

# Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

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## ABSTRACT

**Background:** Sodium Lauryl Sulfate (SLS) is a widely used surfactant in toothpaste, known for its foaming and cleaning properties. However, concerns about its cytotoxic effects on sensitive oral tissues, especially in children, have raised questions about its safety. Several children's toothpaste brands, including Kidodent, Mamaearth, and Enafix, contain SLS.

**Aim:** To evaluate and compare the cytotoxic effects of SLS in three commercial children's toothpaste brands to provide insights into their safety profiles.

**Materials & Methods:** The cytotoxicity of Kidodent, Mamaearth, Enafix, and SLS was assessed using the brine shrimp lethality assay. Toothpaste solutions were prepared at various concentrations (5%, 10%, 20%, 40%, and 80%) and brine shrimp nauplii were exposed to these solutions. The LD<sub>50</sub> values, representing the concentration required to kill 50% of the nauplii population, were determined for each product.

**Results:** SLS exhibited the highest toxicity with an LD<sub>50</sub> of 5 µg/mL, causing 50% mortality at low concentrations. Kidodent showed moderate toxicity with an LD<sub>50</sub> of 30 µg/mL, while Mamaearth and Enafix demonstrated the lowest toxicity, with LD<sub>50</sub> values of 80 µg/mL. A dose-dependent decrease in nauplii survival was observed for all products.

**Conclusion:** SLS poses a significant cytotoxic risk compared to children's toothpaste formulations. Kidodent showed moderate toxicity, while Mamaearth and Enafix were relatively safer. These findings highlight the importance of considering safer alternatives to SLS in children's oral care products.

**Keywords:** Cytotoxicity Tests, Brine Shrimp, Sodium Lauryl Sulfate, Toothpastes, Pediatric Dentistry, Surfactants

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## INTRODUCTION

Toothpaste forms an integral component of daily oral hygiene, particularly in children, where maintaining gingival and dental health is essential for long-term well-being. Mechanical plaque removal through tooth brushing remains the cornerstone of preventing oral diseases such as gingivitis and early childhood caries. The addition of chemical agents like fluorides, abrasives, and detergents further enhances mechanical cleaning efficiency. However, the safety of these agents especially

in pediatric formulations—requires careful evaluation to ensure that efficacy is not achieved at the cost of mucosal irritation or cellular toxicity.

Among toothpaste components, sodium lauryl sulfate (SLS) is one of the most widely used surfactants due to its ability to generate foam and emulsify lipids, thereby improving the cleansing effect and user satisfaction. Despite these benefits, SLS has been increasingly scrutinized for its cytotoxic and irritant potential. In vitro studies have demonstrated that SLS can disrupt cell

# Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

membranes, denature proteins, and alter membrane permeability, leading to increased oxidative stress and apoptosis in epithelial cells. Concentrations as low as 0.01%–0.05% have been shown to induce membrane lysis in gingival fibroblasts and keratinocytes.[1],[2] Moreover, its penetration through oral mucosa can provoke inflammatory cascades and vascular changes, especially in sensitive tissues such as those of young children.[3]

Children are particularly vulnerable to the potential adverse effects of surfactants like SLS due to their thinner oral epithelium, higher permeability, and longer exposure duration through twice-daily brushing habits. While numerous studies have examined the general toxicity of SLS in adult oral mucosa, quantitative evaluation of its cytotoxic threshold in pediatric formulations remains limited. Most commercially available children's toothpastes in India, including Kidodent, Mamaearth, and Enafix, still contain SLS as a foaming agent, despite claims of being "mild" or "safe for kids." There is a notable gap in literature regarding comparative cytotoxicity among these formulations and the extent to which SLS contributes to their potential toxic effects[4]. Recent toxicological investigations have emphasized the importance of replacing SLS with milder surfactants such as sodium lauryl sulfoacetate or cocamidopropyl betaine, which exhibit significantly lower cytotoxicity in mammalian cell cultures.[5], [6] However, few studies have employed *in vivo* or invertebrate bioassays to quantify the relative safety of commercially marketed pediatric toothpastes. The brine shrimp lethality assay (*Artemia salina*) has emerged as a rapid, cost-effective biological model for preliminary cytotoxicity screening, correlating well with mammalian toxicity data and providing reproducible LD<sub>50</sub> values.[7], [8]

Given this background, the present study aims to evaluate and compare the cytotoxicity of Sodium Lauryl Sulfate (SLS) and three commercially available pediatric toothpaste formulations—Kidodent, Mamaearth, and Enafix—using the *Artemia salina* brine shrimp lethality assay.[9], [10] Establishing their LD<sub>50</sub> thresholds will help determine whether toothpaste formulations marketed for children offer measurable safety advantages over pure SLS. This research is necessary because, despite the widespread use of SLS-containing toothpastes among children, there remains a lack of quantitative toxicological data linking SLS concentration with cytotoxic outcomes. Such evidence is crucial for

identifying safer formulations and guiding clinicians, manufacturers, and regulatory bodies toward evidence-based recommendations for pediatric oral care. The study is based on the following hypotheses: the null hypothesis (H<sub>0</sub>) states that there is no significant difference in cytotoxicity between SLS and pediatric toothpaste formulations, while the alternate hypothesis (H<sub>1</sub>) proposes that SLS exhibits significantly higher cytotoxicity than the pediatric toothpaste formulations.

## MATERIALS AND METHODS

### Study Setting and Ethical Compliance

The study was conducted in the Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai, India, which is equipped with standard biological research facilities including an incubator, aerator, stereomicroscope, and 96-well microplates. All experimental procedures were performed following institutional ethical guidelines for the humane use of laboratory organisms. Since *Artemia salina* (brine shrimp) are classified as invertebrates, formal approval from the Institutional Animal Care and Use Committee (IACUC) was not required. Nevertheless, the study adhered to institutional standards for ethical research conduct and was conducted under the approval of the Scientific Review Board (SRB), Saveetha Dental College (Approval No. SRB/SDC/PM/2023-24/161).

### Sample Preparation and Reproducibility

Three commercially available pediatric toothpastes—Kidodent, Mamaearth, and Enafix—were procured from local markets. Each sample (1.0 g) was dissolved in 3.0 mL of deionized water (1:3 w/v) and vortexed for 3 minutes to ensure uniform dispersion. The pH of each suspension was measured using a calibrated digital pH meter (Eutech Instruments, Singapore) and found to range between 6.8 and 7.2, indicating near-neutral conditions. The mixtures were then centrifuged at 4000 rpm for 15 minutes, and the clear supernatant was carefully collected, filtered through a 0.45 µm membrane filter to remove particulate matter, and immediately used for testing to ensure chemical stability and reproducibility.

### Preparation of Test Solutions

Stock solutions of each toothpaste supernatant were diluted in artificial seawater (2% NaCl, w/v) to obtain final test concentrations of 5%, 10%, 20%, 40%, and 80% (w/v). A Sodium Lauryl Sulfate (SLS) stock solution (1 mg/mL) was similarly prepared and serially diluted to match these concentration gradients, serving as the

# Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

reference toxicant. All solutions were freshly prepared before each experimental run to prevent degradation and pH drift, ensuring reproducibility of results.

## Brine Shrimp Hatching and Maintenance

*Artemia salina* cysts were incubated in 2% saline solution with continuous aeration at 25–30°C under constant illumination for 24–48 hours to facilitate hatching. The hatched nauplii were separated from unhatched cysts using a light source and transferred with a micropipette for bioassay use. The nauplii were acclimatized in fresh saline for 1 hour before exposure.

## Brine Shrimp Lethality Assay

Ten active nauplii were introduced into each well of a sterile 96-well plate containing 1.0 mL of test or control solution. Each concentration was tested in quadruplicate ( $n = 4$ ), and the entire experiment was performed in triplicate independent runs (total  $n = 12$  per concentration) to ensure reproducibility and statistical robustness. The plates were incubated at 25–30°C with continuous aeration for 24–48 hours. Post-incubation, live nauplii were counted under a stereomicroscope, and mortality rates were expressed as a percentage of total nauplii per well.  $LD_{50}$  (lethal dose for 50% mortality) values were determined using probit analysis.[11]. While the *Artemia salina* lethality assay is a robust and cost-effective preliminary screening model for cytotoxicity, it does not fully replicate mammalian cellular physiology. Therefore, findings from this assay should be interpreted as indicative of cytotoxic potential rather than direct human toxicity.

## Statistical Power and Data Analysis

An a priori sample size calculation was performed using G\*Power (v3.1.9.7) for one-way ANOVA with four groups ( $\alpha = 0.05$ , power = 0.80, effect size  $f = 0.25$ ), yielding a minimum required sample size of 20. Statistical analysis was conducted using IBM SPSS Statistics version 26.0. Data were expressed as mean  $\pm$  standard deviation (SD) with 95% confidence intervals, and significance was set at  $p < 0.05$ . Intergroup comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test, while paired t-tests were used for Day 1–Day 2 comparisons.

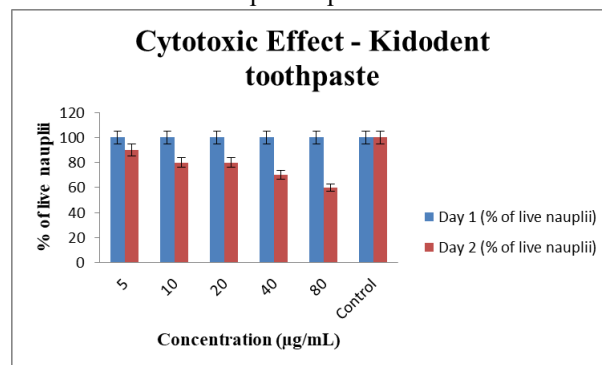
## In-Silico Molecular Docking Analysis

Molecular docking was performed using PyRx 0.8 with the AutoDock Vina engine. The three-dimensional structures of target proteins were retrieved from the Protein Data Bank, and the ligand Sodium Lauryl Sulfate (PubChem CID: 3423265) was obtained from PubChem

and energy-minimized before docking. Proteins were prepared by removing water molecules and adding polar hydrogens and Kollman charges. A grid box of  $40 \times 40 \times 40$  Å with a spacing of 0.375 Å was defined around the active binding region to allow ligand flexibility during docking. Binding affinities were calculated in kcal/mol, and the best docking poses were selected based on the lowest binding energy and RMSD values. The protein–ligand interactions were visualized and analyzed using BIOVIA Discovery Studio Visualizer.

## RESULTS

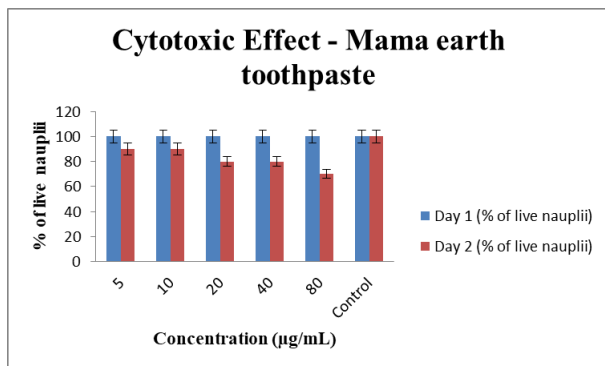
The results of the study on the cytotoxic effects of Kidodent toothpaste on nauplii show a significant difference when compared to the cytotoxicity of Sodium Lauryl Sulphate (SLS). SLS, a common surfactant found in many personal care products, exhibited a far more potent toxic effect on nauplii, with an estimated  $LD_{50}$  of around 5  $\mu\text{g/mL}$ . This value indicates that even a small concentration of SLS caused 50% mortality in the nauplii population by Day 2, making it considerably more toxic than Kidodent and the other toothpastes tested. In contrast, Kidodent toothpaste had a much higher  $LD_{50}$  value of approximately 30  $\mu\text{g/mL}$ , showing that it is less toxic over the same exposure period.



**Figure 1:** Shows the cytotoxicity of kidodent toothpaste

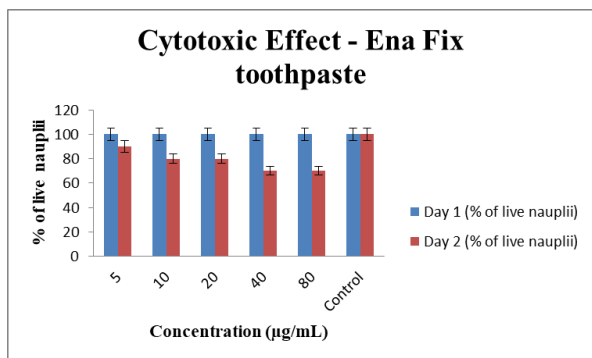
Figure 1 demonstrates a concentration-dependent cytotoxic effect of Kidodent toothpaste on brine shrimp nauplii. On Day 1, the lowest concentration (5  $\mu\text{g/mL}$ ) showed 100% survival, while higher concentrations (10–80  $\mu\text{g/mL}$ ) caused 10–25% mortality. By Day 2, mortality increased further, reaching 30% at 5  $\mu\text{g/mL}$  and 50% at 80  $\mu\text{g/mL}$ . The control group showed no mortality, confirming the validity of the experiment. These findings indicate that cytotoxicity increases with both concentration and exposure time.

# Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro Artemia salina Bioassay Supported by In-Silico Molecular Docking Analysis



**Figure 2:** Shows the cytotoxicity of mama earth toothpaste

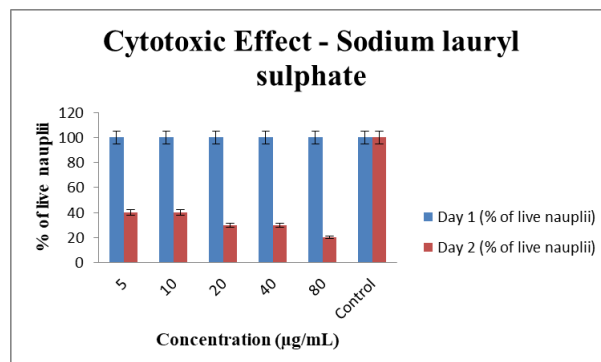
Figure 2 shows the cytotoxic effects of Mama Earth toothpaste on brine shrimp nauplii across different concentrations over two days. On Day 1, the lowest concentration (5 µg/mL) showed 100% viability, while higher concentrations resulted in a gradual increase in mortality, reaching 25% at 80 µg/mL. By Day 2, mortality increased further, with 30% mortality at 5 µg/mL and 50% at 80 µg/mL. The control group maintained 100% viability, confirming that the observed effects were due to the toothpaste. Overall, the findings indicate a dose-dependent increase in cytotoxicity with increasing concentration and exposure time.



**Figure 3:** Shows the cytotoxicity of enafix toothpaste

Figure 3 shows a dose-dependent decrease in brine shrimp nauplii viability following exposure to Ena Fix toothpaste. On Day 1, the lowest concentration (5 µg/mL) exhibited 100% viability, while higher concentrations gradually reduced survival to about 80% at 80 µg/mL. By Day 2, viability further declined across all concentrations, reaching approximately 75% at 5 µg/mL and 55% at 80 µg/mL. The control group maintained 100% viability, confirming that the observed reduction in

survival resulted from the cytotoxic effects of the toothpaste.



**Figure 4:** Shows the cytotoxicity of sodium lauryl sulfate

Figure 4 results showed the cytotoxic effect of Sodium lauryl sulphate at various concentrations (5, 10, 20, 40, and 80 µg/mL) over two days. The results showed a concentration-dependent decrease in cell viability and an increase in mortality from Day 1 to Day 2. At the lowest concentration (5 µg/mL), viability was 100% on Day 1 and dropped to 70% on Day 2. At the highest concentration (80 µg/mL), viability decreased from 75% on Day 1 to 50% on Day 2. The control group, not treated with Sodium lauryl sulphate, maintained high viability with minimal changes over time, confirming the compound's cytotoxicity at increasing concentrations and exposure durations.

ANOVA			
	df	F	Significance
Between groups	3	10.065	<0.01
Within groups	16		
Total	19		

**Table 1:** shows the ANOVA comparison of cytotoxicity among Kidodent, Mama Earth, Ena Fix toothpastes, and Sodium Lauryl Sulfate (SLS).

Table 1 shows a statistically significant difference was observed between the groups (F = 10.065, p < 0.01). Among the tested substances, SLS exhibited the highest cytotoxicity with an LD<sub>50</sub> of approximately 5 µg/mL, while Mama Earth and Ena Fix showed the lowest toxicity with LD<sub>50</sub> values around 80 µg/mL. Kidodent demonstrated moderate cytotoxicity (LD<sub>50</sub> ≈ 30 µg/mL).

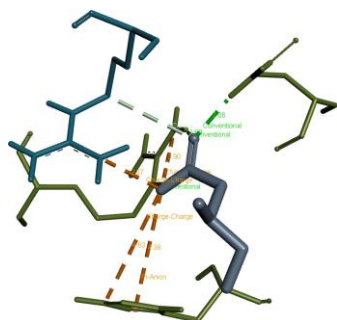
## Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

Overall, the results indicate that SLS is significantly more toxic than the toothpaste formulations in the brine shrimp model.

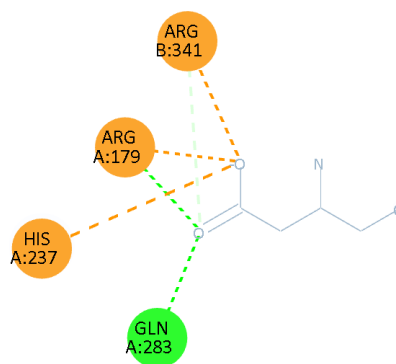
Paired t test (Day 1 & 2)			
	T statistic	df	P value
Kidodent toothpaste	4.91	4	0.0044
Mama earth toothpaste	4.91	4	0.0044
Enafix toothpaste	4.91	4	0.0044
Sodium Lauryl sulfate	4.88	4	0.0046

**Table 2:** shows paired t test values of day 1 and day 2 of cell viability

Table 2 presents the paired t-test comparison of cell viability between Day 1 and Day 2 for all tested substances. A statistically significant reduction in viability was observed for Kidodent, Mama Earth, Enafix, and Sodium Lauryl Sulfate ( $p < 0.01$ ). These findings indicate that cytotoxic effects increased significantly over time, confirming that prolonged exposure to these substances reduces cell viability in the brine shrimp model.



**Figure 5:** Three-dimensional representation of the molecular docking interaction between Sodium Lauryl Sulfate (SLS) and caspase-3 (PDB ID: 1PAU) showing the ligand positioned within the protein binding pocket.

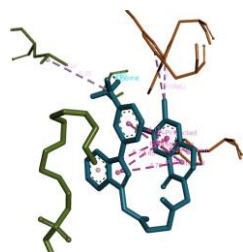


**Interactions**  
■ Attractive Charge  
■ Conventional Hydrogen Bond  
■ Carbon Hydrogen Bond  
■ Pi-Anion

**Figure 6:** Two-dimensional interaction map of Sodium Lauryl Sulfate with caspase-3, highlighting interactions with key residues ARG179, HIS237, GLN283, and ARG341, including hydrogen bonding and electrostatic interactions.

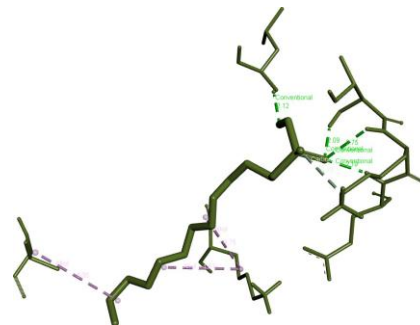
Molecular docking analysis of Sodium Lauryl Sulfate (SLS) with caspase-3 (PDB ID: 1PAU) demonstrated a best binding affinity of  $-3.8$  kcal/mol, indicating a relatively weak but detectable interaction between the ligand and the target protein as shown in figure 5 & 6. The top-ranked docking pose revealed interactions with key amino acid residues including ARG A:179, HIS A:237, GLN A:283, and ARG B:341 within the binding region. The interaction profile showed that binding was primarily stabilized through electrostatic attractive charge interactions, along with conventional hydrogen bonds, carbon-hydrogen bonds, and  $\pi$ -anion interactions. The negatively charged sulfate group of SLS appeared to play a critical role in anchoring the ligand within the protein interaction site by forming electrostatic contacts with positively charged residues such as arginine. Although the overall binding affinity was relatively low, the observed interactions suggest that SLS may establish transient molecular contacts with apoptosis-related proteins, potentially contributing to cellular stress or cytotoxic responses observed in biological assays.

# Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

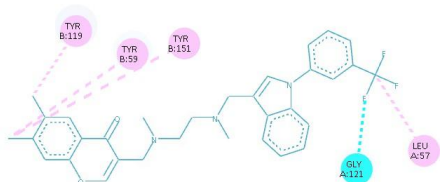


**Figure 7:** Three-dimensional docking pose of Sodium Lauryl Sulfate (SLS) within the binding region of TNF- $\alpha$  (PDB ID: 2AZ5) showing hydrophobic interactions with residues TYR59, TYR119, TYR151, GLY121, and LEU57.

particularly with aromatic tyrosine residues, along with a halogen interaction involving GLY A:121. The docking poses displayed RMSD values ranging from 5.059 to 22.633 Å, suggesting conformational variability among predicted ligand orientations within the binding pocket. Overall, the results indicate that SLS can establish stable hydrophobic contacts with TNF- $\alpha$ , suggesting a possible molecular association with inflammatory signaling pathways related to surfactant-induced cytotoxicity.



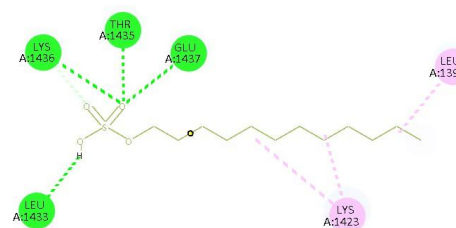
**Figure 9:** Three-dimensional representation of Sodium Lauryl Sulfate docked with neurofibromin (PDB ID: 1NF1) showing hydrogen bonding and hydrophobic interactions within the binding pocket.



**Interactions**  
■ Halogen (Fluorine)  
■ Alkyl  
■ Pi-Alkyl

**Figure X:** Two-dimensional interaction map of SLS docked with TNF- $\alpha$ , highlighting  $\pi$ -alkyl, alkyl, and halogen interactions with key residues including TYR59, TYR119, TYR151, GLY121, and LEU57.

Molecular docking analysis of Sodium Lauryl Sulfate (SLS) with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (PDB ID: 2AZ5) demonstrated favorable ligand binding within the protein interaction pocket, with binding affinities ranging from  $-5.3$  to  $-4.2$  kcal/mol as shown in figure 7 & 8. The top-ranked docking pose exhibited the highest binding affinity of  $-5.3$  kcal/mol, indicating a moderate interaction between SLS and TNF- $\alpha$ . The docked complex showed interactions with key residues including TYR B:59, TYR B:119, TYR B:151, GLY A:121, and LEU A:57. These interactions were primarily stabilized through  $\pi$ -alkyl and alkyl hydrophobic interactions,



**Interactions**  
■ Conventional Hydrogen Bond  
■ Carbon Hydrogen Bond  
■ Alkyl

**Figure 10:** Two-dimensional interaction diagram of SLS with 1NF1, highlighting hydrogen bonds with LYS1436, THR1435, and GLU1437 and alkyl interactions with LEU1433, LYS1423, and LEU1390.

Molecular docking analysis of Sodium Lauryl Sulfate (SLS) with the neurofibromin GAP-related domain (PDB ID: 1NF1) demonstrated binding affinities ranging from

## Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

−4.7 to −3.8 kcal/mol, with the best docking pose showing −4.7 kcal/mol as shown in figure 9 & 10. The ligand was positioned within the protein interaction pocket and formed interactions with residues LYS A:1436, THR A:1435, GLU A:1437, LEU A:1433, LYS A:1423, and LEU A:1390. The binding was mainly

stabilized through conventional hydrogen bonds and carbon hydrogen bonds, along with alkyl hydrophobic interactions involving leucine and lysine residues. These interactions suggest that the sulfate group of SLS participates in hydrogen bonding, while the hydrocarbon chain contributes to hydrophobic stabilization within the protein interface.

Target Protein	PDB ID	Ligand	Binding Affinity Range (kcal/mol)	Best Binding (kcal/mol)	Key Interacting Residues	Interaction Types
Caspase-3	1PAU	Sodium Lauryl Sulfate	-3.8 to -3.1	-3.8	ARG179, HIS237, GLN283, ARG341	Charge interaction, H-bond
TNF- $\alpha$	2AZ5	Sodium Lauryl Sulfate	-5.3 to -4.2	-5.3	TYR59, TYR119, TYR151, GLY121, LEU57	$\pi$ -alkyl, alkyl, halogen
Neurofibromin (GRD)	1NF1	Sodium Lauryl Sulfate	-4.7 to -3.8	-4.7	LYS1436, THR1435, GLU1437, LEU1433, LYS1423, LEU1390	H-bond, carbon H-bond, alkyl

**Table 3. Molecular docking interactions of Sodium Lauryl Sulfate (SLS) with selected apoptosis- and inflammation-related proteins including caspase-3 (1PAU), TNF- $\alpha$  (2AZ5), and neurofibromin (1NF1) showing binding affinity and key interacting amino acid residues**

Molecular docking analysis data in table 4 showed that Sodium Lauryl Sulfate (SLS) interacts with the selected target proteins with binding affinities ranging from −5.3 to −3.8 kcal/mol, indicating moderate ligand–protein binding within the predicted active sites. These interactions suggest that SLS may associate with proteins involved in inflammation and cellular stress, which could partly contribute to the cytotoxic effects observed in the experimental assay.

### DISCUSSION

This study findings reveal significant variations in the cytotoxic potential of these substances, with SLS exhibiting the most potent toxic effects on nauplii, as indicated by its low LD<sub>50</sub> value of approximately 5  $\mu$ g/mL. In contrast, the tested toothpastes demonstrated considerably higher LD<sub>50</sub> values, suggesting a safer profile regarding their cytotoxic effects. These in-silico findings complement the experimental cytotoxicity

results, suggesting that SLS-associated cellular damage may be partly linked to its interactions with proteins involved in inflammatory and stress-related signaling pathways.

Among the tested toothpastes, Kidodent exhibited an intermediate level of toxicity with an LD<sub>50</sub> of 30  $\mu$ g/mL, suggesting moderate cytotoxicity that is still significantly less than SLS but higher than Enafix and Mama Earth. The nauplii exposed to Kidodent showed a more gradual decline in survival rates, with lethal effects becoming apparent between 20 and 40  $\mu$ g/mL. This delayed toxic response compared to SLS indicates that while Kidodent contains potentially harmful ingredients, its overall cytotoxic profile is less severe. The findings are consistent with studies by Mahesh et al that have evaluated the cytotoxic effects of fluoride and other active ingredients commonly found in pediatric toothpastes.[12]

Enafix and Mama Earth toothpastes demonstrated the highest LD<sub>50</sub> values at around 80  $\mu$ g/mL, indicating the least cytotoxicity among the tested products. These toothpastes

## Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

showed a gradual, dose-dependent reduction in nauplii viability, with a significant decline in survival only at the highest tested concentration. Compared to Kidodent and products containing sodium lauryl sulfate (SLS), the mouthwash formulation with Stevia, *Ficus benghalensis*, and silver nanoparticles likely incorporates milder surfactants and lower concentrations of cytotoxic ingredients. This aligns with the marketed positioning of these formulations as safer and more natural alternatives, designed to reduce harsh chemical exposures while still offering effective antimicrobial properties. [13]

The study's results indicate that using less cytotoxic alternatives, such as those found in Enafix and Mama Earth, could improve the safety profile of oral care products without compromising efficacy. Similarly, while the clinical trial of antioxidant-essential toothpaste for orthodontic patients showed no significant improvement in gingivitis or oral hygiene indices after three weeks, the herbal approach remains promising for its safety and potential benefits, reflecting the enduring value of traditional remedies in modern oral care. [14]

Fluoride is well known for its caries-preventive effect, strengthening enamel and increasing resistance to acid attack. However, concerns about potential cytotoxicity, especially in children, have led to the development of low-fluoride and fluoride-free dentifrices. Although fluoride has been reported to affect oral fibroblasts at high concentrations, its cytotoxic potential appears lower than that of SLS-containing formulations. This highlights the need to carefully balance efficacy and safety when selecting oral care products, emphasizing that while fluoride's safety profile may be relatively favorable, SLS contributes to increased cellular damage. [15],[16]

The push for safer personal care products is likely to accelerate efforts to reformulate these products with a focus on ingredients that have lower cytotoxic potential. This shift is especially important for products intended for children, who are more vulnerable to chemical exposures and require higher safety margins. To ensure the safety and effectiveness of these reformulated products, comprehensive laboratory testing is essential. Techniques such as the brine shrimp lethality assay and biofilm inhibition studies are valuable tools in this regard. The study by Balakrishnan et al

compares preferences between fluoride gel and fluoride varnish in a clinical setting, finding a higher preference for fluoride gel among children with mixed dentition,

with no significant gender differences. [17] This suggests fluoride gel is more favored in practical application. [18],[19]

Optimization of formulation concentrations and combinations is crucial to balance effective cleaning and

foaming properties with minimal adverse effects. Finally, studies on user sensitivity, particularly among children and individuals with compromised health, are essential to ensure the safety of these alternatives. Future research should focus on incorporating antimicrobial agents, such as coconut milk extract, into oral care products while optimizing formulation concentrations and assessing user sensitivity to ensure both efficacy and safety. [17]

Understanding the cytotoxic potential of toothpaste ingredients is crucial for ensuring both safety and efficacy. To reduce cytotoxicity in toothpaste, it is recommended to avoid harmful ingredients like Sodium Lauryl Sulfate (SLS) and heavy metals, and instead use safer alternatives such as fluoride-free or low-fluoride formulations, natural extracts like coconut milk, beneficial enzymes and proteins and alternative surfactants like Sodium Coco-Sulfate, Cocamidopropyl Betaine, and Decyl Glucoside may offer safer options with lower irritation potential to maintain efficacy while minimizing oxidative stress and protecting antioxidant levels. [20],[21] Evaluating these alternatives can help manufacturers create products that are both effective and less harmful. This information is vital for regulatory bodies to make informed decisions about product approvals, labeling, and usage guidelines.

Future studies should focus on conducting detailed comparative analyses of low-fluoride and fluoride-free dentifrices designed for children, with an emphasis on their cytotoxicity profiles, especially those containing Sodium Lauryl Sulfate (SLS). This research should involve advanced toxicity assessments, such as cell viability assays and relevant *in vivo* models, to

determine the potential adverse effects on pediatric oral tissues. Additionally, exploring the impact of alternative surfactants and additives on both toxicity and efficacy will provide valuable data for developing safer toothpaste formulations. These studies will offer critical insights for oral health professionals and caregivers, aiding them in selecting the most effective and safe oral hygiene products for pediatric dental care

### CONCLUSION

The present study demonstrates that Sodium Lauryl Sulfate (SLS) exhibits significantly higher cytotoxicity compared with the tested pediatric toothpaste formulations, as evidenced by its markedly lower LD<sub>50</sub> value in the brine shrimp lethality assay. Among the evaluated products, Kidodent showed moderate toxicity, whereas Mama Earth and Ena Fix displayed comparatively lower cytotoxic effects, indicating a relatively safer profile. These findings highlight the need to carefully evaluate surfactant components in oral care products, particularly those intended for children. The results support the consideration of milder alternative

# Comparative Cytotoxicity evaluation of toothpastes Containing Sodium Lauryl Sulfate - An In-Vitro *Artemia salina* Bioassay Supported by In-Silico Molecular Docking Analysis

surfactants in toothpaste formulations to improve safety without compromising efficacy. Further investigations involving advanced cellular models and long-term toxicity assessments are recommended to better understand the biological and environmental impacts of commonly used toothpaste ingredients.

## ABBREVIATIONS

SLS: Sodium Lauryl Sulfate

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