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# In vitro experiments of prokaryotic and eukaryotic antimicrobial peptide cytotoxicity in intestinal epithelial cells

Rawaz Rizgar Hassan[1], Shang Ziyad Abdulaqadir[2], Rzgar Farooq Rashid[3], Abdullah Othman Hassan[4]

[1,3,4]Department of Medical Laboratory Science, College of Science, Knowledge University, Kirkuk Road, 44001 Erbil, Kurdistan Region, Iraq.
[2]Department of Biology, College of Science, Salahaddin University-Erbil, Iraq.

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***Abstract*****—** *These proteinaceous molecules, called antimicrobial peptides (AMPs), are a varied collection of antimicrobial peptides. The ability of AMPs to combat gut infections necessitates further study of the AMP-GI tract interaction. These peptides need to be tested in vitro for cytotoxicity before they may be considered for use in clinical infections. Using the MTT conversion assay, neutral red dye absorption assay, and a comparison to vancomycin, researchers examined the cytotoxicity of gallidermin, nisin A, natural magainin peptides, and melittin in two gastrointestinal cell types (HT29 and Caco-2). Sheep erythrocyte hemolytic activity was also studied, and the influence of AMPs on paracellular permeability was assessed using transepithelial resistance (TEER) and TEM. Gallidermin, nisin A, magainin I, magainin II, and melittin were the least cytotoxic AMPs. To our knowledge, only Melittin and NIS caused considerable hemolysis. There are two distinct ways that melittin and nisin differ in their ability to kill bacteria. It was the only AMP that had an effect on the permeability of the paracellular space. Intestinal tight junctions and cell–cell adhesion were destroyed by long-term melittin therapy, as were microvilli, cell debris, and cell–cell adhesion. Antimicrobial activity and low cytotoxicity make Gallidermin a promising therapeutic drug. The antibacterial properties of Melittin are limited, but its ability to transport poorly bioavailable medicines may be useful.*

***Keywords*****— eukaryotic, antimicrobial peptide cytotoxicity prokaryotic.**

## I. INTRODUCTION

As a new kind of antibiotic, Antimicrobial Peptide (AMP) exhibit strong antimicrobial action against a variety of pathogenic bacteria, including isolates that have developed resistance to previous antibiotic treatments. However, despite their outstanding antibacterial action, these peptides' therapeutic potential is now confined to certain illnesses because of the high production costs they incur and the fact that they are stable in biological fluids (Talandashti, et al., 2019). Even while traditional antibiotics have a high concentration estimated for systemic infections, AMPs are currently not cost-effective (Heath, et al., 2018). Pharmaceutical companies are now producing peptides with more potential for treating systemic infections rather just epithelial and topical diseases (Rashid, et al., 2018).

AMPs can now be investigated for use in gastrointestinal infections because of the development of innovative drug delivery mechanisms for AMP release. The lantibiotic nisin A has been successfully delivered to the site of colonic infections and is currently patented (Wang, et al., 2021). Infectious organisms that are not self-limiting can be treated with a combination of nisin A, gallidermin, and magainin peptides. Bacteria that can withstand antibiotics include E. coli, Clostridium difficile and Helicobacter pylori. To learn more about how these AMPs interact with

the gastrointestinal tract, further research is needed to see if they can be delivered to the right places (Qutb, et al., 2020).

The activity of various peptides has been described using a variety of models. Barrel stave, toroidal pore, carpet, aggregate channel, and wedge models are some of the most famous processes for pore formation (Bulut, et al., 2020). Some peptides may work through more than one of these mechanisms. The general assumption is that AMPs cause cell death by disrupting bacterial plasma membranes, despite the specific debate. Additional antibacterial pathways can be found in AMPs. Peptidoglycan synthesis can be inhibited by Lactococcus lactis nisin A and Gallidermin, while nucleic acids and proteins can be inhibited by pleurocidin from Pleuronectes americanus. These peptides' physiochemical properties, particularly their hydrophobicity (Guan, et al., 2019), suggest that they could have harmful effects on the GI tract's mammalian cell membranes. Mammalian proteins involved in metabolism, as well as the translocation and up-regulation of apoptosis through mitochondrial disruption, have been shown to be particular targets of AMPs (Rice & Wereszczynski, 2017).

As a result, it is imperative that these peptides interact with intestinal epithelial cells before they may be considered for transport to infection sites. It was found that melittin was the most cytotoxic of the three natural magainin peptides tested in this investigation; the others were nisin A, gallidermin and melittin. The toxicity revealed in these cells may be applicable to other epithelial cells because they are regularly exposed to xenobiotics (Rashid & Basusta, 2021). The cytotoxicity of AMPs was assessed using the MTT conversion assay and the neutral red dye uptake assay, while the influence of these peptides on the plasma membrane integrity of gastrointestinal cells was examined using the LDH release assay. It was possible to see the effects of peptides on intestinal epithelial integrity by measuring TEER and looking at them using a scanning electron microscope (TEM) (Shekha, et al., 2013). We also compare the AMPs to vancomycin (Buonocore, et al., 2019), which is commonly used in the treatment of gastrointestinal infections (Salinas, et al., 2021). Pre-clinical and clinical dose of AMPs in gastrointestinal illnesses are expected to be derived from this investigation (Rice & Wereszczynski, 2017).

## II. MATERIALS AND METHODS

### Antimicrobial activity

In order to test the efficacy of several antimicrobials, we used Micrococcus luteus (ATCC 9341). The bacteria (M. luteus or E. coli, ATCC 10536) were planted on microtiter plates with 5 104 cells per well and were exposed to various quantities of freshly produced peptide in tryptic soy broth (TSB) for 21 hours, before the results were collected. After incubation, the absorbance at 550 nm was evaluated in a microtiter plate assay as a measure of cell growth and viability. The IC90 was computed and plotted on the data.

### Cytotoxicity assays

The MTT assay was used to measure the cytotoxic potential of test chemicals after they were incubated with exponentially developing cells. Oxidative enzymes in living cells are used to convert the methylthiazolyldiphenyl-sulfonamide (MTT) salt into the crystalline blue formazan product (Rashid & Basusta, 2021). The quantity of viable cells results in the development of formazan crystals. MTT (5 mg/ml) and 200 ml of fresh culture media were added to 0.1 M PBS, pH 7.4 and incubated for 4 hours at 37 °C in a humid atmosphere with 5 percent CO2 after 24 h of incubation. The test cultures' media were gently aspirated, and 100 ml of dimethyl sulphoxide (DMSO) was added to each well. Absorbance was measured at 550 nanometers in a microtiter plate reader after the plates were agitated for two minutes. It was determined that the IC50 was defined as the quantity of the test chemical that reduced the absorbance of MTT-formazan crystals by half, indicating 50% cell inactivation.

Cytotoxicity was confirmed by using the neutral red dye uptake assay (Sigma–Aldrich, Tox-4).. Cells that are alive and well have lysosomes that are actively transporting neutral red. There is a direct correlation between the quantity of live cells and the uptake of cellular dyes. HT29 and Caco-2 cells were incubated for 3 h or 30 min at 37 8C in a humid environment with 5% CO2 in fresh medium with 10% (w/v) neutral red solution to a final concentration of 0.033 percent for 24 h. Hanks balanced salt solution was used to wash the cells after the media was removed. The dye was then solubilized by adding 100 ml of an aqueous solution comprising 50 percent ethanol and 1 percent acetic acid, both at a volume of volume of volume of volume. After shaking the plates, the microtiter plate reader measured the absorbance at 550 nanometers. Concentration necessary to limit Neutral red dye absorption by 50% was established as IC50 (concentration of the test substance).

The LDH release assay (Tox-7, Sigma–Aldrich) was used to investigate the influence of test substances on the plasma membrane integrity of model cell lines. Analysis of cell supernatants for the activity of LDH, an enzyme that is only found intracellularly under normal conditions, was carried out. There were two graphs: one depicted the medication concentration and the other the percentage of

Triton X100 that had been removed. Using a plate spinner, we centrifuged test cultures produced in phenol red-free culture media for 4 minutes at 250 g. For 25 minutes, enzymatic assay reagent was added to analyze culture supernatant aliquots. 0.1N HCl was used to halt the process. At 490 nm, a microtiter plate reader was used to detect absorbance. The IC50 was defined as the concentration of the test chemical that induced an effect.

**Peptides**

Widely viable AMPs were employed in all cases. Gallidermin, Magainin I and Magainin II, Nisin A, Melittin, and Vancomycin were purchased as lyophilized powders with purity in excess of 95%. Each test chemical was produced in sterile water and stored at a temperature of 20 °C until it was needed. There were positive controls for cytotoxicity experiments in which Daunorubicin (an anthracycline antineoplastic) was utilized.

**Cell culture**

Between passages 30–50, HT29 and Caco-2 cells, both human intestinal epithelial cell lines, were employed. McCoy's 5a media containing 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% (v/v) fetal bovine serum was used to raise HT29 cells. Caco-2 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Sigma–Aldrich, Ireland), 2 mM L-glutamine, 1% (v/v) non-essential amino acids, 100 U/ml penicillin, and 100mg/ml streptomycin. A humidified environment with 5% CO2 was used to culture both cell lines. The exclusion of the vital dye trypan blue showed that the test cells had a vitality of over 99 percent prior to the cytotoxicity testing.

**Haemolysis assays**

A haemoglobin release assay was used to determine the test drugs' spectrophotometric hemolytic potential (Rank, et al., 2020). The erythrocytes were defibrinated, centrifuged, and resuspended in PBS after three PBS rinses (35mM phosphate buffer, 150mM NaCl, pH7.0), followed by 15 minutes at 900 g and 4 percent (v/v) of the original concentration. 96-well microtiter plates were used to plate 100 ml of the solution. Various peptide concentrations were used to treat red blood cells, which were subsequently centrifuged at 1,000 g for five minutes after being incubated at 37°C for an hour. Once the supernatant was transferred to a new 96-well microtiter plate, spectrophotometric measurements were made at 414 nm to determine the amount of hemoglobin released. The following formula was used to determine the percentage of hemolysis: (A414 0.1 percent Triton X100 A414 PBS). In PBS and 0.1 percent Triton X100, we found zero and 100 percent hemolysis, respectively.

**Epithelial integrity**

The transepithelial electrical resistance method was used to evaluate the effects of test substances on intestinal epithelial integrity (TEER). Transwell polycarbonate inserts (Corning Costar Corp., USA) were used to seed Caco-2 cells (P35-45) at a density of 5 105 cells/insert. During the course of 21 days, the cell culture media was replaced twice a day. Hanks balanced salt solution (HBSS) at 37 8C rinsed the differentiated monolayers before treatment with a range of pre-warmed test chemicals given to the apical surface of the monolayers. EVM volt–ohm meters with chopstick-type electrodes were used to measure the TEER over a 24-hour period. subtracting the filter insert's intrinsic resistivity from overall resistivity yielded the monolayer's resistance (monolayer plus filter insert). Percentage of TEER compared to untreated controls at the beginning of the experiment.

*Table 1: Antimicrobial peptide*

| Antimicrobial peptide | E. Coli | M. Luteus |
|---|---|---|
| Melittin | 5.6 | 8.73 |
| Vancomycin | 56.9 | 0.51 |
| Magainin I | 8.01 | 75.8 |
| Magainin II | 18.3 | > 80.0 |
| Nisin A | 19.01 | 0.14 |
| Gallidermin | 19.9 | 0.13 |

Transmission electron microscopy (TEM) was used to examine the effects of the test drug on ultrastructural morphology and intestinal epithelial integrity (Oliva, et al., 2019). For 24 hours, a test chemical with an IC50 twice as high was applied to polarized Caco-2 cells cultured in Transwell polycarbonate inserts. They were promptly fixed with 2.5 percent glutaraldehyde (v/v) and rinsed three times with HBSS. Embedded slices were dyed with uranyl acetate and lead citrate and evaluated after being placed on a 300 mesh copper grid with epoxy glue.

*Table 2:Cytotoxicities of AMPs*

| Drug | Neutral red dye uptake | | MTT conversion | |
|---|---|---|---|---|
| | HT29 | Caco-2 | HT29 | Caco-2 |
| Melittin | 5.8 | 4.8 | | |

| | | | | |
|---|---|---|---|---|
| Vancomycin | 4500 | >6000 | >6000 | >6000 |
| Magainin I | 74.9 | 93.9 | 64.9 | 66.1 |
| Magainin II | >100 | >100 | 80.8 | 80..1 |
| Nisin A | 103.9 | 133.1 | 90.1 | 114.9 |
| Gallidermin | >229 | >229 | 229 | 211 |
| Daunorubicin | 85.9 | 57.9 | 30.9 | 62.2 |

**Statistical analysis**

There were at least three separate runs of each experiment unless otherwise noted, and all results were given as mean and standard deviation. Minitab's analysis of variance was used to establish the study's statistical significance.

## III. RESULTS

**Effect of AMPS on lactate dehydrogenase release**

All cationic AMPs showed a concentration-dependent increase in extracellular LDH, showing that each peptide had a detrimental effect on plasma membrane integrity. On the other hand HT29 and Caco-2 cells were not observed to be affected by Vancomycin up to doses of 6 mM. LDH release was considerably higher in HT29 and Caco-2 cells treated with melittin than in cells treated with any of the other AMPs (P 0.01) or with daunorubicin. When compared to all other peptides, HT29 cells treated with gallidermin released considerably less LDH (P 0.05). Even at lower drug doses than those shown to damage the plasma membranes of HT29 and Caco-2 cells.

*Table 3: Haemolytic Potential of AMPs*

| Drug | Relative hemolysis | | |
|---|---|---|---|
| | 0.5 *IC50 | IC50 | 2 * IC50 |
| Melittin | 1.49±3.6 | 3.99 ± 5.69 | 17.99 ±16.29 |
| Vancomycin | 10.1±1.4 | 1.21±3.6 | 0.61±1.4 |
| Magainin I | 0 | 2.2±3.2 | 2.1±3.9 |
| Magainin II | 0.77±0.58 | 1.44±1.11 | 1.91±1.77 |
| Nisin A | 1.29±2.44 | 2.51±3.52 | 12.11±10.9 |
| Gallidermin | 0.14±0.3 | 1.44±0.7 | 4.11±1.3 |
| Daunorubicin | 0 | 0 | 0 |

**Antimicrobial activities of the AMPs**

Both lantibiotics were at least 100-fold less active against E. coli than gallidermin and nisin A, which had the highest antibacterial activity in M. luteus and activities in the nanomolar range (Table 1). Furthermore, in the NCCLS experiment, these two prokaryotic AMPs were more effective against M. luteus and E. coli than Vancomycin. Antimicrobial activity of melittin, magainins I and II, and gallidermin, eukaryotic peptides, was much lower than that of nisin A and gallidermin. Both indicator organisms were resistant to the antimicrobial effects of melittin at concentrations as low as 1 micromolar. Magainin I and Magainin II showed antibacterial action in the micromolar range against E. coli (IC90 7.89 and 18.7 mM, respectively) with significantly lower potency in the Gram positive M. luteus (IC90 76.1 and >80 mM), respectively.

**Cytotoxicities of AMPs in intestinal epithelial cells in vitro**

Cytotoxicities were comparable in both cell lines, HT29 and Caco-2, for all five AMPs tested. It was found that gallidermin was the least toxic of the three tested cataonic AMPs and was two times less toxic than the FDA-approved nisin A. Gallidermin was also the least hazardous of the eukaryotic peptides magainin I, magainin II, and the positive control (Table 2). Gallidermin had a two-fold cytotoxicity advantage over nisin A, magainin I, and magainin II in the MTT experiment (P 0.01). The in vitro cytotoxicity of magainin peptides (P 0.001) and melittin (P 0.001) was statistically different. MTT and NR assays in HT29 cells exhibited equal IC50 values for nisin A, magainin I and magainin II, however nisin A had a two-fold higher IC50 value than magainin I in Caco-2 cells (Table 2). It was shown that Melittin was 20 times more

cytotoxic than any other peptide in the MTT assay. Daunorubicin, an anticancer medication and well-known cytotoxic agent, was found to be less hazardous than Melittin. When tested in an MTT conversion experiment at a dosage of 6 mM, the standard antibiotic Vancomycin was shown not to be hazardous.

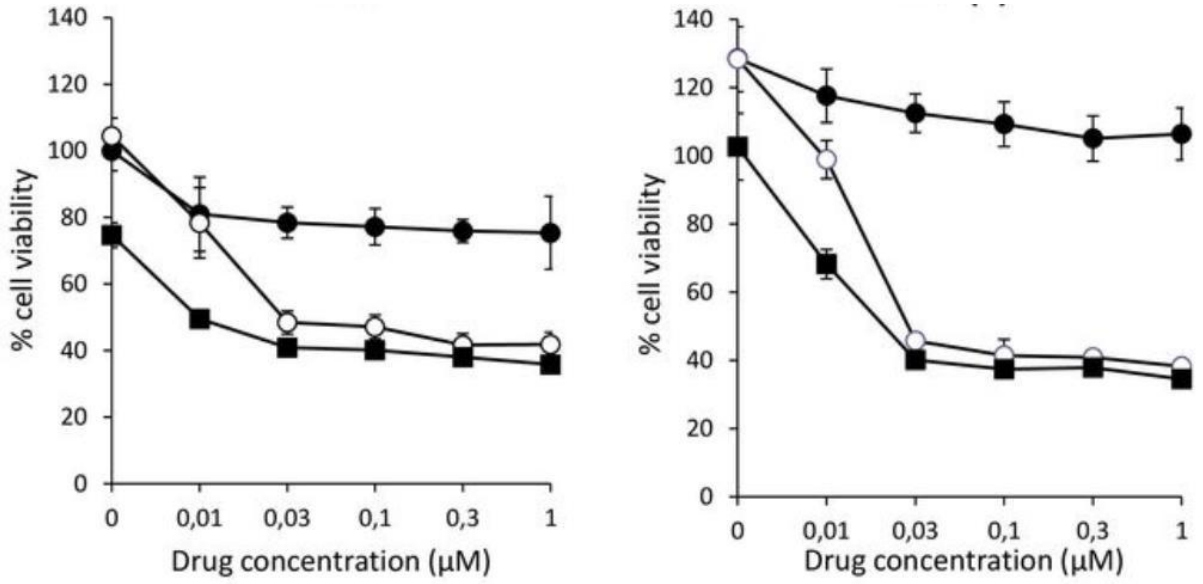


*Fig.1: The MTT assay was used to examine the viability of HT29 (a) and Caco-2 (b) cells after a 24-hour incubation period. Daunorubicin filled circles, melittin clear circles, magain II filled circles, magain I open circles, gallidermin filled squares, nisin A open squares represent responses in HT-29 cells; in Caco-2 cells, the corresponding symbols represent daunorubicin filled circles, melittin clear circles, magain II filled circles, magain I open circles, gallidermin filled squares, nisin A open squares respectively. The mean W S.E.M. of at least three independent tests is shown in the results. Daunorubicin and magainin I were excluded from the MTT and NR assays. Nisin A, magainin II, melittin, and gallidermin have a greater susceptibility to plasma membrane effects, which suggests that a loss of plasma membrane integrity is the predominant route of toxicity.*

**Effect of AMPs on erythrocyte hemolysis**

It was necessary to carry out an erythrocyte hemolysis experiment at concentrations equivalent to those used in the MTT assay on Caco-2 cells in order to correlate the cytotoxicity observed in gut epithelial cells with the lysis of red blood cells. It was discovered that daunorubicin was non-hemolytic at any of the concentrations tested (Table 3). Melittin and nisin A are the only peptides to cause hemolysis at the concentrations studied. Melittin demonstrated 18.16 16.31% relative hemolysis compared with 0.1 percent (v/v) Triton X100 while nisin A treatment resulted in 12.14 10.10% after 1 hour incubation when erythrocytes were treated at doses equivalent to double their respective IC50 values.

**Effect of AMPS on intestinal epithelial integrity as determined by transepithelial resistance measurements**

TEER measurements on differentiated Caco-2 cells were used to assess the impact of AMPs on intestinal epithelial integrity in vitro. The MTT assay was used to detect the cytotoxicity of various test substances in intestinal model organisms (see Table 2). Twenty-one days after plating on filters, the untreated monolayers had an apparent TEER of 1671 95 V cm2. There was only one AMP that had a significant impact on TEER, and that was Melittin In the MTT experiment, melittin reduced TEER by 78 percent within 15 minutes of injection of 0.8 mM (half the IC50 concentration). Over the course of the next 24 hours, the epithelial integrity returned fully. There was an 89 percent drop in TEE with only an intermediate recovery to 46.9 percent of control after 24 hours of treatment with Melittin (IC50 value). When melittin concentrations were doubled to 3.6 mM, monolayers were found to have a 91% loss in epithelial integrity that lasted for 24 hrs. In a concentration-dependent manner, vancomycin had intermediate impacts on TEER readings. Caco-2 cells were unaffected by any of the nisin, magainin I, galli- dermin, magainin II, or daunorubicin concentrations tested. All the properties of polarized Caco-2 cells, such as tight junctions, cell-to-cell adhesion, and micro-villi at the apical surface, were present in Caco-2 cells treated for 24 hours with magainin I, nisin A, and gallidermin. In contrast, 3.6 mM melittin treatment

in Caco-2 cells resulted in loss of microvilli after 24 hours, indicating that melittin therapy alters cell shape significantly. With the opening of tight junctions and the dilatation of cell–cell junctions, melittin (3.6 mM) also compromised barrier integrity. Additionally, Melittin treatment resulted in the breakdown of internal organelles and the accumulation of cellular debris within the cells.

## IV. DISCUSSION

The cytotoxicity of cationic AMPs and their interaction with human cells is not well documented. They could be used in the therapy of a wide range of medical diseases, particularly those related to epithelial barrier dysfunction. In the therapy of GI tract infections, the peptides magainin A and gallidermin have strong effect against gastrointestinal pathogens (Rashid, 2017). Intestinal epithelial cells were exposed to a wide range of bacterial and eukaryotic AMPs for the first time in this investigation. Antimicrobial activity of S. gallinarium's lantibiotic Gallidermin was shown to be one of the most potent of all ribosomally synthesized antimicrobials evaluated in two separate intestinal cell lines. It was also more effective than Vancomycin. For the purpose of comparison, AMPs and an FDA-approved antibiotic used to treat gastrointestinal infections were included in the study. Even though vancomycin is classified as an AMP, its monocyclic structure, reduced molecular weight, non-ribosomal production, and absence of pore formation distinguish it from the other peptides in this study. Both HT29 and Caco-2 cells were unaffected by vancomycin at doses of up to 6 mM in the MTT experiment. Gallidermin, an FDA-approved food preservative, was shown to be considerably more hazardous than nisin A (P 0.05) in both cell lines. It's possible that nisin A's higher cytotoxicity is due to its higher hydrophobicity, but the precise mechanism through which toxicity differs merits more research. A number of studies have shown that nisin A is cytotoxic to epithelial cells in the vaginal, colon, and kidney. Over 48 hours, Vero cells showed considerable toxicity between 0.85 and 3.4 mM, but vaginal epithelial cells showed no significant toxicity up to 95 mM (Talandashti, et al., 2021). Some of the observed toxicity discrepancies may be due to the use of various cell lines, contaminants in the nisin A preparations, or even purifying loss (Torres, et al., 2020). Food-grade nisin, which has been partially purified, was employed in both of the previous trials. Nisin A purity was not mentioned in each trial, making it difficult to compare results. Human lung fibroblast cells were exposed to 75 mM nisin A for 13 minutes, resulting in 90% efflux of intracellular ATP and 75% efflux of intracellular LDH, which is significantly higher than the concentration required for 75 percent efflux of intracellular LDH in intestinal epithelial cells after 24 hours of incubation. However, this may be attributable to a variety of factors, including cell type, exposure length, assay variation, and the medium used to apply the peptides. On both HT29 and Caco-2 cells, we found that nisin A was less hazardous than magainin I or magainin II in an MTT conversion experiment. It has previously been shown that magainin I and II are cytotoxic to a variety of cell lines and solid tumors, but direct comparisons to nisin are challenging. Magainin I is more sensitive to intestinal epithelial cells than U937 cells (24 h versus 72 h) in a shorter incubation period (Paray, et al., 2021). Comparatively, it appears that intestinal cells are less toxic to magainin II over a shorter length of time than U937 cells (24 h versus 72 h). Cellular cytotoxicity in intestinal epithelial cells (IC50 100 mg/ ml, 48 hours) was equivalent to that in lung epithelial cells (Bulut, et al., 2021). Haimovich and Tanaka conducted additional cytotoxicity tests on magainin II, in which the cytotoxicity assay relied on the exclusion of propridium iodide. In both normal and trans-formed cells, toxicity was found in excess of 500 mg/ml in this investigation. There have been studies done on Magainin analogues in cervical epithelial cells with the purpose of developing a contraceptive, with particular focus on Magainin A. Non-systemic infections, such as skin and gut infections, can be treated using non-systemic antimicrobials such as magainin, which have greater cytotoxicity and lower antimicrobial potency than lantibiotics, but may still be utilized as therapies. It is thought that the 'torroidal pore' process of pore creation is used by melittin and magainin. Their cytotoxic effects in vitro are markedly differing (P 0.001). The honey bee venom of the European honey bee contains the most cytotoxic component, Melittin (Apis meliffera). It has been shown that melittin is also a calcium-dependent antagonist of the multifunctional protein calmo-dulin, as well as an inhibitor of the Na+ K+ ATPase pump. According to the study, melittin is more cytotoxic than other AMPs. This indicates that melittin has numerous toxicity mechanisms in addition to its known cytolytic action. Negative controls were utilized as positive controls for cell toxicity experiments, including daunorubicin, an anthracycline that can inhibit topoisomerase II and cause cell necrosis. At high doses, it may lead to necrosis of the plasma membrane, resulting in a loss of membrane integrity. A 24-hour exposure to each AMP/control caused cell death in intestinal epithelial cells. Although the peptides' loss of plasma membrane integrity suggests a lethal necrosis, the possibility of a secondary necrosis occurring after an apoptosis is now being investigated. Antimicrobial activity against indicator bacteria (Table 1) and a wide spectrum of gastrointestinal pathogens (Enoki, et al., 2018) is much

lower than the cytotoxicity in HT29 and Caco-2 cells, particularly in the case of the lantibiotics nisin A and gallidermin, respectively. Given their ability to be poisonous just to certain types of bacteria, these peptides may have clinical application. Cytotoxicity and therapeutic index can be estimated by measuring the hemolytic activity of membrane-active amps on red blood cells. According to this comparison, all peptides were shown to have less than 2% hemolysis when compared to human intestinal epithelial cells, which is less hazardous to sheep than to humans. This may be due to the fact that normal and transformed mammalian cells have different plasma membrane compositions (Feng, et al., 2020), metabolic activity, and the specific assay employed for comparison. At a dose of 130 mM (313 mg/ml), two times the IC50 value in Caco-2 cells, Magainin I was shown to be non-hemolytic. Different plasma membrane compositions, including varied cholesterol [48] concentrations, may explain this lesser toxicity in two different cell types. Previously, it was reported that magainin I and magainin II did not cause hemolysis up to 150 mg/ml (Feng, et al., 2020), but we have now shown that magainins I and II cause mild hemolysis up to 313 and 400 mg/ml, respectively. At a concentration of 2.4 mM, melittin caused hemolysis in sheep erythrocytes at a rate of 18.16 16.31%. Compared to Tosteson et al. [50], with human cells, this hemolysis is significantly lower, but comparable to that seen by Zasloff (Pala, et al., 2021). This is most likely due to the previous study's use of lower amounts of phosphate (Pardhi, et al., 2020). When sodium is replaced with anionic phosphate, the maximal lysis extent is reduced, but hemolysis rate is unaffected (Rashid, et al., 2021). E. coli and M. luteus have IC90s comparable to the concentration required to produce hemolysis in eukaryotic cells for melittin's antibacterial action. At the antibacterial concentration where hemolysis was detected, melittin was found to be ineffective in the treatment of infections. The IC90 concentration of nisin A was 1000 times higher than the concentration needed to trigger 12.14 percent hemolysis. Because of the peptide's large selective toxicity, hemolysis is not a cause for worry. Red blood cell lysis by nisin A was shown to be much less harmful by Kordel and Sahl, with a toxicity level of only 6% after 30 min of incubation with 1 mM Nisin (Henderson, et al., 2019). However, these discrepancies may have been caused by different nisin A preparations. To trigger hemolysis, nisin concentrations much exceed the AMP's antibacterial activity in either circumstance. Due to its lack of pore-forming properties, vancomycin was found to be non-hemolytic up to 12 mM, as predicted. In the NCCLS assay, gallidermin administration caused just 4% hemolysis at a dose 3850 times higher than its IC90 value. It has a high degree of selective toxicity, indicating that it could be used as a therapeutic agent in the future. TEER has been found to be linked with changes in cell monolayer paracellular permeability (Almasia, et al., 2017). If AMPs are to be used in the treatment of bacterial infections of epithelial barriers, they must have an influence on cell–cell adhesion and tight junction integrity. Harm to either TJ integrity or cell-to-cell adhesion in the gastrointestinal tract can result in substantial and irreparable damage. The absence of effect of magainin I, magainin II, gallidermin, and nisin A on intestinal epithelial integrity implies that these peptides may be suited for the treatment of gastrointestinal tract infections. Polycations had previously been proven to open tight junctions (Rashid & Saler, 2020), thus this was a surprise. Importantly, TEM verified visibly that the epithelial barrier had not been disrupted. It is clear that melittin is unsuitable for treating either topical or systemic infections due to the harm it causes to the integrity of the epithelium at various concentrations. However, it is notable that melittin reduced TEER significantly more than two previously examined cell penetrating peptides (CPP), transportan and penetratin, and that effect was reversible (Zahedifard & Rafati, 2018). As paracellular permeability enhancers, these molecules are extremely effective in the transfer of medications across different epithelia (PPEs). Melittin has also been shown by Ma, et al., (2020), to mediate drug absorption in the current study. To find out if an ideal, non-toxic concentration can be achieved with sufficient tight junction opening and timely closure, more research is needed. There is some evidence that melittin or analogues may be effective in the delivery of weakly bioavailable hydrophilic medicines, according to these findings.

## V. CONCLUSION

Overall, we have proven that gallidermin contains the greatest amount of selective toxicity among the investigated AMPs. This implies potential for gallidermin as a therapeutic antimicro- bial drug. Furthermore nisin, and magainin peptides, despite showing lesser selective toxicity than gallidermin, also demonstrate modest cytotoxicity in gastrointestinal cells and hence may be beneficial, alone or in combination, against variety of clinical infections of the GI tract. Furthermore the potent cytotoxicity and ability of melittin to modify intestinal epithelial integrity suggests the AMP is not suitable for therapeutic use as an antimicrobial agent, but its ability to act as a paracellular permeability enhancer warrants further studies, where melittin derivatives of reduced toxicity may maintain a permeability enhancing role.

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