Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 16, Issue 4, 2026, 105
https://doi.org/10.33263/BRIAC164.105

Polystyrene Nanoparticles Attenuate Brevinin-1 E8.13-induced Cytotoxicity and Alter Nanoparticles-Peptide Interactions In Human Lung Epithelial Cells

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Received: 25.04.2026; Accepted: 28.05.2026; Published: 15.08.2026

Abstract: Antimicrobial peptides (AMPs) are promising therapeutic agents; however, their biological activity can be significantly influenced by interactions with nanomaterials present in biological environments. In this study, we investigated the cytotoxic effects of the peptide Brevinin-1 E8.13 and its interaction with polystyrene nanoparticles (PS-NPs) in human lung epithelial A549 cells. PS-NPs (50 nm) were characterized by transmission electron microscopy and dynamic light scattering. Cell viability and membrane integrity were assessed using MTT and lactate dehydrogenase (LDH) assays, respectively. Brevinin-1 E8.13 exhibited dose-dependent cytotoxicity, significantly reducing cell viability and increasing LDH release at concentrations ≥10 μM, indicating membrane-disruptive activity. In contrast, PS-NPs alone showed minimal cytotoxicity at the tested concentration (100 μg/mL). Notably, co-exposure to PS-NPs significantly attenuated Brevinin-1 E8.13-induced cytotoxicity, as evidenced by improved cell viability and reduced LDH release. Physicochemical analysis revealed an increase in hydrodynamic size and a shift in zeta potential of PS-NPs after incubation with the peptide, suggesting possible interaction between Brevinin-1 E8.13 and nanoparticle surfaces. These interactions may reduce the availability of free peptide, thereby diminishing its membrane-disruptive effects. This study highlights the critical role of nano–biointerface interactions in modulating AMP activity and suggests that nanoparticles may influence the efficacy and safety of peptide-based therapeutics.

Keywords: Brevinin-1 E8.13; antimicrobial peptide; polystyrene nanoparticles; nano-peptide interaction; cytotoxicity, membrane damage.

1. Introduction

Antimicrobial peptides (AMPs) are small, naturally occurring molecules that play a crucial role in the innate immune defense system of various organisms [1,2]. They exhibit broad-spectrum activity against Gram-positive, Gram-negative bacteria, fungi, and viruses, mainly by interacting with microbial membranes or inhibiting nucleic acid and protein biosynthesis [3-5]. Due to their rapid mode of action and low propensity for inducing microbial

resistance, AMPs have been widely investigated as promising alternatives to conventional antibiotics [6,7].

Among AMPs, Brevinins are a family of cationic amphipathic peptides isolated from frog skin secretions, including Brevinin-1 (approximately 24 amino acid residues in length) and Brevinin-2 (approximately 33-34 residues) [8]. These peptides typically adopt α-helical conformations in a membrane-mimetic environment and exert biological activity by interacting with lipid bilayers. Their cationic and amphipathic nature facilitates binding to negatively charged membranes of bacteria, cancer cells, or erythrocytes, leading to membrane destabilization and cell death [8]. In addition to antimicrobial activity, Brevinins have also been reported to exhibit anticancer and wound-healing properties [9,10]. However, like many AMPs, Brevinins may induce cytotoxicity in mammalian cells at elevated concentrations due to their membrane-disruptive properties, limiting their biomedical applications.

Nanoparticles are increasingly present in biological and environmental systems and are known to interact with biomolecules at the biointerface. Non-specific physicochemical forces, including hydrophobic interactions and electrostatic attraction, primarily govern these interactions. Upon exposure to biological fluids, nanoparticles can adsorb biomolecules, such as proteins and peptides, from their surfaces, forming a protein corona that alters the biological identity, stability, and activity of the adsorbed molecules [11,12]. As a result, nanoparticle–biomolecule interactions can significantly influence cellular responses, including uptake, signaling, and cytotoxicity [13]. Polystyrene nanoparticles (PS-NPs) are widely used as model nanomaterials in nanotoxicology studies due to their well-defined size and surface properties [14,15]. Their hydrophobic surface makes them particularly suitable for interacting with amphipathic peptides such as Brevinins. Such interactions may reduce the availability of free peptide molecules in solution and consequently modulate their biological activity. Despite increasing interest in nanobio interactions, the impact of nanoparticles on the cytotoxicity of antimicrobial peptides in mammalian cells remains poorly understood. Understanding these interactions is important for evaluating potential risks associated with nanomaterials and for designing peptide-based therapeutic systems.

In this study, we investigated the effect of non-functionalized polystyrene nanoparticles on Brevinin-1 E8.13-induced cytotoxicity in A549 human lung epithelial cells. We further explored the underlying mechanisms by assessing membrane damage and peptide-nanoparticle interactions. We hypothesized that hydrophobic adsorption of Brevinin-1 E8.13 onto PS-NPs reduces the concentration of membrane-active free peptide, thereby attenuating peptide-induced cytotoxicity.

2. Materials and Methods

2.1. Materials.

Brevinin-1 E8.13 peptide (24 amino acids; sequence: $_{1}$FLGALFKVASKLVPAAICSFSKKC$_{24}$ was synthesized using standard Fmoc-based solid-phase peptide synthesis (SPSS) with a purity of > 90%. The amino acid sequence was derived from the nucleotide sequence of Brevinin-1 E8.13 isolated from the skin secretion of Sylvirana guentheri, a frog species indigenous to Ha Giang Province, Vietnam, as previously reported in a study [16].

Fluorescently labeled PS NPs with a diameter of 50 nm were purchased from Polysciences, Inc. (Warrington, PA, USA). Dulbecco's Modified Eagle Medium (DMEM),

fetal bovine serum (FBS), and penicillin–streptomycin were obtained from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The MTT reagent was obtained from Merck (Darmstadt, Germany).

2.2. Characterization of PS-NPs.

The morphology and particle size of PS-NPs were characterized by transmission electron microscopy (TEM). Briefly, a drop of nanoparticle suspension was deposited onto a carbon-coated copper grid, air-dried at room temperature, and analyzed at an accelerating voltage of 80–200 kV.

The hydrodynamic size distribution and zeta potential of PS-NPs before and after incubation with Brevinin-1 E8.13 were measured using an electrophoretic light scattering instrument (ELSZ-2000 ELS, Otsuka Electronics, Osaka, Japan).

2.3. Cell culture.

Human lung epithelial A549 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified incubator containing 5% CO_2. Cells were subcultured every 2–3 days when they reached approximately 70–80% confluence.

2.4. Cell viability assay.

Cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A549 cells were seeded in 96-well plates at a density of $1x10^5$ cells/mL and allowed to attach overnight at 37°C. Cells were then treated with PS-NPs (100 µg/mL), Brevinin-1 E8.13 (15 µM), or a combination of both for 24 h. Untreated cells served as the control. Following treatment, cells were incubated with 10 µl of MTT solution (Merck, Darmstadt, Germany) per well for 3 h. Subsequently, the medium was aspirated, and 100 µl of DMSO (Merck, Germany) was added to dissolve the formazan precipitate. The absorbance was determined at 570 nm with a microplate reader (Synergy HT, Biotek, USA). Cell viability was expressed as a percentage relative to the control group.

2.5. LDH release assay.

Cell membrane integrity was evaluated by measuring lactate dehydrogenase (LDH) release into the culture medium. Cells were treated with PS-NPs (100 µg/mL), Brevinin-1 E8.13 (15 µM), or their combination for 24 h. Untreated cells served as the control. After treatment, culture supernatants were collected and analyzed using a commercial LDH assay kit (Sigma-Aldrich) according to the manufacturer's instructions. Absorbance was recorded at 450 nm, and LDH release was expressed as absolute optical density (OD) values.

2.6. Morphological observation.

Cellular morphology was observed using phase-contrast microscopy. A549 cells were exposed to 100 µg/mL PS-NPs, 15 µM Brevinin-1 E8.13, or their combination for 24 h. Morphological changes, including cell rounding, detachment, and membrane disruption, were qualitatively assessed.

2.7. Statistical analysis.

All data are presented as mean ± standard deviation (SD) from three independent biological experiments (n = 3). Differences among groups were examined using a one-way ANOVA followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant, and exact p-values are provided in the figure legends when applicable.

3. Results and Discussion

3.1. Characterization of PS-NPs.

Transmission electron microscopy (TEM) analysis revealed that PS-NPs exhibited a uniform spherical morphology with smooth surfaces and good dispersion, with no aggregation observed (Figure 1). In addition, dynamic light scattering (DLS) analysis confirmed that PS-NPs exhibited an average hydrodynamic diameter of 50 nm with a low polydispersity index (PDI = 0.053). The particles displayed a strongly negative surface charge (-56.27 mV), suggesting good colloidal stability in an aqueous environment. These observations confirm the nanoparticles' nanoscale size and morphological homogeneity.

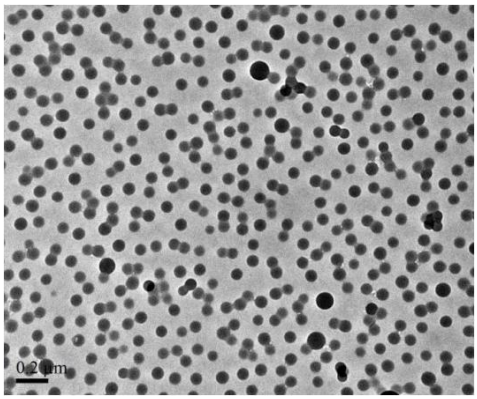

Figure 1. Morphology and size of 50 nm PS-NPs observed by TEM.

3.2. Cytotoxic effects of Brevinin-1 E8.13 on A549 cells.

The cytotoxicity of Brevinin-1 E8.13 in A549 cells was evaluated after 24 h of exposure. As shown in Figure 2, the peptide induced a dose-dependent reduction in cell viability.

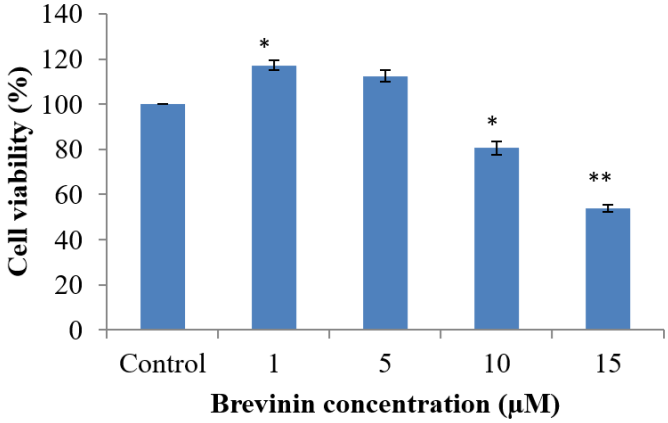

Figure 2. Dose-dependent cytotoxicity of Brevinin-1 E8.13 in A549 cells. Cells were treated with different concentrations of Brevinin-1 E8.13 (1, 5, 10, and 15 µM). Untreated cells served as a control. Data represent mean ± SD (n = 3). *p < 0.05, **p < 0.01 vs control.

At the lowest concentration (1 µM), cell viability increased significantly (to 117.26 ± 2.20%) compared to the control, but only a slight increase was observed at the 5 µM concentration. In contrast, treatment with higher concentrations (10 and 15 µM) significantly decreased cell viability to 80.57 ± 2.91% and 53.98 ± 1.55%, respectively, compared to the control group ($p < 0.05$).

Cytotoxicity was further evaluated by measuring lactate dehydrogenase (LDH) release into the medium following treatment with Brevinin-1 E8.13. Consistently, LDH release increased significantly at 10 and 15 µM to 157.35 ± 3.06% and 153.79 ± 3.98%, respectively, compared to the control (Figure 3), indicating loss of membrane integrity.

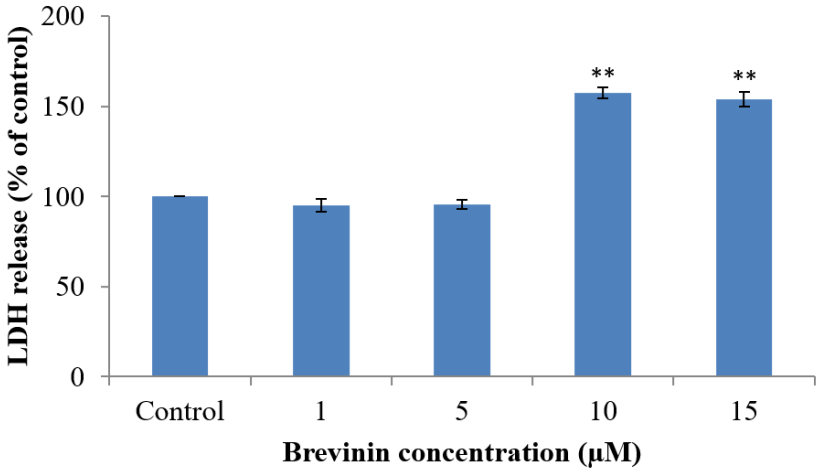

Figure 3. LDH release in A549 cells treated with Brevinin-1 E8.13. Cells were treated with different concentrations of Brevinin-1 E8.13 (1, 5, 10, and 15 µM). Untreated cells served as a control. Data represent mean ± SD (n = 3). **$p < 0.01$ vs control.

Brevinin peptides, which are isolated from frog skin, are well known for their potent antimicrobial activity but may also exert cytotoxic effects in mammalian cells due to their membrane-disruptive properties. Some AMPs exert anticancer effects by recruiting immune cells to kill tumor cells, inducing necrosis or apoptosis, inhibiting angiogenesis to deprive tumors of nutrients and prevent metastasis, and interfering with gene transcription and translation [17,18]. In this study, Brevinin-1 E8.13 (24 amino acids, net charge +4, ~62% hydrophobic residue) exhibited pronounced cytotoxicity at higher concentrations, as evidenced by decreased cell viability and increased LDH release. LDH is a cytosolic enzyme released upon membrane disruption and is widely used as a marker of necrotic cell death [19,20]. The elevated LDH levels observed here suggest that Brevinin-1 E8.13 primarily induces membrane damage rather than early apoptosis, where membrane integrity is largely preserved.

The amphipathic nature of Brevinin-1 E8.13, characterized by abundant hydrophobic residues (Leu, Val, Phe, Ile) and a positive charge, facilitates strong interactions with the negatively charged membranes of cancer cells. This promotes membrane insertion and pore formation, leading to cytotoxicity. These findings are consistent with previous reports showing that Brevinin peptides preferentially target malignant cells and can induce cell death via membrane disruption or lysosomal–mitochondrial pathways [21,22].

3.3. Cytotoxicity of PS-NPs on A549 cells.

Exposure of A549 cells to PS-NPs for 24 h demonstrated a concentration-dependent effect on cell viability. Preliminary experiments evaluating multiple PS-NP concentrations (12.5 µg/mL to 200 µg/mL) showed that the highest tested concentration induced noticeable

reductions in cell viability, indicating intrinsic nanoparticle-mediated cytotoxicity. In contrast, PS-NPs at 100 µg/mL did not result in significant changes in cell viability (94.33 ± 2.98) or LDH release (to 97.16 ± 2.95%) compared to the control group (Figure 4), indicating low cytotoxicity at this concentration. Therefore, 100 µg/mL was selected for subsequent co-exposure experiments to minimize direct nanoparticle-induced toxicity.

These results suggest that PS-NPs exhibit relatively low acute toxicity, consistent with previous studies reporting minimal cytotoxic effects of similar nanoparticles at comparable concentrations Phuc *et al*. [23]. However, despite their low cytotoxicity, PS-NPs are known to modulate cellular functions, including inflammatory responses and nutrient transport. For example, PS-NPs have been reported to interfere with iron absorption in intestinal epithelium (Mahler *et al*. [24]) and to activate inflammasome pathways in immune cells (Lunos *et al*. [25]). Thus, while PS-NPs alone may not significantly affect cell survival, their interactions with biomolecules may lead to indirect biological effects. Notably, their influence on antimicrobial peptides remains insufficiently explored.

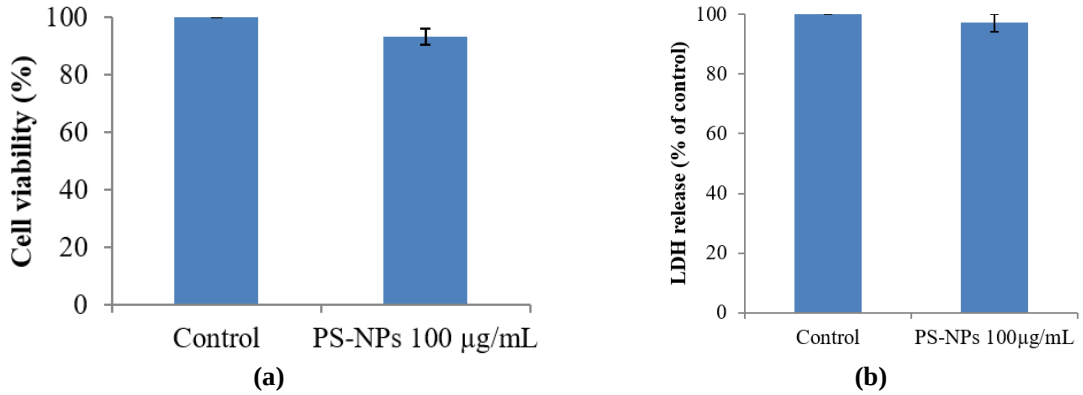

Figure 4. Effect of PS-NPs on **(a)** cell viability; **(b)** LDH release in A549 cells. Cells were exposed to 100 µg/mL of PS-NPs for 24 h. Untreated cells served as a control. Cell viability was assessed by MTT assay, while LDH release into the medium was measured with an LDH assay kit. Data represent mean ± SD (n = 3).

3.4. PS-NPs attenuate Brevinin-1 E8.13-induced cytotoxicity.

Co-exposure of A549 cells to PS-NPs and Brevinin-1 E8.13 resulted in a significant attenuation of peptide-induced cytotoxicity. As shown in Figure 5, at low concentrations of Brevinin-1 E8.13 (1 and 5 µM), PS-NPs did not significantly affect LDH release. In contrast, at higher peptide concentrations (10 and 15 µM), LDH release was significantly reduced in cells co-treated with PS-NPs and Brevinin-1 E8.13 compared with cells treated with Brevinin-1 E8.13 alone ($p < 0.05$).

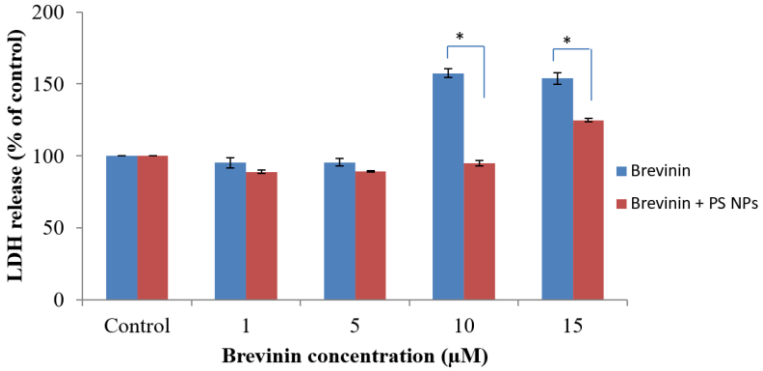

Figure 5. Effect of PS-NPs on Brevinin-1 E8.13-induced cytotoxicity in A549 cells. Cells were treated with Brevinin (1, 5, 10, 15 µM), or a combination of PS-NPs (100 µg/mL) + Brevinin-1 E8.13 (1, 5, 10, 15 µM) for 24h. Following treatment, LDH release into the medium was analyzed. Data represent mean ± SD (n = 3). * p < 0.05 vs Brevinin-1 E8.13 treatment alone.

Morphology observations by phase-contrast microscopy further supported these findings (Figure 6). Cells treated with Brevinin-1 E8.13 alone exhibited typical features of membrane damage, including cell shrinkage and membrane blebbing. In contrast, co- treated cells maintained better cellular morphology and integrity, similar to control cells.

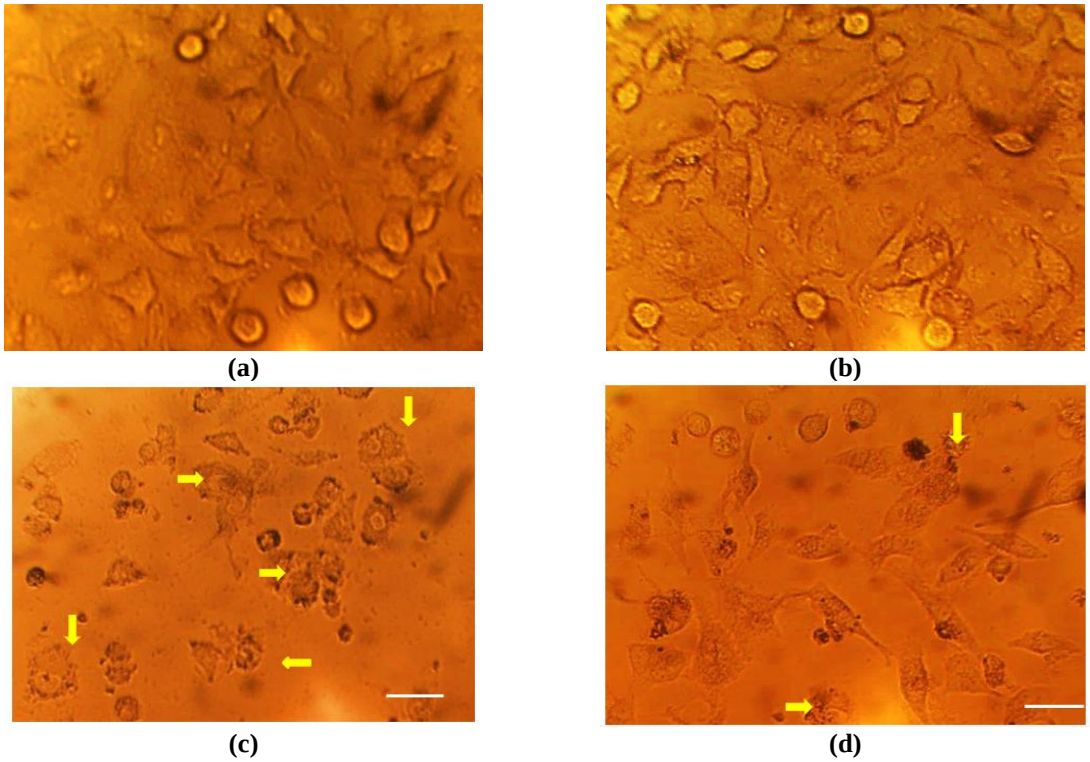

Figure 6. Morphological changes in A549 cells following treatments. **(a)** Control cells; **(b)** cells treated with PS-NPs; **(c)** cells treated with Brevinin-1 E8.13; **(d)** cells treated with PS-NPs + Brevinin-1 E8.13. Brevinin-treated cells exhibited membrane blebbing and cell shrinkage, as indicated by the yellow arrow, whereas co-treatment reduced these alterations. Scale bar = 100 µm.

The reduced cytotoxicity observed in the presence of PS-NPs suggests that nanoparticle–peptide interactions may modulate the peptide's bioactivity. One possible explanation is that the amphipathic nature of the peptide promotes its association with the hydrophobic surface of PS-NPs, leading to partial sequestration of the peptide (Figure 7).

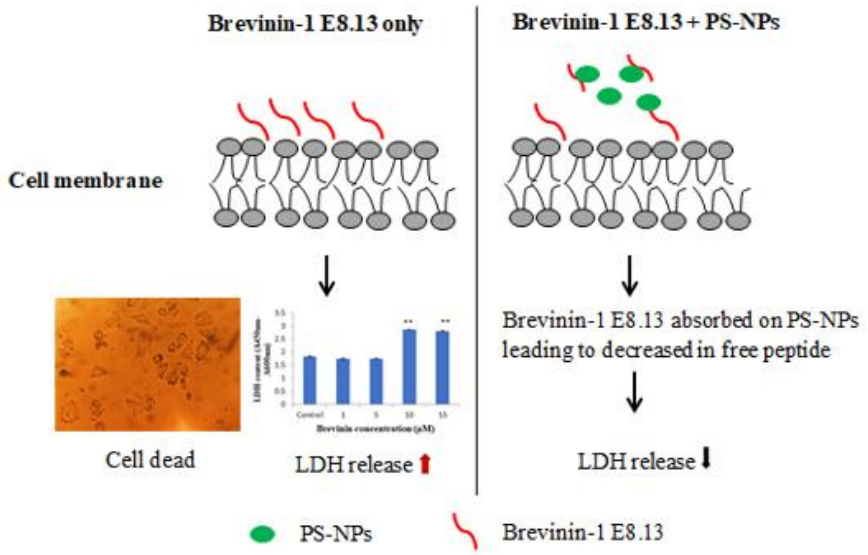

Figure 7. Hypothesis explaining the mechanism of PS-NPs-mediated attenuation of Brevinin-1 E8.13 cytotoxicity. When PS-NPs possess a small part of Brevinin-1 E8.13 absorbed on their surface, leading to a reduction in the concentration of membrane-active free peptide, thereby attenuating cytotoxic effects.

This interaction likely reduces the concentration of free peptide available to interact with cell membranes, thereby diminishing membrane disruption and cytotoxic effects. Given that peptide-induced toxicity is concentration-dependent, even partial adsorption may significantly alter biological outcomes.

Such behavior is consistent with the well-established concept of protein corona formation, in which nanoparticles adsorb biomolecules, altering their structure and function [26, 27]. In this context, nanoparticle binding may restrict peptide conformational flexibility or mask hydrophobic domains required for membrane insertion, thereby reducing cytotoxic activity.

3.5. Evidence for peptide–nanoparticle interaction.

Further evidence of peptide–nanoparticle interaction was obtained from dynamic light scattering (DLS) and zeta potential measurements (Figure 8). The hydrodynamic diameter of PS-NPs slightly increased after incubation with Brevinin-1 E8.13, accompanied by a shift in zeta potential toward less negative values (from −56.27 mV to −46.68 mV), indicating adsorption of positively charged peptides onto the nanoparticle surface.

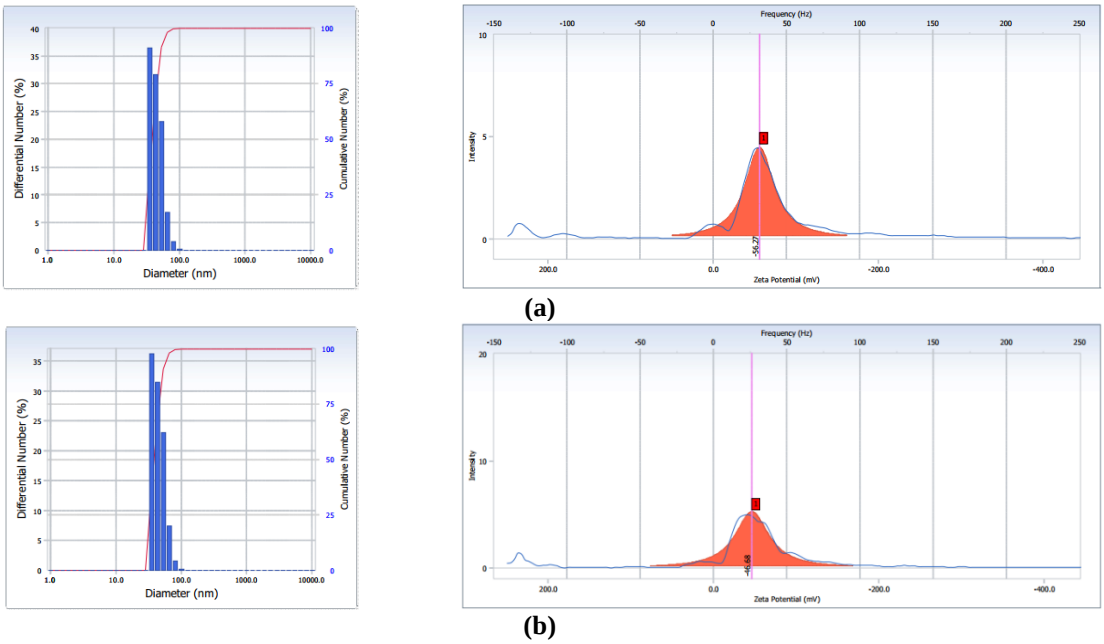

Figure 8. Size distribution and zeta potential of PS-NPs: **(a)** before; **(b)** after incubation with Brevinin-1 E8.13.

These findings highlight that even non-functionalized nanoparticles can significantly influence peptide activity through physicochemical interactions. While this effect may be beneficial for reducing cytotoxicity, it may also compromise the therapeutic efficacy of antimicrobial peptides.

However, several limitations should be noted. First, this study was conducted using only a single cell line (A549), which may limit the generalizability of the findings to other cell types or biological systems. Second, the interaction between Brevinin-1 E8.13 and PS-NPs was assessed indirectly via physicochemical measurements and biological responses rather than through direct adsorption or binding assays. Future studies should focus on direct quantification of peptide adsorption, structural characterization of peptide–nanoparticle complexes, and validation across multiple cell models better to understand the implications of these interactions in biomedical applications.

4. Conclusions

This study demonstrates that Brevinin-1 E8.13 induces dose-dependent cytotoxicity in A549 cells primarily through membrane disruption, as reflected by decreased cell viability and increased LDH release. While polystyrene nanoparticles alone exhibited minimal cytotoxicity, their presence significantly attenuated peptide-induced cell damage. Physicochemical changes observed in DLS and zeta potential measurements after incubation of PS-NPs with Brevinin-1 E8.13 are consistent with possible interactions between the peptide and nanoparticle surfaces. These interactions may reduce the availability of free membrane-active peptides and consequently diminish their cytotoxic effects. These findings suggest that even non-functionalized nanoparticles can modulate the biological activity of antimicrobial peptides through nano–biointerfacial interactions. Such effects should be carefully considered in the development of peptide-based therapeutics and in the assessment of nanoparticle safety in biological systems.

Author Contributions

Conceptualization, P.T.M.L. and H.T.L.; methodology, D.T.N. and H.H.H.; formal analysis, D.T.N. and T.L.N.; investigation, D.T.N. and T.L.N.; writing—original draft preparation, T.H.L. and H.H.H.; writing—review and editing, P.T.M.L.; funding acquisition, P.T.M.L. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research was funded by the Vietnam Academy of Science and Technology (VAST), grant number VAST02.02/24-25.

Acknowledgments

We thank the members of the Department of Cell Biotechnology and Bioassay, Institute of Biology, VAST, for their technical assistance.

Conflicts of Interest

The authors declare no conflict of interest.

Role of Funders

The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
AMPs	Antimicrobial Peptides
PS-NPs	Polystyrene Nanoparticles
MTT	
LDH	Lactate Dehydrogenase
DMSO	Dimethyl Sulfoxide
OD	Optical Density
SD	Standard Deviation

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