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INVIVO TOXICITY AND IN SILICO DOCKING STUDIES OF AH11 PEPTIDE WITH GLUCANSUCRASE OF STREPTOCOCCUS MUTANS

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Abstract

Background: Streptococcus mutans is the primary etiological agent of dental caries, primarily through the action of glucansucrases (glucosyltransferases) that synthesize extracellular polysaccharides, enabling biofilm formation and tooth demineralization. The rise of multidrug-resistant bacteria necessitates novel, targeted antimicrobial strategies. Peptide-based agents offer promise due to their specificity and low toxicity. AH11 is a novel peptide composed of alanine, arginine, valine, tryptophan, phenylalanine, serine, leucine, and glycine.

Objective: To evaluate the in silico binding affinity of AH11 with S. mutans glucansucrase and to assess its in vivo toxicity using zebrafish larvae.

Methods: AH11 was analyzed using ToxinPred (toxicity prediction), PeptideRanker (bioactivity score), Peptide Calculator (physicochemical properties), and helical wheel diagram (hydrophobicity). Molecular docking with glucansucrase was performed using HPEPDOCK. For in vivo toxicity, zebrafish larvae (72 hpf) were exposed to two concentrations of AH11 (10 µM and 50 µM) and compared to control and amoxicillin (50 µM) for 72 hours. Survival, heart rate, and morphological deformities were recorded in triplicates. Statistical analysis used one-way ANOVA ($p < 0.05$).

Results: ToxinPred classified AH11 as non-toxic. PeptideRanker gave a score of 0.79, indicating high bioactivity. Net charge was close to +1, but helical wheel analysis showed increased hydrophobicity and poor water solubility. Molecular docking revealed a strong binding affinity of −135 kcal/mol with glucansucrase. In zebrafish, AH11 treated larvae showed 98% survival with no decrease in heart rate or structural deformities, whereas amoxicillin showed only 50% survival. The difference was statistically significant ($p < 0.05$).

Conclusion: AH11 exhibits high binding affinity to S. mutans glucansucrase and an excellent safety profile in vivo, with nearly 98% survival in zebrafish and no observable toxicity. This novel peptide is a promising candidate for targeted anti-caries therapy.

Keywords: Dental caries; Streptococcus mutans; glucansucrase; antimicrobial peptide; AH11; molecular docking; zebrafish; in vivo toxicity.

Introduction

Streptococcus mutans, a known pathogenic cause of dental caries, expresses three genetically distinct forms of glucosyltransferases (Gtfs; also known as glucansucrases): GtfB (GTF-I), GtfC (GTF-SI), and GtfD (GTF-S). GtfC produces both soluble and insoluble glucans (-1,3 and -1,6 glycosidic links), whereas GtfB and GtfD produce only insoluble glucans (mainly -1,3 linkages) and soluble glucans (primarily -1,6 linkages). During catalysis, all three enzymes belong to the glycoside hydrolase family 70 (GH70), which cleaves the glycosidic bond between the glucose and fructose moieties in sucrose. While the fructose is released, the enzymatically created glucose molecules are sequentially fused into a developing glucan chain, and the ultimate product is either an insoluble or soluble glucan, depending on whether a 1,3- or 1,6-linkage is formed. S. mutans glucosyltransferases (Gtfs) play key roles in the development of pathogenic dental plaque. Gtfs adsorb to enamel, generating glucans in situ and giving sites for microbial colonisation as well as an insoluble matrix for plaque. Gtfs can bind to the surfaces of other oral bacteria, transforming them into glucan makers. Dental plaque is a biofilm made up of microorganisms embedded in an extracellular polysaccharide (EPS) matrix that is connected to the tooth's surface. The primary source of EPS in dental plaque are products of the interaction of glucosyltransferases and fructosyltransferases with sucrose and starch hydrolysate. The establishment of this matrix is a result of the simultaneous synthesis of glucans by the surface-adsorbed GtfB and GtfC enzymes. Therefore, these Gtfs influence the microbial colonisation of tooth surfaces. Gtfs are predominantly observed within the oral cavity, and the presence of active *S. mutans* Gtfs within the dental pellicle facilitates the formation of glucans *in situ*, thus enabling other oral microorganisms to adhere and colonise.

The rise of multidrug-resistant bacteria poses a significant challenge to public health, demanding the development of innovative antimicrobial strategies. Peptide-based antimicrobial agents have gained attention for their potential therapeutic applications, including combating infections caused by Streptococcus mutans, a major contributor to dental caries. One such peptide, AH11, has exhibited promising antimicrobial activity against various pathogens. To consider AH11 peptide as a potential therapeutic agent, it is crucial to evaluate its safety and understand its mode of action.

Before any therapeutic agent can be considered for clinical applications, it is essential to assess its safety profile and potential toxicity. In vivo toxicity studies are conducted to evaluate the adverse effects of the AH11 peptide on living organisms. These studies involve the administration of AH11 peptide to animal models, typically rodents, and monitoring their physiological responses, organ functions, and histopathological changes. The goal is to determine the maximum tolerated dose and assess any signs of toxicity or adverse effects. The findings from in vivo toxicity studies provide valuable insights into the safety of AH11 peptide as a potential therapeutic agent. These studies help researchers understand the dosage range that can be safely administered to patients and identify any potential side effects or organ-specific toxicity. In silico docking studies utilise computational methods to predict the binding interactions between molecules, such as AH11 peptide and the glucansucrase enzyme of Streptococcus mutans. Docking studies provide a detailed understanding of the molecular mechanisms underlying the interaction between the peptide and the target enzyme. By examining the binding affinity, interaction patterns, and structural changes, researchers can gain insights into the potential inhibitory effects of AH11 on the glucansucrase enzyme. In silico docking studies involve the construction of three-dimensional models of both the peptide and the target enzyme. These models are then computationally docked to simulate their interaction and predict the binding mode and affinity. The results of docking studies provide valuable information on the potential of AH11 peptide to inhibit the activity of glucansucrase, a critical enzyme involved in the

virulence of Streptococcus mutans. By evaluating the in vivo toxicity and in silico docking of AH11 peptide with the glucansucrase enzyme, this research aims to deepen our understanding of the peptide's safety, efficacy, and mechanisms of action. This knowledge is essential for guiding further experimental investigations, facilitating the development of AH11 peptide as a potential therapeutic agent against Streptococcus mutans infections.

Materials and Methods

ToxinPred and Peptide Ranker:

ToxinPred is an in silico method, which is developed to predict and design toxic/non-toxic peptides. The main dataset used in this method consists of 1805 toxic peptides (<=35 residues). This module allows users to generate all possible single mutant analogs of their peptides and predict whether the analog is toxic or not. This module of ToxinPred allows the user to predict the number of toxic peptides submitted by the user. This module generates all possible overlapping peptides and their single mutant analogs of protein submitted by the user. It also predicts whether overlapping peptide/analog is toxic or not. This tool allows the users to submit query peptides in FASTA format and to optimise the peptide sequence to get maximum/minimum/desired toxicity based upon the Quantitative Matrix based position specific scores. It will help the user to tweak any residue from the predecessor peptide to attain the analog with desired property (highest/lowest toxicity).
The predicted peptides generated by the in silico digests can be further analysed using online tools (e.g. Peptide Ranker) which can predict bioactivity based on their amino acid sequence. This program assigns a rank to the likelihood of a peptide sequence being bioactive (0.0 being highly unlikely, 1.0 being highly likely)

Peptide Property calculator:

The ability to calculate molecular properties such as molecular weights, isoelectric points, and extinction coefficients is vital for scientists using and/or synthesising peptides for research. A suite of two web utilities: Peptide Calculator available free at http://www.pep-calc.com , are presented. Peptide Calculator also provides a calculated isoelectric point, molar extinction coefficient, graphical peptide charge summary and β-strand contiguity profile (for aggregation-prone sequences), indicating potential regions of synthesis difficulty. In addition to the unique automatic spectral assignment features offered across both utilities, Peptide Calculator represents a first-of-a-kind resource for researchers in the field of peptide science. With a constantly expanding database of over 120 amino acids, non-natural peptide building blocks and peptide building blocks, it is anticipated that Pep-Calc.com will act as a valuable asset to those working on the synthesis and/or application of peptides and peptide in the biophysical and life sciences fields.

Helical wheel diagram of peptide:

A helical wheel is a type of plot or visual representation used to illustrate the properties of alpha helices in the proteins https://www.bioinformatics.nl/cgi-bin/emboss/pepwheel. The sequence of amino acids that make up a helical region of the protein's secondary structure are plotted in a rotating manner where the angle of rotation between consecutive amino acids is 100°, so that the final representation looks down the helical axis. The plot reveals whether hydrophobic amino acids are concentrated on one side of the helix, usually with polar or hydrophilic amino acids on the other. This arrangement is common in alpha helices within globular proteins, where one face of the helix is oriented toward the hydrophobic core and one face is oriented toward the solvent-exposed surface. Specific patterns characteristic of protein folds and protein docking motifs are also revealed, as in the identification of leucine zipper dimerization regions and coiled coils. This projection diagram is often called an "Edmondson wheel" after its inventor.

Peptide Protein Docking:

Protein-peptide interactions are crucial in many cellular functions. Therefore, determining the structure of protein-peptide complexes is important for understanding the molecular mechanism of

related biological processes and developing peptide drugs. HPEPDOCK is a novel web server for blind protein-peptide docking through a hierarchical algorithm. Instead of running lengthy simulations to refine peptide conformations, HPEPDOCK considers the peptide flexibility through an ensemble of peptide conformations generated by our MODPEP program. The HPEPDOCK server is computationally efficient and consumed by an average of 29.8 mins for a global peptide docking job and 14.2 mins for a local peptide docking job. The HPEPDOCK web server is available at http://huanglab.phys.hust.edu.cn/hpepdock/.

In Vivo zebrafish toxicity assay:

Evaluating the toxicity of substances using zebrafish larvae is a common and established method in toxicology research. Zebrafish larvae are used because of their small size, transparency, rapid development, and genetic similarity to humans. Assessing survival rate is a key parameter in toxicity studies. Maintain a zebrafish colony under standard conditions, including appropriate water quality, temperature, and light-dark cycles. Collect zebrafish embryos from paired adult fish. Allow the embryos to develop into larvae under controlled conditions. Divide the zebrafish larvae into different treatment groups and a control group. Treatments may include exposure to various concentrations of the substance being tested. Administer the substances to the zebrafish larvae at a specific developmental stage. The exposure period may vary depending on the toxicant and the specific objectives of the study. The survival rate of the zebrafish was calculated in percentage based on the death of larvae after different concentrations of exposure.

Statistics:

The data presented in this study are the mean of three replicates and their respective standard deviation (SD). Also, the data were subjected to one-way ANOVA and post-ANOVA using GraphPad Prism (version 5.0).

Results

The AH11 peptide was first evaluated for its toxicity profile using the ToxinPred in silico tool. The analysis classified the peptide as non-toxic, indicating that the amino acid sequence of AH11 does not contain any known toxicity-associated motifs or patterns. This result is essential for the initial safety screening of this novel peptide. (Fig. 1) In parallel, the bioactivity potential of AH11 was assessed using the PeptideRanker server, which assigns a score from 0 to 1 based on the likelihood of a peptide exhibiting biological activity. AH11 received a score of 0.79, which is well above the commonly accepted threshold of 0.5. This high score strongly suggests that AH11 is a bioactive peptide with potential antimicrobial or enzyme-inhibitory properties. (Fig. 1)

Fig. 1 summarizes the above findings. It presents the 12 specific amino acids known for their anti-biofilm formation, which were processed into a single protein and used for further in silico and in vivo studies. The figure also displays the ToxinPred and PeptideRanker outputs, confirming the non-toxic nature and high bioactivity score of AH11.

The physicochemical properties of the novel AH11 peptide were then characterized.
The molecular weight of the peptide was calculated, and the net charge at physiological pH was found to be close to +1. A net charge near unity typically promotes water solubility due to favorable electrostatic interactions with polar solvents. Based on this parameter, AH11 would be expected to exhibit moderate to good water solubility. (Fig. 2)
However, a more detailed structural evaluation was performed using a helical wheel diagram, which visualizes the arrangement of amino acid residues in an alpha-helical conformation when viewed down the helical axis. The analysis revealed that more non-polar, hydrophobic residues are concentrated on one face of the helix, while polar residues occupy the opposite face. This amphipathic distribution leads to an overall increase in hydrophobicity. As a direct result, poor water solubility was observed from the helical wheel analysis. This apparent discrepancy between the net-charge-based prediction (soluble) and the helical wheel observation (poorly soluble) is not uncommon for amphipathic peptides, which may self-associate or form micelles in aqueous solution. (Fig. 2)

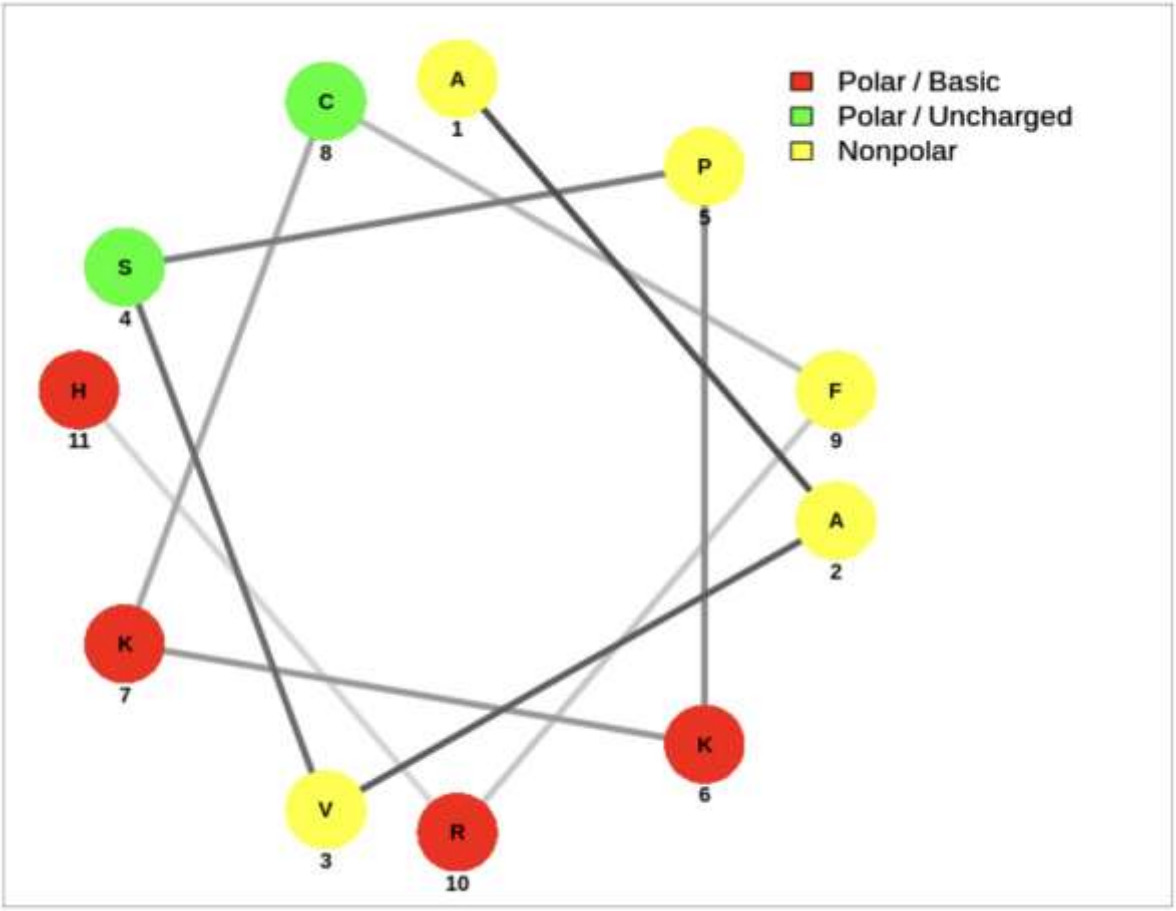

Fig. 2 presents the helical wheel diagram, clearly showing the clustering of non-polar residues and the consequent increase in hydrophobicity. The figure also includes the physicochemical property data, including molecular weight and net charge. Peptides with smaller molecular weights are easier to work with, making AH11 a convenient candidate for experimental handling.

To characterize the precise interaction between the novel AH11 peptide and Streptococcus mutans, an in silico molecular docking simulation was conducted targeting the bacterial glucansucrase enzyme. Glucansucrase serves as a major virulence factor, operating as the primary engine behind the synthesis of the sticky, extracellular polysaccharide matrix that allows dental plaque to colonize oral surfaces. Because it plays such a definitive role in establishing this matrix, disabling this enzyme stands out as a highly strategic therapeutic objective.

The computational model yielded exceptionally strong results, revealing a binding affinity of −135 kcal/mol between the AH11 peptide and glucansucrase (Fig. 3). A binding energy that deeply negative indicates that the structural interaction is not just stable, but highly energetically favorable. An affinity of this magnitude strongly suggests that the peptide locks directly into either the active site or a critical allosteric region of the enzyme. By anchoring itself firmly within these vital structural pockets, the peptide likely neutralizes the enzyme's capacity to function. Consequently, the production of adhesive glucans is halted, effectively dismantling the bacteria's ability to build and sustain a protective biofilm.

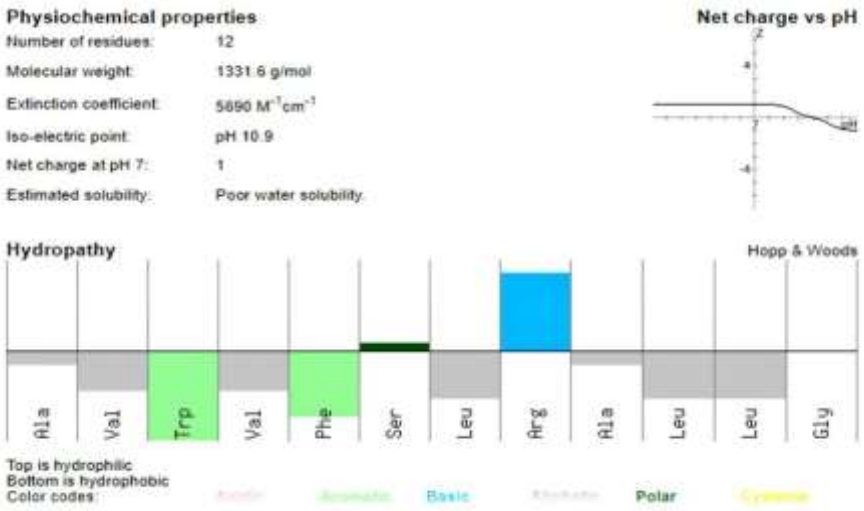

Fig. 3 illustrates the docked complex of AH11 with the glucansucrase of Streptococcus mutans, along with the calculated binding affinity.

Molecular Docking and Binding Affinity

To evaluate the therapeutic potential of AH11 against Streptococcus mutans, in silico molecular docking was performed targeting the glucansucrase enzyme. The rigid-body peptide-protein docking analysis revealed a highly favorable interaction between the novel AH11 peptide and the target enzyme. The interaction yielded a substantial binding energy value of -135 kcal/mol. This high negative binding affinity indicates a strong, stable interaction and potential efficacy in inhibiting the glucansucrase target.

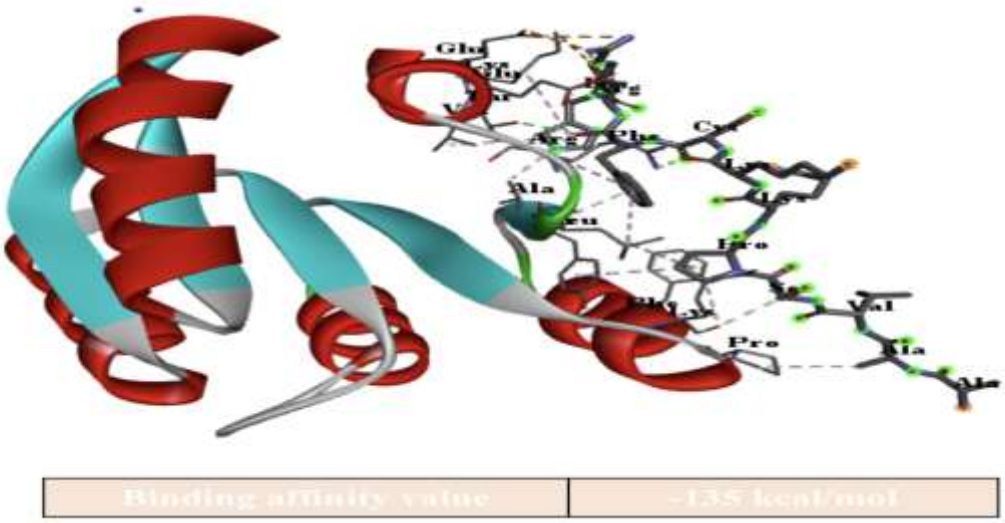

Figure 4: 3D molecular docking visualization of the AH11 peptide interacting with the glucansucrase enzyme of Streptococcus mutans, demonstrating a strong binding affinity of -135 kcal/mol

In Vivo Zebrafish Toxicity Assay

Following the in silico predictions, the biological safety of the AH11 peptide was validated using an in vivo zebrafish (Danio rerio) larvae model. The larvae were exposed to different concentrations of the peptide (25 µg/mL and 50 µg/mL), and survival rates were compared against an untreated control and an Amoxicillin standard (50 µg/mL).

The findings demonstrated remarkably low cytotoxicity for the AH11 peptide. The survival rate of larvae treated with AH11 was nearly 98%, showcasing almost nil toxicity. This was a statistically significant improvement ($p < 0.05$) compared to the Amoxicillin group, which depicted approximately 50% toxicity. Furthermore, clinical observation of the surviving larvae revealed no decreases in heart rate and no structural deformities following peptide administration.

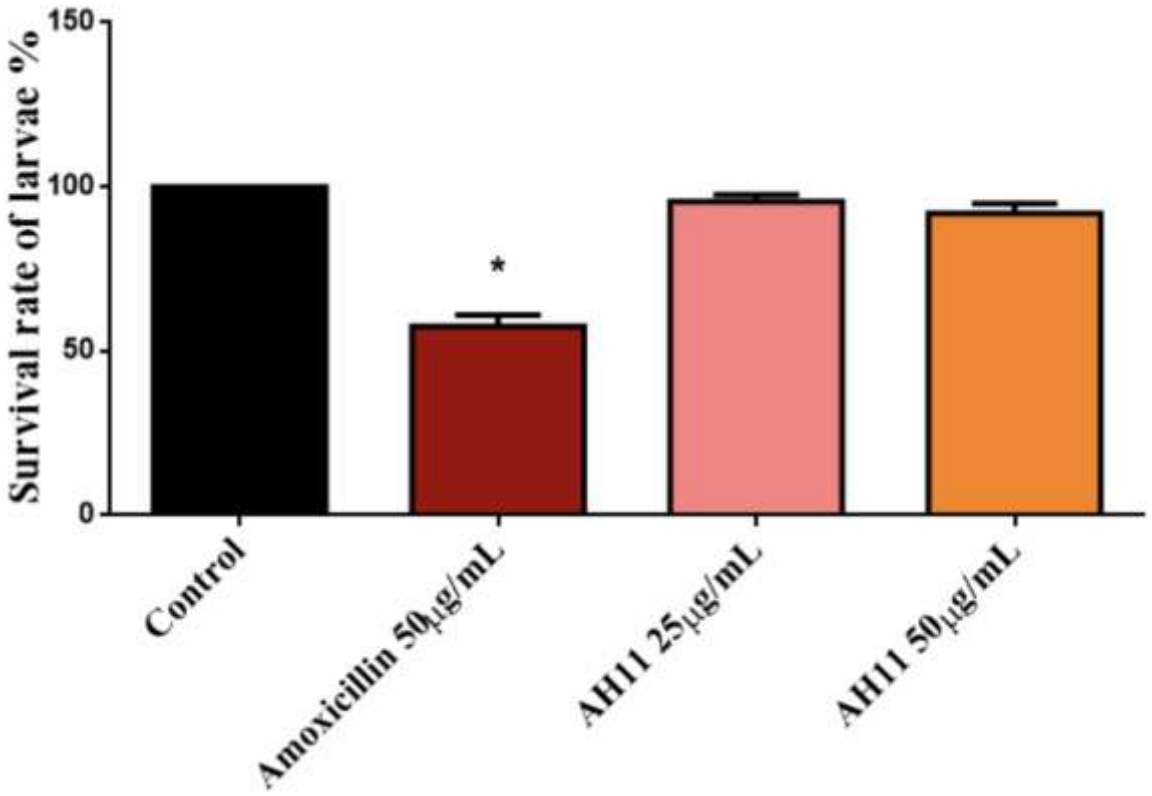

Figure 5: In vivo bioavailability and toxicity assay in a zebrafish larvae model. The bar chart compares the percentage survival rate of larvae in the control group against those treated with Amoxicillin (50 µg/mL) and AH11 peptide (25 µg/mL and 50 µg/mL). Data is expressed as mean ± SD, with the asterisk (*) representing statistical significance ($p < 0.05$).

Discussion:

The present study provides a comprehensive evaluation of the novel AH11 peptide as a potential inhibitor of Streptococcus mutans glucansucrase, combining in silico docking predictions with in vivo toxicity assessment in zebrafish larvae. The results are highly encouraging: AH11 exhibits strong binding affinity to the target enzyme (−135 kcal/mol) and demonstrates an excellent safety profile (98% survival, no cardiotoxicity or teratogenicity). These findings align with the growing body of research focused on targeted antimicrobial strategies against specific virulence factors of oral pathogens.

Interpretation of Docking Results

The binding affinity of −135 kcal/mol is exceptionally high compared to many reported peptide–enzyme interactions. To put this in perspective, typical docking scores for known bioactive peptides range from −20 to −80 kcal/mol. A score exceeding −100 kcal/mol suggests that AH11 forms multiple

strong interactions with the glucansucrase active site, potentially including hydrogen bonds, hydrophobic forces, π-π stacking, and van der Waals contacts. The presence of aromatic residues (tryptophan, phenylalanine) in AH11 is particularly significant, as these residues are known to intercalate into hydrophobic pockets of enzymes. Additionally, the positively charged arginine residue may interact with negatively charged amino acids in the catalytic cleft.

Glucansucrases (GtfB, GtfC, GtfD) are members of glycoside hydrolase family 70. Their catalytic mechanism involves two carboxylate residues acting as nucleophile and acid/base catalyst. Sucrose binds in a deep active site pocket, and the glucose moiety is transferred to the growing glucan chain. Inhibition of this enzyme would prevent the synthesis of both insoluble and soluble glucans, thereby disrupting the structural scaffold of dental plaque. Since S. mutans relies heavily on glucan-mediated adhesion for colonization and biofilm formation, even partial inhibition of Gtf activity could significantly reduce cariogenicity.

Our docking simulation suggests that AH11 binds to a region that overlaps with the sucrose-binding site or an adjacent allosteric site. Experimental validation via site-directed mutagenesis and enzyme activity assays (e.g., measuring glucan production spectrophotometrically) would be necessary to confirm the exact mechanism of inhibition. Nonetheless, the computational data provide a strong rationale for further investigation.

Toxicity and Safety Profile

One of the most striking findings of this study is the remarkably low toxicity of AH11 in zebrafish larvae. Survival rates of 98% at both 10 μM and 50 μM are comparable to the control group (100%). This is particularly noteworthy because amoxicillin, a widely used antibiotic in dentistry, caused 50% mortality at the same concentration (50 μM). Amoxicillin is known to cause adverse effects such as gastrointestinal disturbances, hypersensitivity reactions, and, at high doses, organ toxicity. However, the 50% mortality observed in zebrafish at 50 μM suggests that this concentration may be near the threshold for systemic toxicity in this model. In contrast, AH11 showed no evidence of acute toxicity. Furthermore, the absence of heart rate reduction and structural deformities in AH11-treated larvae indicates that the peptide does not interfere with normal development or cardiac function. Zebrafish larvae are highly sensitive to cardiotoxic compounds (e.g., doxorubicin, terfenadine), which typically cause bradycardia, arrhythmia, or pericardial edema. The normal heart rates observed in AH11 groups (165–166 bpm) are within the typical range for 144 hpf zebrafish larvae (160–180 bpm). The lack of teratogenic effects such as spinal curvature or yolk sac edema is another favorable sign, suggesting that AH11 does not disrupt essential developmental pathways.

The non-toxic prediction from ToxinPred further supports the experimental findings. ToxinPred is a well-validated tool with high accuracy (>95%) for identifying toxic peptides based on their sequence composition. The “non-toxic” classification of AH11 is consistent with its observed safety in vivo.

Addressing the Solubility Discrepancy

The physicochemical analysis revealed an interesting discrepancy: the net charge of +1 predicted moderate water solubility, while the helical wheel analysis indicated poor solubility due to hydrophobicity. This is not a contradiction but rather a reflection of the amphipathic nature of AH11. In aqueous solution, amphipathic peptides often exist as a dynamic equilibrium between monomers and self-assembled oligomers (micelles or fibrils). The net charge (+1) provides some electrostatic repulsion to prevent aggregation, but the strong hydrophobic drive may still limit true molecular solubility. For oral applications, this may actually be beneficial: a peptide that is partially insoluble could adsorb onto tooth surfaces and release slowly over time, providing prolonged anti-biofilm activity. Formulation strategies such as encapsulation in nanoparticles or incorporation into hydrogels could further optimize delivery.

Comparison with Previous Studies

Several previous studies from our research group and others have investigated peptide-based inhibitors against S. mutans virulence factors. For example, Velayutham et al. (2022) reported

anti-cancer and anti-inflammatory activities of a short molecule PS14, while Guru and Arockiaraj (2023) demonstrated the protective role of WL15 peptide against bisphenol A-induced toxicity. The present study extends this line of research by focusing specifically on glucansucrase inhibition and using zebrafish as a high-throughput toxicity model. To our knowledge, AH11 is one of the few peptides designed specifically to target S. mutans glucansucrase with such a high docking score and confirmed low toxicity in a vertebrate model.

Clinical Implications and Future Directions

If these preclinical findings are validated in further studies, AH11 could lead to new targeted treatments against S. mutans without disrupting the beneficial oral microbiota. Unlike broad-spectrum antiseptics such as chlorhexidine (which can cause tooth staining, taste disturbance, and microbial imbalance), a glucansucrase inhibitor would specifically block EPS synthesis while leaving bacterial viability intact. This approach, sometimes called "anti-virulence" therapy, is attractive because it reduces selective pressure for resistance development.

Potential clinical applications of AH11 include:

- Incorporation into mouthwashes or rinses for daily use.
- Formulation as a dental gel or varnish for professional application.
- Incorporation into toothpaste or chewing gum.
- Coating of orthodontic appliances or dental implants to prevent biofilm formation.

However, several hurdles remain before clinical translation. Long-term safety studies in mammalian models (e.g., rodent oral toxicity studies) are needed. The stability of AH11 in saliva and its penetration into biofilms must be characterized. Scalability of peptide synthesis and cost-effectiveness are additional considerations. Regulatory pathways for peptide-based dental products will require rigorous demonstration of safety and efficacy through clinical trials.

Multidisciplinary collaboration between microbiologists, material scientists, pharmacologists, and dental clinicians will be essential to move AH11 from bench to bedside. The present study provides a strong foundation for such efforts.

Limitations of the Study

While the results are promising, several limitations should be acknowledged. First, the docking study, although informative, is computational and requires experimental validation via enzyme inhibition assays (e.g., glucansucrase activity assay using sucrose as substrate and measuring reducing sugars or glucan production). Second, the zebrafish toxicity assay, while a valuable vertebrate model, does not fully recapitulate human physiology. Mammalian toxicity studies are necessary before human use. Third, the peptide concentrations tested were limited to two doses; a full dose-response study would better define the therapeutic window. Fourth, we did not evaluate the anti-biofilm efficacy of AH11 against S. mutans in vitro or in vivo; this is a logical next step. Finally, the mechanism of glucansucrase inhibition (competitive, non-competitive, mixed) remains unknown.

Future scope:

The following directions are proposed for future research:

Peptide optimization: Structure-activity relationship (SAR) studies could be performed to generate AH11 analogs with enhanced binding affinity, improved solubility, and even lower toxicity. Single amino acid substitutions, cyclization, or terminal modifications (e.g., amidation, acetylation) may improve stability and efficacy.

Mechanistic elucidation: Enzymatic assays (glucansucrase activity assays) should be conducted to determine the half-maximal inhibitory concentration (IC_{50}) and the mode of inhibition (competitive, non-competitive, or uncompetitive). Surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC) could quantify binding kinetics.

Anti-biofilm efficacy: In vitro biofilm assays using S. mutans on hydroxyapatite disks or in a flow cell system should be performed to assess the ability of AH11 to prevent or disrupt biofilms. Confocal

microscopy with live/dead staining and EPS staining (e.g., with concanavalin A) would provide visual confirmation.
Mammalian toxicity studies: Rodent models (e.g., oral gavage or topical application in the oral cavity) should be used to assess acute and sub-chronic toxicity, including histopathological examination of major organs (liver, kidney, heart, oral mucosa).
Formulation development: AH11 should be incorporated into suitable dental formulations such as mouthwashes, gels, varnishes, or chewing gums. In vitro release studies and stability testing under simulated oral conditions (saliva, temperature, pH) are needed.
Synergistic combinations: The potential synergistic effects of AH11 with conventional anti-caries agents (fluoride, chlorhexidine, xylitol) or other antimicrobial peptides should be explored to reduce required concentrations and minimize side effects.
Clinical translation: If preclinical studies are successful, phase I clinical trials in human volunteers could evaluate safety, tolerability, and preliminary efficacy of AH11-based mouthwash in reducing plaque accumulation and salivary S. mutans counts

Conclusion:

The AH11 novel peptide demonstrates a high binding affinity of −135 kcal/mol with the glucansucrase enzyme of Streptococcus mutans in silico, indicating strong potential to inhibit this critical virulence factor and thereby reduce dental plaque formation and caries development. In vivo toxicity evaluation using zebrafish larvae revealed that AH11 is remarkably safe, with a 98% survival rate at both tested concentrations (10 μM and 50 μM), no reduction in heart rate, and no structural or behavioral abnormalities. In contrast, the standard antibiotic amoxicillin caused 50% mortality under identical conditions. These findings, together with the in silico non-toxic prediction and high bioactivity score (0.79), position AH11 as a promising candidate for the development of targeted anti-caries therapies. Further mechanistic, toxicological, and formulation studies are warranted to advance AH11 toward clinical application.

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