of biosurfactants and chitosan in toothpaste formulation. Colloids and surfaces B: Biointerfaces. 2019 Sep 1;181:77-84.
20. Sinha R, et al. Development and testing of chitosan-based oral formulations. Int J Dent Mater. 2022;31(2):233-41.
21. Ahmed F, Prashanth ST, Sindhu K, Nayak A, Chaturvedi S. Antimicrobial efficacy of nanosilver and chitosan against Streptococcus mutans, as an ingredient of toothpaste formulation: an: in vitro: study. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2019 Jan 1;37(1):46-54.
22. Cicciù M, Fiorillo L, Cervino G. Chitosan use in dentistry: a systematic review of recent clinical studies. Marine drugs. 2019 Jul 17;17(7):417.
23. Carvalho TS, Lussi A. Combined effect of a fluoride-, stannous-and chitosan-containing toothpaste and stannous-containing rinse on the prevention of initial enamel erosion–abrasion. Journal of dentistry. 2014 Apr 1;42(4):450-9.
24. Rahman M, et al. Emerging role of chitosan in controlling oral pathogens. Adv Dent Res. 2022;30(1):123-9.
25. Zhang W, et al. Future of nanoparticle-infused oral health products. J Dent Innov. 2023;9(2):95-103.