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A Pleurocidin-Like Peptide from Poecilia Mexicana Fish Induces Selective Cytotoxicity in Leukemia Jurkat Cells Through The Apoptosis Pathway

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Abstract

Objective: Some cationic anti-microbial peptides show a wide range of cytotoxic action versus malignant cells, which may lead to developing a novel group of antitumor medications. In the present study, the anticancer activity of pleurocidin-like peptide WF3 isoform X2 (AMP-WF3), from the Poecilia Mexicana fish, against leukemic cell line Jurkat was evaluated, and the cytotoxicity compared with the effects on normal cells, including peripheral blood mononuclear cells (PBMCs) and human dermal fibroblast (HDF) cells.

Materials and Methods: In this experimental study, cells were treated with various dosages of AMP-WF3 for 24 hours. Using methyl thiazole tetrazolium salt reduction (MTT test), the effects of the AMP-WF3 on cell viability and toxicity were evaluated. The impact of this peptide on apoptotic pathways was examined using flow cytometry and Annexin V-PI stains. Additionally, the relative expression of the *P53, P21*, and *BCL-2* genes was evaluated using a real-time polymerase chain reaction.

Results: The Jurkat cell line was more susceptible to AMP-WF3 cytotoxicity [half-maximal inhibitory concentration (IC_{50})=50 μM], while normal cells (PBMCs and HDF) were less susceptible. Flow cytometry verified that the apoptotic activity of AMP-WF3 on Jurkat cells was significantly higher than that of HDF and PBMCs. Peptide-treated Jurkat cells were associated with increased expression of *P21*, and *P53* genes. In contrast, the changes in *P21, P53*, and *BCL-2* genes differed in PBMCs and HDF cells. In HDF cells, simultaneous increase of *P21, P53*, and *BCL-2*, and in PBMCs, only the increase of *BCL-2* was observed.

Conclusion: Our research showed that AMP-WF3 could be developed as a novel treatment agent with minimum side effects for ALL patients.

Keywords: Acute Leukemia, Apoptosis, Cationic Peptide

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Introduction

Despite considerable advances in cancer research and treatment, the illness continues to be one of the major causes of death worldwide. In 2018, approximately 18,000,000 instances of cancer were reported, with 9,000,000 dead as a result (1). Leukemia, caused by the uncontrolled growth of the hematopoietic cell lineage, is one of the most prevalent kinds of cancer. Multiple causes, including genetics, infections, toxins, and radio waves, can result in leukemia (2). In recent decades, there have been advancements in treating childhood leukemia with stem cell transplantation. Still, chemo drug continues to be the dominant therapeutic option for adults, with less treatment response, particularly in those aged over 50 years (3).

Chemotherapeutic medicines used to treat leukemia are nonspecific, affecting both malignant and healthy cells and organs (4). Some medications, such as Doxorubicin and Arsenic trioxide, have serious side effects (5). Doxorubicin causes oxidative stress, which harms vital organs like the heart, kidneys, and brain (6). Arsenic, one of the components used in the chemotherapy regimen for refractory leukemia treatment, can induce mandibular bone necrosis (7). Due to the challenges connected with current therapies, such as many mental and physical problems in patients and the inefficacy of these treatments in reducing cancer cells, new safe and effective medications for leukemia treatment must be developed as early as possible (8).

Cationic anti-microbial peptides (CAPs) are polypeptides that typically contain between 5 and 100

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amino acids (9). These peptides, which are made up of alkaline amino acids, play a significant role in the host immune response (10). CAPs are classified as a-helical, b-sheet, loop, or extended peptides based on the secondary structure they develop while interacting with biological membranes (11). These peptides can stimulate the secretion of cytokines, decrease inflammation, and destroy cancer cells and bacteria (12). CAPs were shown to kill malignant cells in experimental and clinical investigations (13). Some of the targets these peptides interact with to kill cancer cells include phosphatidylserine, heparan sulfate proteoglycans, chondroitin sulfate, and O-sialoglycoproteins (14). Pleurocidins are a novel family of CAPs resembling cathelicidins in structure and function (15). This peptide was first discovered in the winter flounder's skin-secreted mucus (pleuronectes americanus) (16). In 2003, 20 types of NRCs of pleurocidin-like peptides were discovered in various species of Flanders (17). These peptides, like magainin 2, create pores in lipid membranes. In addition, Pleurocidins have weak hemolytic and moderate antibacterial activity (18). When these peptides interact with biological membranes, they form an alpha-helix (19). These peptides' apoptosis mechanism is twofold: i. Membranolytic targets: they cause cell death by reacting with extracellular receptors or creating instability in the plasma membrane, ii. Non-membranolytic targets: they cause cell death by reacting with intracellular proteins and inducing apoptosis (12). The amount of negatively charged on the outer layer of different malignant cell types determines the mode of action of these peptides. In investigations on pleurocidin's anti-cancer potential, the peptides NRC-07 and NRC-03 from the pleurocidin family were found to be effective in killing multiple myeloma and breast cancer cells (20). Additionally, it was demonstrated in the recent study that NRC-03 may trigger apoptosis in oral squamous cell carcinoma by activating the CypD-mPTP axis, which is brought on by mitochondrial oxidative stress (21).

In the present studies, we evaluated the cytotoxicity of a pleurocidin-like peptide WF3 (AMP-WF3) isoform X2 from Poecilia Mexicana fish against Jurkat cells, as well as its impact on *P21*, *P53*, and *BCL-2* expression.

Materials and Methods

Database search

The AMP-WF3 signal sequences MKCATLFLVLSM-VVLMAEPGDA were predicted as a query for searching against fish in NCBI (https://www.ncbi.nlm.nih.gov) using the parameters of the BLOSUM62 matrix method, Gap Costs, existence 11, and extension 1.

Sequence analysis

The coding DNA sequence was identified using the ORF finder (https://www.ncbi.nlm.nih.gov/orffinder). The amino acid sequence of pleurocidin-like WF3 isoform X2 (XP 014834597.1) was provided in P 4.0 (http://www.cbs.dtu.dk/services/SignalP/) and (https:/www.genome.jp/tools/motif/) to find the peptide signals and motifs. CAMP (http://www.camp.bicnirrh.res.in/predict) predicted the position of mature anti-microbial peptide (AMP) on its precursor. Anti-microbial characteristics were predicted using an anti-microbial peptide calculator and predictor (APD3) (http://aps.unmc.edu/AP/prediction/prediction main.php), and most AMPs that were comparable to prospective AMPs were discovered. The protein was provided in the helix rotation scheme (http://rzlab.ucr.edu/scripts/wheel/wheel.cgi) to forecast the helix and the hydrophilic interaction and hydrophobicity on the secondary structure of the peptide, the peptide sequence, and the amino acid sequence. The peptide's 3D structure was predicted using I-TASSER (http://zhanglab.ccmb.med.umich.edu/ I-TASSER /), an online protein structure prediction site.

Peptide synthesis

AMP-WF3 (amino acid sequence: FLKSLWRGVKAIFNGARQGYKEHKN) from Poecilia Mexicana fish (Pepmic, Suzhou, China) was produced using a solid phase technique based on Fmoc (9-fluorenyl-methoxycarbonyl) chemistry. This peptide was isolated using a SHIMADZU Inertsil ODS-SP (4.6 250 mm 5 m) column in RP-HPLC. For 30 minutes, the peptide was eluted with a 0-100% H_2O/acetonitrile gradient containing 0.1 percent trifluoroacetic acid (TFA). The peptide was homogeneous in an analytical high-performance homogeneity experiment utilizing an Inertsil ODSSP column, indicating a purity of 95%. The atomic mass of isolated peptides was successfully determined using mass spectrometry (MS).

Cell culture

The Pasteur Institute provided us with Jurkat and human dermal fibroblast (HDF) cell lines (Tehran, Iran). Jurkat cell lines were maintained in RPMMI1640 medium with L-glutamine (Caisson, USA), and HDF cell lines were maintained in DMEM.F12 medium (Caisson, USA) and 1% non-essential amino acid solution (Sigma, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Caisson, USA) in 5% CO_2 environment at 37˚C. Cells were passaged for optimum development and proven free of Mycoplasma and Endotoxin contamination. The cells were only employed if trypan blue exclusion revealed that more than 95% of them were viable. Healthy human peripheral blood mononuclear cells (PBMCs) were extracted from heparinized blood using Ficoll and utilized immediately.

Isolation of PBMCs via Ficoll

Whole blood was obtained in 10 mL EDTA tubes to isolate PBMCs. Phosphate Buffer Saline was added to

fresh blood in equal amounts. In a sterile tube, phosphate buffer saline was mixed with an equal amount of fresh blood. Whole blood with PBS was added to a 50 ml canolical tube in a 2: 1 ratio, and centrifuged in a swinging bucket rotor without brake for 30 minutes at 20˚C at 800 g. The middle layer was slowly pouredinto a new 50 ml falcon, then washed twice with PBS at 300 g for 10 minutes with the break. After washing twice, the formed pellet was dissolved in 1 ml of RPMI1640 medium, and the cells were counted.

MTT assay

Different concentrations of AMP-WF3 were incubated with Jurkat cells, PBMCs, and HDF cells, and cytotoxic activity was measured using the MTT test (ROTH, USA). In brief, 2×10^4 Jurkat cells and PBMCs were seeded in 96-well plates in medium with different concentrations of AMP-WF3 (6.25, 12.5, 25, 50 µM), and 1×10^4 HDF cells were sown in 96-well plates in media with varying concentrations of AMP-WF3 (6.25, 12.5, 25, 50 µM). After 24 hours, 10 µL MTT was added to each well, and the plates were incubated at 37˚C for 4 hours, and then the formazan crystals were subsequently solubilized in dimethyl sulfoxide (DMSO, Bioscience, USA, 100 µl/well). The optical absorbance was measured at 570 nm after 30 minutes. The final findings were calculated using an average of at least three repeated testings, and control cell survival was assumed to be 100%. The half-maximal inhibitory concentration (IC_{50}) value for Jurkat cells was the peptide dosage that resulted in a 50% drop in absorbance compared to the control. The viable cell percentage was estimated using the formula: absorbance sample - absorbance blank/absorbance control - absorbance blank ×100.

RNA extraction, cDNA synthesis, and real-time polymerase chain reaction

The TRIzol reagent was used to extract total RNA from cultivated cells in each group (Yekta Tajhiz Azma, Iran). Gel electrophoresis and the Nanodrop spectrometer were used to assess the RNA quality and quantity. Complementary DNA was produced according to the manufacturer's instructions using a random hexamer primer and the reverse transcriptase M-MLV (Yekta Tajhiz Azma, Iran). Bonbiotech (Bonbiotech, Inc., Iran) synthesized primers for polymerase chain reaction (PCR) amplification, as shown in Table 1. Step One Plus Real-Time PCR devices were used to assess the mRNA expression in the cells (Applied Biosystems, USA). According to the manufacturer's instructions, a 2X YTASYBR Green PCR Master Mix kit (Yekta Tajhiz Azma, Iran) was used to evaluate the expression of p53, p21, and BCL-2 mRNAs in Jurkat cells, PBMCs, and HDF cells. Briefly, 2 µl of cDNA product, 1 µl of each primer, 10 µl of 2X YTASYBR Green, and 7 µl of DEPC water were diluted to a final volume of 20 µl. (Sina Gene, Iran). An initial denaturation phase at 95˚C for 2 minutes was followed by 40 cycles of denaturation at 95˚C for 5 seconds, annealing, and extension at 60˚C for 30 seconds. HPRT was used as a reference gene to assess the mRNA expression levels of *P21*, *P53*, and *BCL-2*.

Table 1: Real-time polymerase chain reaction primer sequences

Target gene	Primer sequence (5´-3´)	Product size (bp)
HPRT	F: GGACAGGACTGAACGTCTTGC	88
	R: ATAGCCCCCCTTGAGCACAC	
P21	F: GAGGCCGGGATGAGTTGGGAGGAG	221
	R: CAGCCGGCGTTTGGAGTGGTAGAA	
P53	R: TAACAGTTCCTGCATGGGCGGC	121
	R: AGGACAGGCACAAACACGCACC	
BCL-2	F: GGTGGGGTCATGTGTGTGG	89
	R: CGGTTCAGGTACTCAGTCATCC	

Apoptosis assay

Apoptosis was determined using a bicolor flow cytometric technique with an Annexin V/PI testing kit. In summary, 2×10^4 cells were inoculated into 6-well plates with or without AMP-WF3 in the medium. Following overnight incubation, the cells were collected, washed in PBS, and re-suspended in 500 µL of binding buffer. The sample was combined with FITC-conjugated Annexin V and PI, and the combination was then incubated for 10 minutes on ice in the dark. Following flow cytometry examination of the labeled cells, the percentage of apoptotic cells was calculated using Cell Quest software.

Statistical analysis

All experiments were performed in triplicate, and results were reported as mean and standard deviation. The results were analyzed by the Wilcoxon rank test and ANOVA test by Graph Pad Prism version 9 (GraphPad Software Inc, USA) and SPSS v.22 software (IBM SPSS Statistics, USA). The significance level of statistical tests was considered to be 0.05 ($P<0.05$).

Ethical issue

This investigation has been approved by the Ethical Committee of Bushehr University of Medical Sciences (IR.BPUMS.REC.1398.013).

Results

Database search

When the pleurocidin peptide signal was used as a query in BLASTP against fish, a precursor to a new AMP, the

pleurocidin-like peptide (XP_014834597.1) was found. The output list was checked for conserved peptide signal, and a score (81.82% detection score, 100% coverage score) was presented in the amino acid sequence as a potential, pleurocidin-like peptide (XP_014834597.1). After BLASTP, the results were as follows: The signaling region of pleurocidin-like peptide is 1 to 22 amino acids, and the cut-off region is between 22 and 23 amino acids (Fig.1). The desired sequence used in the research is as follows: FLKSLWRGVKAIFNGARQGYKEHKN, which is one of the amino acids from 23 to 44 (Table 2).

Jurkat cell line viability was inhibited by an AMP-WF3

The MTT assay was used to test the peptide's anti-cancer efficacy in Jurkat cells. After treatment with 6.25 µM pleurocidin-like peptide, more than 90% of Jurkat cells survived, and the viability of Jurkat cells decreased substantially as the peptide concentration increased. The IC_{50} of the pleurocidin-like peptide was 50 µM in Jurkat cells (Fig.2).

The AMP-WF3 was less cytotoxic against PBMCs and HDF cells compared with Jurkat cells

MTT test was used to determine the cytotoxicity of the pleurocidin-like peptide against normal cells (PBMCs and HDF cells). Following treatment with 6.25 µM of the pleurocidin-like peptide, more than 90% of the cells remained alive, and after treatment with 50 µM, more than 70% remained viable, demonstrating that this pleurocidin-like peptide had minimal cytotoxicity against PBMCs and HDF cells (Fig.2).

Table 2: The score of each sequence is ranked according to the sequence position with anti-microbial properties (AMP)

Seq ID	Position	Sequence	Class	AMP probability
1	1-25	FLKSLWRGVKAIFNGARQGYKEHKN	AMP	0.969
1	2-26	LKSLWRGVKAIFNGARQGYKEHKNQ	AMP	0.853
1	3-27	KSLWRGVKAIFNGARQGYKEHKNQR	AMP	0.761
1	4-28	SLWRGVKAIFNGARQGYKEHKNQRR	AMP	0.786
1	5-29	LWRGVKAIFNGARQGYKEHKNQRRE	AMP	0.661
1	6-30	WRGVKAIFNGARQGYKEHKNQRREE	AMP	0.536
1	7-31	RGVKAIFNGARQGYKEHKNQRREEK	AMP	0.595
1	8-32	GVKAIFNGARQGYKEHKNQRREEKL	AMP	0.575
1	9-33	VKAIFNGARQGYKEHKNQRREEKLA	AMP	0.566
1	10-34	KAIFNGARQGYKEHKNQRREEKLAN	AMP	0.533
1	11-35	AIFNGARQGYKEHKNQRREEKLANA	AMP	0.500
1	12-36	IFNGARQGYKEHKNQRREEKLANAK	AMP	0.515
1	13-37	FNGARQGYKEHKNQRREEKLANAQ	AMP	0.484
1	14-38	NGARQGYKEHKNQRREEKLANAKD	AMP	0.450
1	15-39	GARQGYKEHKNQRREEKLANAKDM	AMP	0.358
1	16-40	ARQGYKEHKNQRREEKLANAKDMQ	AMP	0.327
1	17-41	RQGYKEHKNQRREEKLANAKDMQD	AMP	0.350
1	18-42	QGYKEHKNQRREEKLANAKDMQDQ	AMP	0.366
1	19-43	GYKEHKNQRREEKLANAKDMQDQQ	AMP	0.404

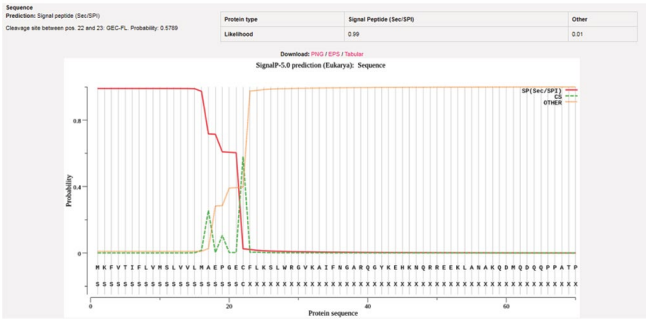

Fig.1: Peptide signal and cleavage area were predicted using SignalP 5.0 server. Positions 22 and 23 are the protein sequences of the cleavage area.

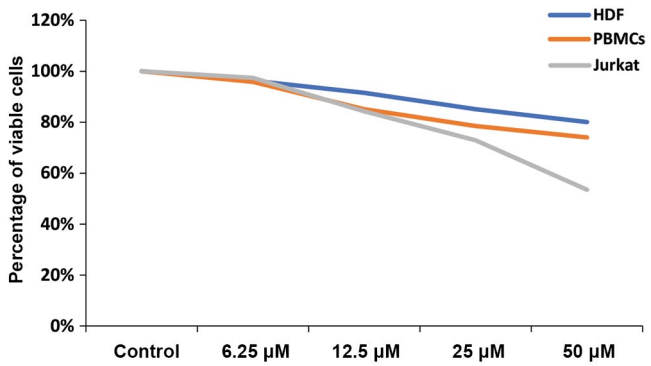

Fig.2: Viability of Jurkat, PBMCs, and HDF cells after treatment with pleurocidin-like peptide (24 hours). PBMCs; Peripheral blood mononuclear cells and HDF; Human dermal fibroblast.

Examining necrosis and apoptosis in leukemia cells treated with peptide versus normal cells treated with peptide

A red fluorescent dye called PI was used to precisely mark necrotic cells. A dual marking with Annexin V-FITC/PI was used to examine apoptosis and necrosis. Treatment of Jurkat cells with 50 µM Pleurocidin resulted in 13% early apoptosis, 24% late apoptosis, and 14% necrosis (Fig.3A, B). While HDF cells also showed about 14% early apoptosis, about 16.5% late apoptosis, and 2% necrosis after Pleurocidin treatment (Fig.3C, D). Additionally, around 16.5 % early apoptosis, 9% late apoptosis, and 10% necrosis were observed in PBMCS treated with peptide (Fig.3E, F).

Expression of genes *P53*, *P21*, and *BCL-2* in JURKAT cells in response to the AMP-WF3

qRT PCR was used to evaluate the expression levels of *P21*, *P53*, and *BCL-2* in Jurkat cells treated with the AMP-WF3 at a concentration of 50 µM versus untreated cells. *P21* and *P53* mRNA levels rose considerably after 24 hours in the pleurocidin-like peptide-treated group compared with the untreated group. *P21* and *P53* gene expression rose by 175.095 and 10.33 folds, respectively (Fig.4A, B).

The treated group's *BCL-2* gene expression was enhanced 3.655-fold compared to the untreated, but this was not statistically significant (Fig.4C).

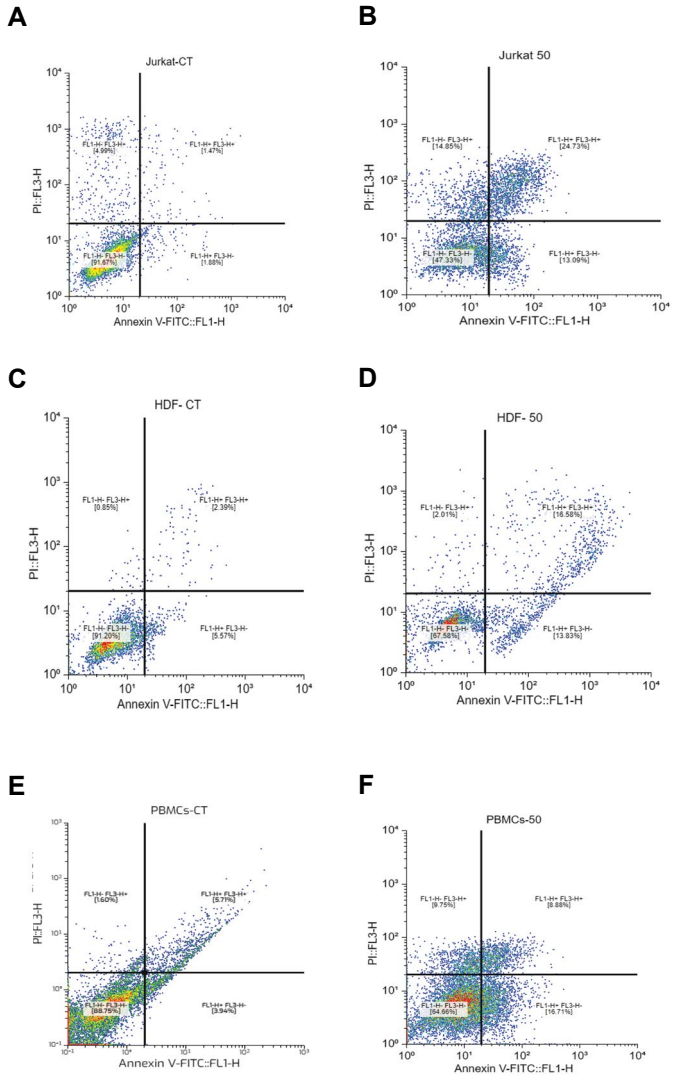

Fig.3: Based on the annexin assay. **A.** Flow cytometry of Jurkat cells in the control group, **B.** Jurkat cells treated with Pleurocidin peptide, **C.** HDF cells in the control group, **D.** HDF cells treated with Pleurocidin peptide, **E.** PBMC cells in the control group, and **F.** PBMCs cells treated with Pleurocidin peptide. PBMCs; Peripheral blood mononuclear cells and HDF; Human dermal fibroblast.

Effect of the AMP-WF3 on *P53*, *P21*, and *BCL-2* gene expression in PBMCs

The expression levels of *P21*, *P53*, and *BCL-2* in PBMCs treated with peptide at a concentration of 50 µM versus control pBMCs were evaluated using qRT PCR. After 24 hours, the expression of *P53* and *P21* genes in the treated groups did not change compared to the control group, but the expression of *BCL-2* in the treated group increased compared to the untreated group (Fig.4D-F).

Effect of the AMP-WF3 on *P53*, *P21*, and *BCL-2* gene expression in HDF cell lines

qRT PCR was used to compare the expression levels of *P53*, *P21*, and *BCL-2* in HDF cell lines treated

with a 50 μM dose of AMP-WF3 to untreated cells. *P21*, *P53*, and *BCL-2* expression were significantly higher in the peptide-treated groups than in the control group. *P21*, *P53*, and *BCL-2* gene expression levels were elevated by 21.67, 68.97, and 112.98 times, respectively, as a result of this (Fig.4G-I).

The comparison of apoptotic and anti-apoptotic gene expression in peptide-treated leukemia and peptide-treated normal cells

P21 gene expression rose 37-fold in the peptide-treated Jurkat cells compared with the peptide-treated PBMCs and 8-fold in the peptide-treated fibroblast cells, both of which were statistically significant (P=0.032 and P=0.026, respectively). *P53* gene expression increased 9-fold in the peptide-treated Jurkat cells compared with the peptide-treated PBMCs (P=0.057) and decreased 6.67-fold compared with the peptide-treated fibroblast cells, which was statistically significant (P=0.043). *BCL-2* expression of genes raised 7.64-fold in the peptide-treated Jurkat cells compared with the peptide-treated PBMCs (P=0.110) and 1.36-fold in the peptide-treated fibroblast cells (P=0.842) and was not statistically significant.

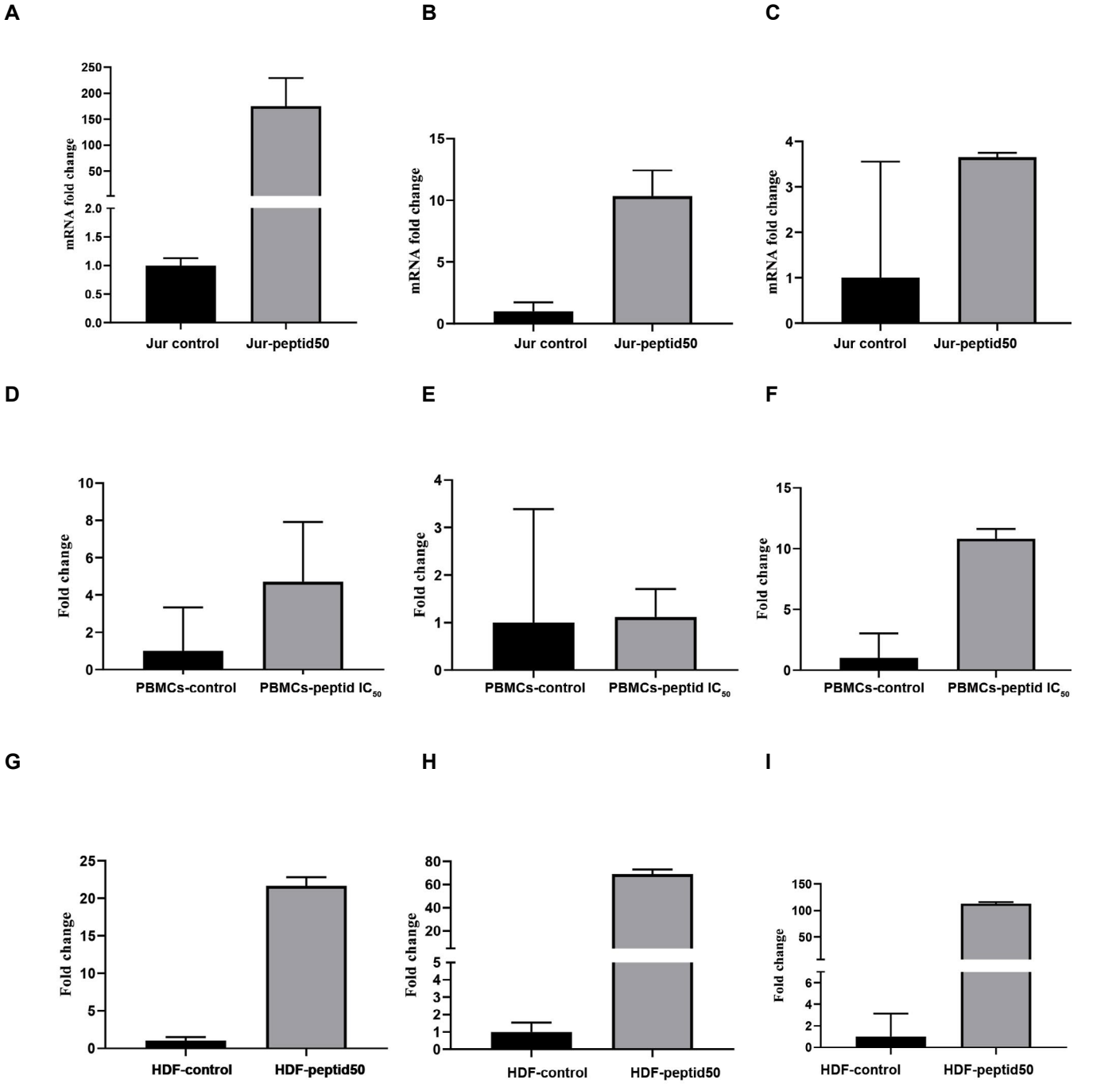

Fig.4: mRNA expression of apoptotic and anti-apoptotic genes. **A.** *P21* in Jurkat cells, **B.** *P53* in Jurkat cells, **C.** *BCL-2* in Jurkat cells, **D.** *P21* in PBMCs, **E.** *P53* in PBMCs, **F.** *BCL-2* in PBMCs, **G.** *P21* in HDF cells, **H.** *P53* in HDF cells, and **I.** *BCL-2* in HDF cells. PBMCs; Peripheral blood mononuclear cells and HDF; Human dermal fibroblast. Error bars denote standard deviation.

Discussion

Current anti-cancer medications have many side effects and must be absorbed into the cell to work, so cancer cells can build resistance to them by pumping them out of the cell. Some AMPs, on the other hand, appear to have unique anti-cancer capabilities, making them promising new anti-cancer options. In this study, the effects of the pleurocidin-like peptide on Jurkat cells as a target group and HDF cells and PBMCs as control groups were investigated.

We found that changes in the dose of AMP-WF3 were associated with the lethality of Jurkat cells and that the peptide IC_{50} in Jurkat cells was 50 µM. Furthermore, this peptide exhibited decreased toxicity in normal PBMC and HDF cells.

The structure of the cell membrane is important in the function of peptides because AMP interaction with cancer cells can disrupt the cell membrane or penetrate the cell and influence intracellular proteins (22).

The cytotoxicity of pleurocidin family members such as NRC-03 and NRC-07 in multiple myeloma cells was studied, and it was shown that these cells were more vulnerable to NRC-03 than to NRC-07 (20). On the other hand, HL60 cell lines were similarly sensitive to different types of peptides NRC of this family (23). Furthermore, a previous study on the effect of pleurocidin peptide on breast cancer cells revealed that, in comparison to leukemia cells, a higher peptide concentration is required to achieve an effective rate of cell death in breast cancer cells, which is likely because breast cancer cells are larger than leukemia cells (20). According to recent research, NRC-03 suppresses OSCC cell proliferation at concentrations between 15 and 75 µg/ml throughout the course of the therapy. While NRC-03 at higher doses, 60 and 75 µg/ml, mainly causes cytotoxic in normal Human Oral Keratinocytes cells (21).

One of the leading causes for the differences in peptide effects on cancer cell types is the negative charge on the cell membrane surface. The outer lipid membrane of Jurkat cells contains high levels of cholesterol, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol, all of which are associated with poor prognosis, while the outer surface of normal cell membranes of PBMCs and HDF is mainly made of Phosphatidylcholine and Sphingomyelin, which has zwitterionic properties (24).

The strong bonding and selective breakdown of cancer cell membranes are thought to be promoted by electrostatic adsorption between negatively charged cancer cell components and positively charged AMPs. The cationic anti-microbial peptide concentration is another factor involved in peptide's action on target cells; at toxic concentrations, the peptide disrupts cancer cell membranes, but at lower concentrations, it enters the cell and activates apoptotic signaling pathways (13).

Although the permeability system of this peptide in the Jurkat cell membrane has not been studied, the behavior of the pleurocidin family in two planar lipid layers has shown that ion channels are formed based on the toroidal model (25).

The combined peptides and lipids generate well-defined pores in the toroidal model. In contrast, the hydrophilic portions of the head groups of peptides and phospholipids face the center of the pores and produce aqueous pores. Due to the tilt of lipid molecules, the membrane bends inward, generating a hole surrounded by lipid head groups and filled by peptides (26). The findings show that peptides combine and establish a channel-like hole throughout the membrane after 2.5 µs of simulations (27).

Resistance to apoptotic processes is a major strategic factor in pathogenesis of acute lymphoblastic lukemia (ALL). As a result, many medications aim to stimulate apoptosis to accomplish the desired outcomes (28). Understanding the molecular processes that generate leukemia might lead to the development of novel treatments that extend the lives of patients (29). Most alpha-helical AMPs primarily inhibit cancer by inducing necrosis and apoptosis (30). P53 protein levels rise in response to DNA-damaging anti-cancer stimulation, and rising p53 levels, in turn, lead to apoptosis. In cells undergoing either p53-mediated G1 arrest or apoptosis, the p21 protein has been observed to be induced (31). P21 is an essential regulator of cell growth arrest, especially when the genome is harmed by damaging agents. However, because P21 plays such a critical function in cell cycle regulation, mutations in this gene are extremely rare (32). Some cationic anti-microbial peptides, such as LL37, are structurally and functionally similar to pleurocidins (15). This peptide inhibits colon cancer by initiating a caspase-independent apoptotic cascade driven by p53 (33). Significantly, treatment of LL-37 with induction of caspase-independent apoptosis kills Jurkat T leukemia cells (34). LL-37 is paradoxically involved in spreading cancers of the breast, ovaries, lungs, prostate, and pancreas (35). Another cationic anti-microbial peptide is Buforin IIb alpha-helix, which leads to the death of prostate cancer cells by decreasing BCL2 and increasing P21 and P53 (36). Another peptide that has an anti-cancer role is temporin-1CE, which in high concentrations, disrupts the plasma membrane and causes the lysis of breast cancer cells. In contrast, its lower levels cause cell death by intracellular mechanisms (37). The pleurocidin-like peptide WF3 was linked to elevated levels of *p53* and *p21* gene expression in this study, indicating that Jurkat cell growth was reduced.

In earlier studies, p53 activation was shown as a result of DNA damage caused by the CAP reaction and a lack of DNA repair (38).

Bcl-2 is an inhibiting apoptosis protein that promotes cell survival and proliferation by inhibiting the function of pro-apoptotic proteins. In leukemia, the expression of

inhibiting apoptosis proteins such as BCL2 is increased (39). The Bcl-2 family of proteins has long been thought to be essential regulators of drug-induced mitochondrial apoptosis. Furthermore, elevated BCL-2 levels have been linked to poor treatment response (40). Our data further showed that in Jurkat cells, AMP-WF3 did not affect the *BCL2* gene.

This is the first research to look at the influence of the pleurocidin-like peptide WF3 isoform X2 on the expression of the *p53, p21*, and *Bcl-2* genes, as well as apoptosis and cell growth inhibition in the Jurkat cell line.

Conclusion

In brief, the present investigation demonstrated that AMP-WF3 exhibited good cytotoxicity versus acute lymphoblastic cell line but had lower cytotoxicity toward normal cells. The apoptotic and necrotic activity of AMP-WF3 on Jurkat cells was much higher than that of HDF and PBMCs, according to Annexi V-PI. In Jurkat cells, the IC50- AMP-WF3 activates signaling pathways P21 and P53, leading to cell cycle arrest and cell apoptosis, and also did not affect the *BCL-2* gene. However, the effect of the peptides on HDF cells showed a high expression of *BCL-2* anti-apoptotic protein along with an increase in P53 and P21 protein compared to control cells. The effect of peptides on PBMC cells in a healthy individual did not change the expression of *P21* and *P53*, but the expression of *BCL-2* in the treated group showed an increase compared to the untreated group.

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Authors' Contributions

Gh.R.Kh., M.M., N.O., M.E.; Participated in study design, contributed to all experimental work, data and statistical analysis, interpretation of data, and reviewed the literature for the manuscript. S.A.M; Contributed extensively to the interpretation of the data and the conclusion. Gh.R.Kh.; Performed editing and approved the final version of this manuscript for submission and approved the final draft. All authors read and approved the final manuscript.

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