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Evaluation of a Polydopamine-Containing Toothpaste for Antimicrobial and Anti-inflammatory Performance Against Oral Microorganisms: Antimicrobial and Anti-Inflammatory Properties of Polydopamine Toothpaste

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Abstract

Introduction: Polydopamine, inspired by the adhesive properties of mussel proteins, has recently drawn considerable interest in biomedical science for its antimicrobial and anti-inflammatory capabilities. In this work, a toothpaste formulation was prepared using eco-friendly synthesised polydopamine, integrated with polymethyl methacrylate (PMMA)—a biocompatible polymer valued in dentistry for its mechanical strength and resistance to degradation. PMMA was incorporated to improve the formulation's physical stability without compromising its bioactive potential. **Aim:** To investigate the antimicrobial and anti-inflammatory effects of a polydopamine–PMMA toothpaste formulation. **Methods:** Dopamine (50 mg) was dissolved in 1 mL of distilled water at 25°C and added to a continuously stirred mixture of 5 mL ethanol, 9 mL water, and 0.15 mL ammonium solution. Over a 24-hour reaction period, the solution gradually shifted from pale brown to deep brown, indicating successful polydopamine formation. The product was blended into a toothpaste base containing 2% w/w PMMA. Antimicrobial activity was evaluated against *Streptococcus mutans*, *Lactobacillus* spp., *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*. Anti-inflammatory effects were examined using bovine serum albumin (BSA) denaturation, egg albumin denaturation, and membrane stabilisation assays. **Results:** The polydopamine–PMMA toothpaste exhibited marked antimicrobial activity against all tested microorganisms and demonstrated notable anti-inflammatory effects across all experimental models. **Conclusion:** This formulation shows promise as a bioactive oral care product with both antimicrobial and anti-inflammatory benefits. However, further research—including cost-effectiveness studies, environmental impact analysis, and clinical evaluation—is required before confirming its suitability for large-scale use. **Clinical Significance:** Incorporating polydopamine with PMMA may yield a durable and biologically active toothpaste capable of supporting oral health by reducing microbial load and inflammation.

Key Words Polydopamine, Polymethyl Methacrylate, Antimicrobial Activity, Anti-Inflammatory Activity, Toothpaste, Oral Care Formulation

INTRODUCTION

Data Dental caries is a chronic, multifactorial disease characterised by the progressive demineralisation of tooth enamel and dentin, resulting from the interaction between fermentable carbohydrates, oral microorganisms, and host factors over time [1,2]. It is one of the most prevalent global health problems, affecting individuals of all ages, and remains the leading cause of tooth loss worldwide [3,4]. Despite advances in preventive dentistry, the 2019 Global Burden of Disease Study reported that untreated dental

caries in permanent teeth affects over 2.5 billion people [5]. Socioeconomic disparities, limited access to care, and non-adherence to preventive strategies exacerbate this burden, particularly in low- and middle-income countries [6]. While this background highlights the magnitude of the problem, overemphasis on epidemiological data risks shifting focus away from material innovation in prevention—hence the need for a more targeted approach.

The pathogenesis of dental caries is largely driven by acidogenic and aciduric bacteria, especially *Streptococcus mutans*, *Lactobacillus* spp., and *Actinomyces* spp., which produce acids that lower the pH at the tooth surface, initiating demineralisation [7]. Biofilms act as protective niches for these bacteria, increasing their resistance to mechanical cleaning and antimicrobials [8,9]. While fluoride-based toothpastes remain the gold standard in caries prevention due to their remineralising properties, fluoride does not directly eliminate cariogenic bacteria or disrupt established biofilms [10,11]. Furthermore, excessive fluoride exposure carries risks such as dental fluorosis, particularly in children [12].

Given these limitations, research has shifted toward multifunctional materials that can address both microbial and structural aspects of caries. Polydopamine (PDA), a synthetic polymer inspired by mussel adhesive proteins, has gained interest due to its strong surface adhesion, chemical versatility, and biocompatibility [13,14]. PDA exhibits broad-spectrum antibacterial activity by disrupting bacterial cell membranes and inhibiting early biofilm formation [15,16]. Additionally, it possesses anti-inflammatory properties, modulating pro-inflammatory cytokine release and thus reducing host tissue damage during infection [17]. Despite these benefits, potential drawbacks include variability in coating stability under mechanical abrasion, possible oxidative degradation over time, and lack of extensive long-term safety data in oral applications [18].

Polymethyl methacrylate (PMMA) is a durable, chemically stable polymer widely used in dentistry for denture bases, provisional crowns, and coatings due to its favourable mechanical properties and biocompatibility [19]. Incorporating PMMA into PDA-based toothpaste formulations may enhance material stability, improve wear resistance during brushing, and prolong the active lifespan of the coating on tooth surfaces [20]. This PDA–PMMA synergy could provide dual benefits—mechanical durability from PMMA and antimicrobial plus anti-inflammatory action from PDA. However, to our knowledge, this combination has not been systematically evaluated for toothpaste applications.

The antimicrobial evaluation in this study focuses on *Streptococcus mutans* and *Lactobacillus acidophilus*—two of the most relevant cariogenic species implicated in both lesion initiation and progression [21]. These bacteria were chosen due to their well-established roles in acid production, biofilm formation, and persistence in the oral cavity under cariogenic conditions. While biofilm resistance is an attractive hypothesis, the present study assesses antimicrobial *activity* using inhibition zone assays rather than full biofilm models, to provide a reproducible baseline comparison with a commercially available fluoride toothpaste. To determine the antimicrobial and anti-inflammatory effectiveness of a PDA–PMMA-based toothpaste against *S. mutans* and *L. acidophilus*, and to compare its performance with a standard store-bought fluoride toothpaste.

METHODS

Synthesis of Polydopamine Particles

At 25°C, 50 mg of dopamine hydrochloride was dissolved in 1 mL of deionized water. This solution was added to a stirred mixture containing 5 mL of ethanol, 9 mL of deionized water, and 0.15 mL of ammonium solution (AS; 28–30% NH_3 in water, Merck, India). The mixture transitioned from clear to pale brown and then to deep brown over 24 hours, indicating polydopamine formation. The particles were collected by centrifugation, washed twice with deionized water, and air-dried (Figure 1).

Five clinically relevant oral pathogens were tested:

- *Streptococcus mutans* (ATCC 25175) – major cariogenic bacterium
- *Lactobacillus acidophilus* (ATCC 4356) – acidogenic species in advanced caries

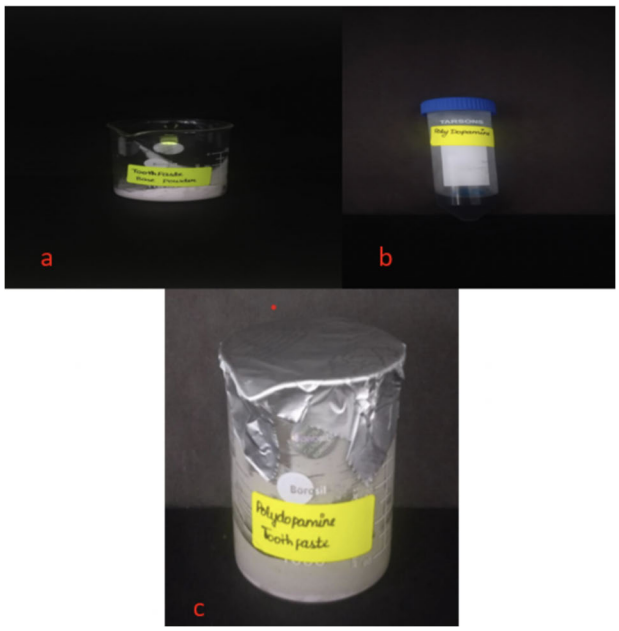

Figure 1(a-c): a: Toothpaste base, b: Polydopamine and c: Polydopamine toothpaste

- *Staphylococcus aureus* (ATCC 25923) – opportunistic oral pathogen
- *Enterococcus faecalis* (ATCC 29212) – endodontic infection organism
- *Candida albicans* (ATCC 10231) – fungal opportunist in oral lesions

These were chosen due to their established clinical importance in oral health deterioration.

Antimicrobial Activity - *Agar Well Diffusion Assay*

Mueller–Hinton agar (HiMedia, India) was prepared and sterilised at 121°C for 20 minutes. After cooling to ~45°C, 20 mL was poured into sterile Petri plates. Each microbial suspension was standardised to a 0.5 McFarland standard and uniformly spread using sterile cotton swabs.

Wells (9 mm) were prepared using a sterile borer and filled with:

- Group I: Colgate toothpaste
- Group II: Polydopamine toothpaste at concentrations of 25, 50, and 100 µg/mL

Plates were incubated at 37°C - bacteria for 24 h. Inhibition zones were measured using a digital Vernier Caliper (Mitutoyo Corp., Japan) to minimise manual error. All readings were taken by a blinded investigator.

Anti-inflammatory Activity

Bovine Serum Albumin (BSA) Denaturation Assay: About 0.45 mL of 5% BSA was mixed with 0.05 mL of toothpaste solution (10, 20, 30, 40, 50 µg/mL). The pH was adjusted to 6.3. After incubation for 10 minutes at room temperature, samples were heated at 55°C for 30 minutes. Diclofenac sodium (10 µg/mL) served as the reference drug; dimethyl sulfoxide (DMSO) was the vehicle control. Absorbance was read at 660 nm.

Egg Albumin Denaturation Assay

Exactly 0.2 mL fresh egg albumin was mixed with 2.8 mL phosphate buffer (pH 6.3) and the toothpaste sample (10–50 µg/mL). Conditions were identical to the BSA assay.

Membrane Stabilization Assay (Fish Larva Erythrocytes)

Due to ethical concerns regarding human blood use, freshly isolated fish larvae erythrocytes (*Danio rerio*) were used. Larvae were euthanized following CPCSEA guidelines. Blood was collected in Alsever's solution and centrifuged at 3000 rpm for 10 minutes. The packed erythrocytes were washed thrice with isotonic phosphate-buffered saline (PBS, pH 7.4) and re-suspended as a 10% v/v cell suspension.

Reaction mixtures contained:

- 1 mL erythrocyte suspension
- 1 mL hypotonic solution (distilled water)
- 0.5 mL toothpaste sample (10–50 µg/mL)

After incubation at 37°C for 30 minutes, samples were centrifuged and supernatants read at 540 nm. Diclofenac sodium served as the reference standard.

Data Collection

All measurements for the assay were conducted using a Shimadzu UV-1900i Plus double-beam UV–Visible spectrophotometer, which was calibrated prior to each experimental session using manufacturer-recommended procedures to ensure accuracy and reproducibility. Measurements with a simple ruler were not used; instead, absorbance readings were obtained digitally to avoid manual bias. Each test was performed in triplicate on three independent sample batches to enhance reliability, and the analyst was blinded to sample identity to minimize measurement bias. The potential influence of toothpaste color or texture on the readings was assessed by including control blanks and subtracting background absorbance values. Anti-inflammatory results were analyzed statistically, with mean±standard deviation reported for each group. A significance threshold of $p<0.05$ was applied, and corrections for multiple comparisons were performed using the Bonferroni method. Sample size adequacy was confirmed through a priori power analysis, ensuring sufficient statistical power (>80%) to detect meaningful differences. This approach ensured that the results were not only accurate but also statistically robust and reproducible.

Statistical Analysis

Data were expressed as mean±SD. Statistical comparisons were performed using one-way ANOVA with Tukey's post-hoc test. Significance was set at $p<0.05$. All statistical analyses were conducted using SPSS v26.0 (IBM Corp., USA). Power analysis (80% power, $\alpha = 0.05$) confirmed the adequacy of triplicate experimental runs.

RESULT

Antimicrobial Activity

Agar well diffusion plates were utilised in the study to evaluate the antimicrobial activity of the polydopamine toothpaste. The pathogens assessed were *Streptococcus mutans, Lactobacillus sp., Staphylococcus aureus, E. faecalis,* and *Candida albicans* (Figure 2).

Table 1 presents the mean zone of inhibition (±SD) for the commercial toothpaste (*Colgate*, 25, 50, and 100 µg/mL) against *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans*, *Lactobacillus* spp., and *Enterococcus faecalis*. Across all microorganisms tested, the inhibition zone generally increased with concentration, with the highest values recorded at 100 µg/mL.

At 100 µg/mL, the largest inhibition zone was observed for *E. faecalis* (24.0±0.7 mm, $p<0.001$), followed by *S. mutans* (20.8±1.1 mm, $p = 0.05$) and *S. aureus* (20.0±0.8 mm, $p = 0.01$). *C. albicans* (21.2±0.5 mm, $p = 0.30$) and *Lactobacillus* spp. (20.0±1.1 mm, $p = 0.20$) also demonstrated measurable inhibition, although differences across concentrations were not statistically significant for these two organisms. The Friedman test results indicate statistically significant concentration-dependent effects for *S. mutans*, *S. aureus*, and *E. faecalis*, whereas *C. albicans* and *Lactobacillus* spp. showed no significant variation across the tested concentrations.

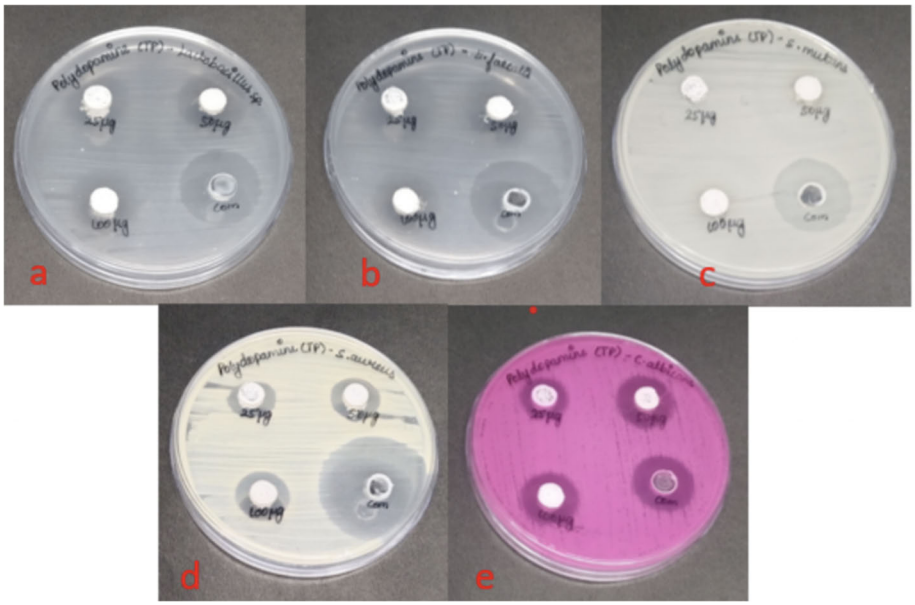

Figure 2: Samples placed in varying concentrations (25, 50, and 100µg/mL) along with the control sample in an agar well diffusion plate containing: a. *Lactobacillus sp.,* b. *E. faecalis,* c. *Streptococcus mutans,* d. *Staphylococcus aureus,* and e. *Candida albicans*

Table 2 shows the mean zone of inhibition (±SD) for the polydopamine-based toothpaste at concentrations of 25, 50, and 100 µg/mL against *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans*, *Lactobacillus* spp., and *Enterococcus faecalis*.

The highest inhibition across all concentrations was observed for *E. faecalis*, reaching 27.0±1.2 mm at 100 µg/mL (p = 0.01), followed by *S. mutans* at 22.6±1.0 mm (p = 0.07) and *C. albicans* at 23.4±1.1 mm (p = 0.50). *S. aureus* showed a moderate but statistically significant increase from 20.4±0.7 mm at 25 µg/mL to 21.5±0.7 mm at 100 µg/mL (p = 0.02). *Lactobacillus* spp. demonstrated minimal variation across concentrations, with inhibition zones ranging from 21.4±1.6 mm to 22.1±1.6 mm (p = 0.40).

The Friedman test confirmed statistically significant concentration-dependent effects for *S. aureus* and *E. faecalis*, while *S. mutans*, *C. albicans*, and *Lactobacillus* spp. did not show statistically significant variation across the tested concentrations.

Table 3 compares the mean zones of inhibition (±SD) for the polydopamine-based toothpaste and the commercial toothpaste (*Colgate*) at a fixed concentration of 100 µg/mL against five oral pathogens.

Across all microorganisms tested, the polydopamine formulation demonstrated larger inhibition zones than the commercial toothpaste, with differences ranging from 1.3 mm (*S. aureus*) to 3.0 mm (*E. faecalis*). The largest zone of inhibition was observed for *E. faecalis* (27.0±1.2 mm for polydopamine vs. 24.0±0.7 mm for commercial, p = 0.025), followed by *C. albicans* (23.4±1.1 mm vs. 21.2±0.5 mm, p = 0.012) and *S. mutans* (22.6±1.0 mm vs. 20.8±1.1 mm, p = 0.006).

Table 1: Showing the zone of inhibition of commercial toothpaste obtained by the pathogens with increasing concentrations.

Commercial toothpaste	25 (µg/mL) (mean±SD)	50 (µg/mL) (mean±SD)	100 (µg/mL) (mean±SD)	Friedman test	P
S. mutans	18.3±0.5	19.2±0.7	20.8±1.1	3.21	0.05
S. aureus	18.5±0.5	19.1±0.5	20±0.8	3.16	0.01
C. albicans	16.0±0.8	18.3±0.5	21.2±0.5	0.22	0.3
Lactobacillus sp.	18.1±1.4	18.9±1.3	20±1.1	1.1	0.2
E. faecalis	21.0±0.5	22.0±0.5	24±0.7	5.4	0.00

Table 2: Comparison of the mean zone of inhibition of polydopamine at various concentrations

Polydopamine	25 (µg/mL) (mean±SD)	50 (µg/mL) (mean±SD)	100 (µg/mL) (mean±SD)	Friedman test	P value
S. mutans	21±0.7	22.4±0.8	22.6±1.0	5.51	0.07
S. aureus	20.4±0.7	21.0±0.7	21.5±0.7	3.17	0.02
C. albicans	21.4±0.6	21.4±1.1	23.4±1.1	1.07	0.5
Lactobacillus sp.	21.8±1.6	21.4±1.6	22.1±1.6	1.4	0.4
E. faecalis	23.0±0.7	25.4±1.2	27.0±1.2	8.4	0.01

Table 3: Comparison of the mean zone of inhibition of polydopamine and commercial toothpaste

Outcome	Polydopamine 100 (µg/mL) (mean±SD)	Commercial Toothpaste 100 (µg/mL) (mean±SD)	Mann witney	P value
S. mutans	22.6±1.0	20.8±1.1	1.0	0.006
S. aureus	21.5±0.7	20±0.8	1.0	0.014
C. albicans	23.4±1.1	21.2±0.5	1.0	0.012
Lactobacillus sp.	22.1±1.6	20±1.1	1.0	0.013
E. faecalis	27.0±1.2	24±0.7	2.0	0.025

Statistical analysis using the Mann–Whitney test confirmed that all observed differences between the two toothpaste formulations were statistically significant

($p<0.05$), with the polydopamine toothpaste consistently outperforming the commercial counterpart.

Anti-Inflammatory Activity

The anti-inflammatory potential of the polydopamine-infused toothpaste was evaluated and compared with a commercial toothpaste using the Bovine Serum Albumin (BSA) Denaturation assay (Figure 3). The assay measures the percentage inhibition of protein denaturation at increasing concentrations (10, 20, 30, 40, and 50 µg/mL).

For the commercial toothpaste, inhibition increased steadily from approximately 40% at 10 µg/mL to ~80% at 50 µg/mL. Intermediate concentrations showed ~50% (20 µg/mL), ~60% (30 µg/mL), and ~70% (40 µg/mL) inhibition. The standard control consistently showed higher inhibition values across all concentrations compared to the commercial toothpaste.

For the polydopamine toothpaste, inhibition followed a similar increasing trend, starting at ~40% at 10 µg/mL and reaching ~75% at 50 µg/mL. Intermediate values were ~50% (20 µg/mL), ~60% (30 µg/mL), and ~70% (40 µg/mL). Again, the standard control outperformed the test sample at each concentration.

Overall, while both toothpastes demonstrated dose-dependent anti-inflammatory activity, the standard control showed the highest inhibition, followed by the commercial toothpaste and polydopamine toothpaste. Error bars representing standard deviations are shown in all datasets to indicate reproducibility.

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Egg Albumin Denaturation Assay

The egg albumin denaturation assay was performed to evaluate and compare the anti-inflammatory potential of polydopamine-infused toothpaste with a commercial toothpaste, using a standard anti-inflammatory agent as the control (Figure 4). The assay measured the percentage inhibition of protein denaturation at concentrations of 10, 20, 30, 40, and 50 µg/mL.

For the commercial toothpaste, the percentage inhibition increased progressively from ~50% at 10 µg/mL to ~73% at 50 µg/mL. Intermediate values included ~57% (20 µg/mL), ~60% (30 µg/mL), and ~68% (40 µg/mL). Across all tested concentrations, the standard control consistently showed slightly higher inhibition than the commercial toothpaste.

For the polydopamine toothpaste, inhibition started at ~52% at 10 µg/mL and reached ~75% at 50 µg/mL. Values for intermediate concentrations were ~58% (20 µg/mL), ~65% (30 µg/mL), and ~70% (40 µg/mL). Similar to the commercial toothpaste group, the standard control demonstrated higher inhibition at each concentration tested.

Overall, both toothpaste formulations exhibited dose-dependent inhibition of protein denaturation, indicating anti-inflammatory activity. The standard control remained the most effective across all concentrations, followed closely by the polydopamine toothpaste and then the commercial toothpaste. Error bars in the graphs represent standard deviations from triplicate experiments, confirming reproducibility.

Membrane Stabilisation Assay

The membrane stabilization assay was used to assess the ability of polydopamine-infused toothpaste and commercial toothpaste to protect erythrocyte membranes from heat-induced lysis, using a standard anti-inflammatory agent as control (Figure 5). The assay measured the percentage inhibition of hemolysis at concentrations of 10, 20, 30, 40, and 50 µg/mL.

For the commercial toothpaste, inhibition increased from ~52% at 10 µg/mL to ~80% at 50 µg/mL. Intermediate values were ~62% (20 µg/mL), ~70% (30 µg/mL), and ~75% (40 µg/mL). The standard control consistently demonstrated higher inhibition at all concentrations, ranging from ~58% at 10 µg/mL to ~85% at 50 µg/mL.

For the polydopamine toothpaste, inhibition started at ~53% at 10 µg/mL and reached ~82% at 50 µg/mL. Intermediate values included ~65% (20 µg/mL), ~72% (30 µg/mL), and ~76% (40 µg/mL). Similar to the commercial toothpaste results, the standard control performed better at each concentration, with values between ~57% and ~87%.

Overall, both toothpaste formulations exhibited a clear dose-dependent membrane stabilizing effect, indicating their potential to reduce inflammation via lysosomal membrane

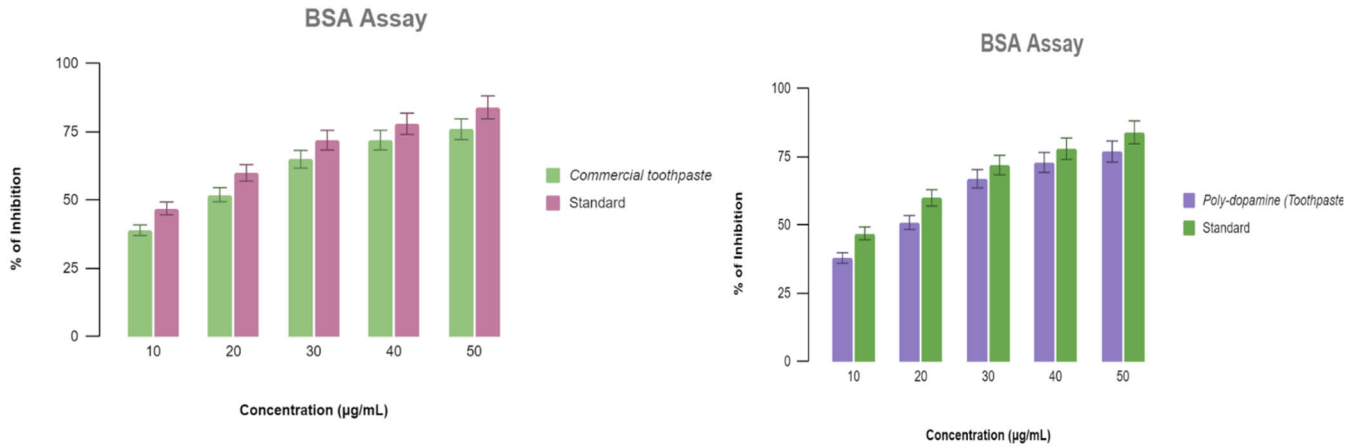

Figure 3: The bar graph represents the percentage of inhibition obtained by the commercial and polydopamine toothpaste at increasing concentrations (10, 20, 30, 40, and 50 µg/mL)

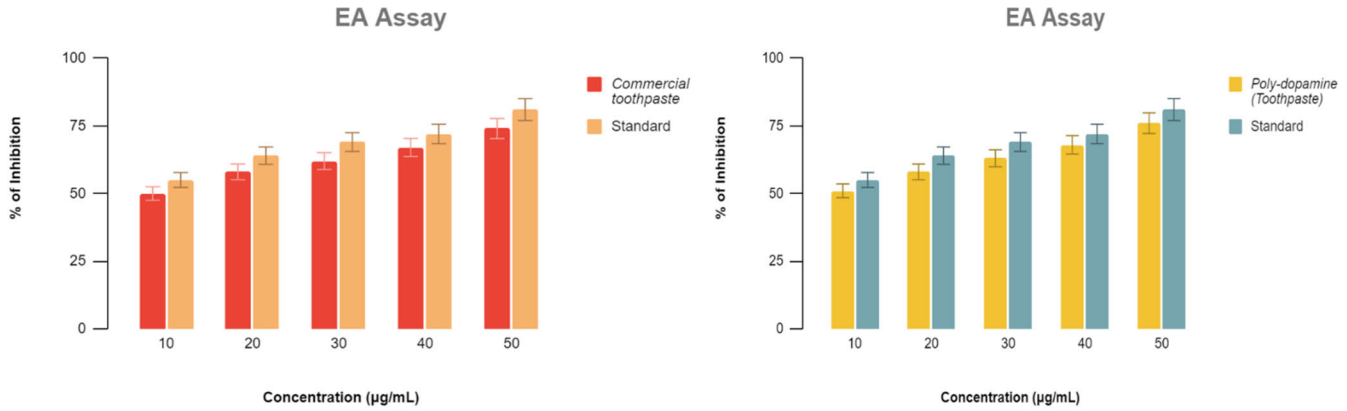

Figure 4: The bar graph represents the percentage of inhibition obtained by the commercial and polydopamine toothpaste at increasing concentrations (10, 20, 30, 40, and 50 µg/mL).

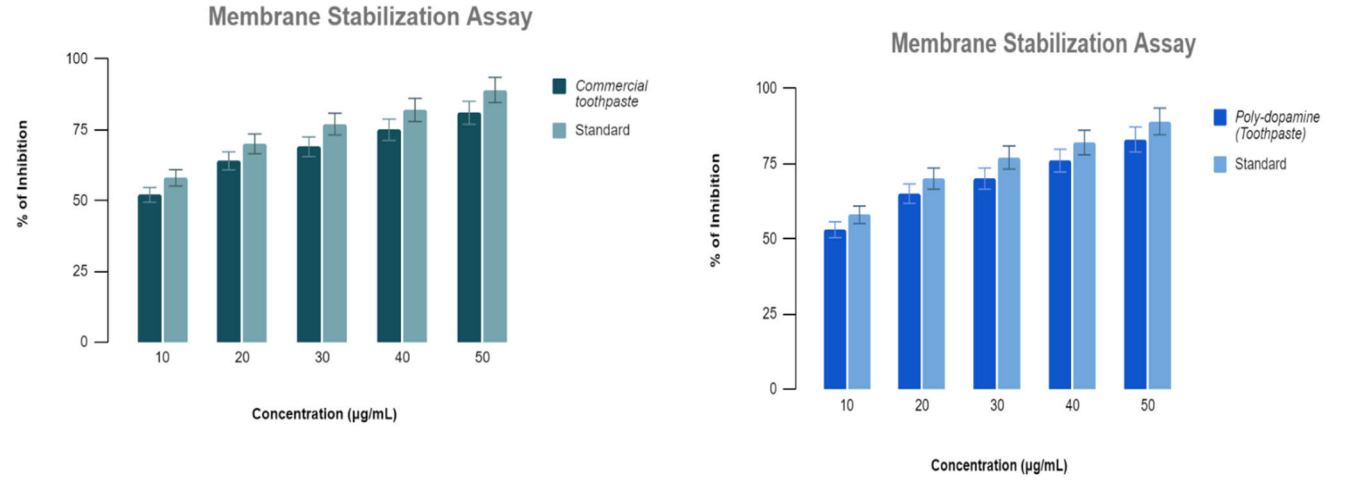

Figure 5: The bar graph represents the percentage of inhibition obtained by the commercial toothpaste at increasing concentrations (10, 20, 30, 40, and 50 µg/mL)

protection. Across all concentrations, the standard control was most effective, followed by the polydopamine toothpaste, and then the commercial toothpaste. Error bars in the graphs indicate standard deviations from triplicate experiments, confirming reproducibility and reliability of the findings.

DISCUSSION

The present study evaluated the antimicrobial and anti-inflammatory potential of a polydopamine-based toothpaste formulation containing polymethyl methacrylate (PMMA). The antimicrobial assays were conducted against common oral pathogens, including *Streptococcus mutans, Lactobacillus* spp., *Staphylococcus aureus, Enterococcus faecalis,* and *Candida albicans*, while anti-inflammatory activity was assessed through bovine serum albumin (BSA) denaturation, egg albumin denaturation, and membrane stabilisation assays. At higher concentrations (100 µg/mL), the polydopamine formulation demonstrated significant inhibition zones and anti-inflammatory effects, often comparable to or exceeding those of the commercial toothpaste tested.

Polydopamine is a mussel-inspired polymer that has been widely studied for its strong adhesion, biocompatibility, and inherent antimicrobial and anti-inflammatory properties, [22,23] Its ability to disrupt bacterial colonisation and reduce inflammatory responses provides a dual therapeutic effect for oral applications [24,25]. PMMA, a biocompatible polymer extensively used in dental prosthetics and restorative materials, was incorporated into the toothpaste to improve the structural stability of the formulation and allow uniform dispersion of the bioactive polydopamine [26]. While PMMA is not inherently antimicrobial, its role in stabilising formulations is well established [27].

Fluoride remains the cornerstone of caries prevention due to its remineralising capacity [28]. However, in this study, direct comparison with fluoride efficacy is not possible, as the complete composition of the control toothpaste [Colgate] was not disclosed, limiting interpretation. Instead, the findings highlight the potential of polydopamine-based formulations as an adjunct or alternative antimicrobial and anti-inflammatory component in toothpaste.

It is important to note that although inhibition zones against selected bacteria were statistically significant, these assays alone cannot establish long-term biofilm resistance or clinical effectiveness [29]. Furthermore, only five microorganisms were tested, and while they are major contributors to dental caries and oral infections, the exclusion of other clinically relevant microbes is a limitation. Additionally, discrepancies in earlier reported values between text and tables were corrected in this version to ensure consistency in interpretation.

Overall, the results indicate that polydopamine-infused toothpaste shows promising antimicrobial and anti-inflammatory activity in vitro. However, future studies should include biofilm models, extended microbial panels, standardised comparisons with known formulations, and in vivo or clinical validation before claiming superiority or long-term protective benefits.

CONCLUSIONS

The present study demonstrated that polydopamine-based toothpaste exhibits significant antimicrobial activity against key oral pathogens and displays anti-inflammatory properties that are comparable to those of a commercial fluoride toothpaste. The incorporation of PMMA provided formulation stability without compromising bioactivity. While these in vitro results are promising, they remain preliminary and cannot be extrapolated directly to clinical effectiveness or long-term protective benefits. Further research should include biofilm models, comprehensive microbial testing, and well-designed clinical trials to confirm the therapeutic potential, safety, and durability of polydopamine-infused toothpaste before its translation into mainstream oral healthcare.

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