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INVESTIGATION OF THE RESVERATROL EFFECT ON IL-6 RECEPTOR GENE EXPRESSION LEVEL IN ACUTE MYELOID LEUKEMIA CELL

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ABSTRACT

Resveratrol has been extensively investigated as a natural compound with promoising anticancer properties. the present study evaluated whether resveratrol modulates IL-6R gene expression in the AML derived KG-1 a cell line. cells were exposed to different concentrations of resveratrol for varying incubation period and cytotoxicity was determined using MTT assay to detect the inhibitory concentration (IC50). RNA was isolated from treated cells, and IL-6R expression was evaluated using RT*qPCR. The expression of IL-6R was quantified using real-time reverse transcription polymerase chain reaction (RT-PCR). The Results appeared the resveratrol significantly reduced KG1-a cell viability in a dose- and time-dependent manner, with IC50 values ranging from 30 to 40 µM. RNA integrity was confirmed by the presence of distinct 28S and 18S rRNA bands. Gene expression analysis demonstrated a significant downregulation of IL-6R mRNA levels following treatment with the IC50 concentration of resveratrol for 72 hours ($P < 0.001$), showing an approximately 1.5-fold decrease compared with untreated controls. This study conclude that Resveratrol exhibited significant anti-leukemic activity against KG1-a cells by reducing cell viability and suppressing IL-6R gene expression.

KEYWORDS: Acute myeloid leukemia, IL-6 receptor, KG1-a Cells, Resveratrol, RT-PCR.

1. INTRODUCTION

Acute Myeloid Leukemia (AML a rapidly progressive malignancy of the hematopoietic system resulting from the transformation of myeloid precursor cells. The disease is characterized by the excessive production of immature myeloid blasts that accumulate within the bone marrow, leading to suppression of normal blood cell formation. AML is considered a genetically heterogeneous disorder because it is associated with numerous molecular and chromosomal abnormalities that influence disease behavior and clinical outcome.[1] AML arises from either hematopoietic stem cells or myeloid progenitor cells, which undergo aberrant clonal expansion and differentiation arrest. This results in the rapid accumulation of leukemic blasts within the bone marrow, severely disrupting normal hematopoiesis. As healthy blood cell production declines, patients often present with anemia, thrombocytopenia, and neutropenia, manifesting clinically as fatigue, weakness, bleeding tendencies, susceptibility to infections, bleeding episodes, and enlargement of organs such as the spleen and liver.[2,3] IL-6 is a pleiotropic cytokine that regulates multiple physiological processes, including immune responses, inflammation, and hematopoiesis In AML, IL-6 contributes to disease progression by promoting leukemia cell growth, inhibiting apoptosis, and modifying the bone marrow microenvironment.[4] Resveratrol (RV) is a prominent polyphenolic compound

found in various fruits and vegetables, such as peanut sprouts, grapes, and peanuts. It was initially isolated from the white hellebore plant, veratrum grandiflorum. In recent years, it has gained considerable attention from medical chemists, nutritionists, and health professionals due to its wide range of benefits, including antiangiogenic, immunomodulatory, antimicrobial, neurological, anticancer, antidiabetic properties, and prevention of cardiovascular diseases.[5] Resveratrol is recognized as a valuable complementary medicine for preventing and treating various cancers, including those affecting the eyes, breast, cervix, kidneys, liver, prostate, blood, brain, bladder, thyroid, ovaries, esophagus, stomach, lungs, skin, neck, and bones.[6] the goal of this paper is study the effects of resveratrol on the expression levels of the IL-6 receptor gene in AML cell lines were investigated and this study aimed to determine how varying concentrations of resveratrol were found to influence IL-6 receptor expression over time where mechanistic insights were explored by analyzing the signaling pathways involved in the modulation of IL-6 receptor expression, particularly focusing on pathways such as STAT3 and NF-κB and functional outcomes were assessed to understand how changes in IL-6 receptor expression impacted key cellular functions ,including proliferation, apoptosis and inflammatory responses in AML cells and broader implications of resveratrol as a complementary treatment in AML therapy were also evaluated, emphasizing its potential role in modulating inflammatory signaling pathways.

2. MATERIALS AND METHODS

2.1 Cell Culture

KG1-a cells were obtained from Pasteur Cell Bank of Iran and transferred to the laboratory under appropriate temperature-controlled conditions. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cell cultures were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO_2.

2.2 Cell viability assay

The MTT assay was performed to evaluate the cytotoxic effect of the studied compound. The assay is based on the ability of metabolically active (viable) cells to reduce the yellow tetrazolium salt, MTT, in to insoluble purple formazan crystals through the action of as mitochondrial succinate dehydrogenases enzymes, the amount of formazan produced is directly proportional to the number of viable cells and is used as an indicator of cell viability and cytotoxicity.

3.1. Gene expression evaluation via real time RT-PCR

For investigating the expression of IL-6R gene RNA extraction, quantitative and qualitative analysis of the extracted RNA, cDNA synthesis and Real Time PCR reaction were done**.**

3.1.1 RNA extraction from treated and untreated KG1-a cells

Following a 72-hour incubation period, the RNA samples were isolated from both untreated and treated KG1-a cells using RNX-plus reagent obtained from Cinna gen in Iran.

3.1.2 Quantitative and qualitative analysis of RNA

Then after extracting RNA, its quantity was checked with NanoDrop device and its quality was checked by loading in agarose gel. For this purpose, 1µL of RNA is placed inside the NanoDrop device to measure its absorbance (represent of concentration) and OD at 260 nm. In order to check the quality of RNA, 2 µl of RNA were loaded in 1.5% agarose gel and electrophoresis was performed for 30 minutes with a voltage of 100 mV. A good-quality RNA sample should indicate three bands on the gel including 28s RNA, 18s RNA, and 5.8s RNA, respectively and the absence of clear 28s and 18s bands indicate RNA degradation.

3.1.3 cDNA synthesis

To evaluate gene expression total RNA was reverse-transcribed into complementary DNA (cDNA). For cDNA synthesis 2 µg of RNA was first poured into the microtube and 1µL of DNase I and its buffer were added to the microtube and then it was placed in a thermocycler for 30 minutes at 37 °C and then, in order to inhibit the activity of DNase I enzyme, 1µL of EDTA enzyme was added to the samples and placed in a thermocycler at 65°C for 10 minutes. In the next step, 3.5 microliters of the cDNA synthesis kit mixture were added to the samples according to the company's instructions and was placed at 37°C and 95°C for one hour and 5 minutes, respectively.

3.1.4 Primer design

In this research, the expression of IL-6R gene was investigated by quantitative Real-Time PCR. For this purpose, the β-actin gene was considered as a housekeeping gene. In this study, the primers were designed by the NCBI database, Primer Blast, and Gene Runner program, and the specificity of the primers was insured by using Oligo 7 software version 4.0.3 (Beta). All primers, with sequences detailed in Table 1.

Table 1: Specific primer with the following sequences used in reverse transcription real-time quantitative polymerase chain reactions.

β-actin	F	AGAGCTACGAGCTGCCTGAC	20	184 bp
	R	AGCACTGTGTTGGCGTACAG	20	
IL6R	F	5'-GGCTGGAATGGCGACTTA-3'	18	150 bp
	R	5'-ATCACCTCGGCTTGATGA-3'	18	

3.1.5 Quantitative real-time PCR

Following a 72-hour incubation period, the RNA samples were isolated from both untreated and treated KG1-a cells using RNX-plus reagent sourced from Cinnagen in Iran. Subsequently, the total RNA underwent reverse transcription to produce cDNA via the Reverse Transcriptase kit from Parstoos Company and the process of qRT-PCRs was conducted on the Light Cycler 96 Instrument following the protocol outlined in table 2, and via the utilization of Real Q Plus 2x Master Mix Green High ROX™, provided by Amplicon in Denmark. Gene expression level of IL-6R was normalized to β-actin and compared to untreated cells. All reaction ingredients with amounts were listed in table 3.

Table 2: Real-time PCR schedule for target gene and β-actin gene.

Steps	Temp(°C)	Time	Cycles
Initial denaturation	95 °C	8 min	1
Denaturation	95 °C	30 sec	
Annealing	60 - 64°C	30 sec	40
Extension	72 °C	30 sec	
Final extension	72 °C	5 min	1

Table 3: Required materials to perform Real time PCR.

GENE	Master	ddH2O	Primer Forward	Primer Reverse	cDNA
β-actin	5 µl	2.4 µl	0.3 µl	0.3 µl	2 µl
IL-6R	5 µl	2.4 µl	0.3 µl	0.3 µl	2µl

4. Statistical analysis

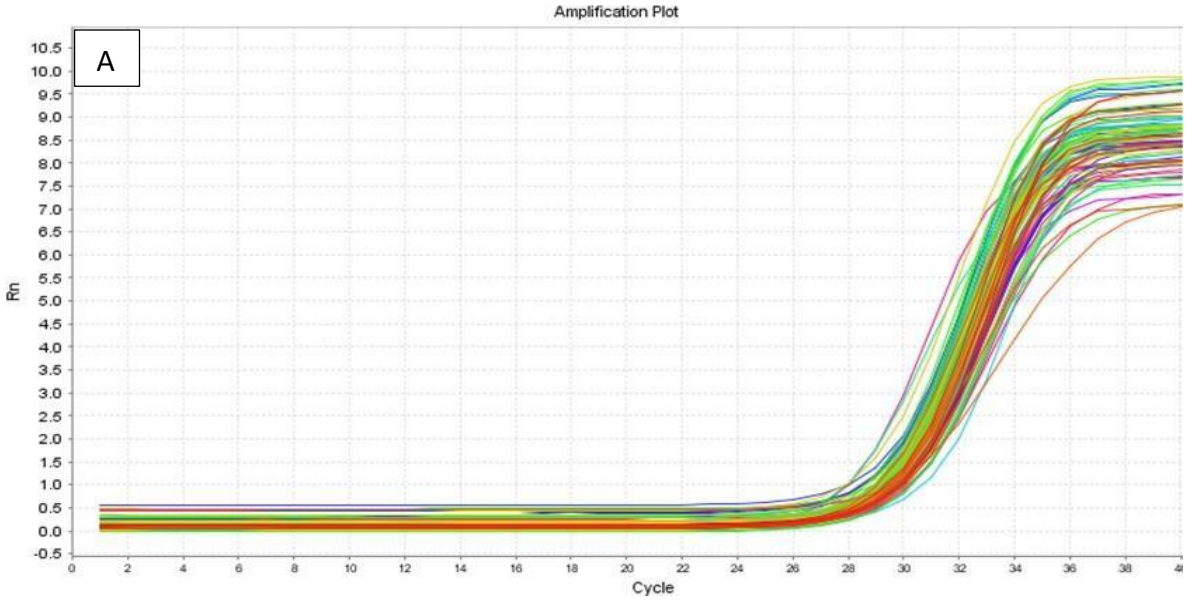
All experiments were performed in triplicate, and the results are presented as the mean ± standard deviation (SD). Statistical analyses were conducted using one-way or two-way analysis of variance (ANOVA), as appropriate, with GraphPad Prism software (version 6.02; GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $P < 0.05$ (), $P < 0.01$ (), $P < 0.001$(),and$P<0.0001$().**

5. RESULTS

5.1 Real-time PCR reaction results

Following the real-time PCR assay, amplification and melting curves were generated to evaluate the efficiency and specificity of the reactions. The amplification curves demonstrated the real-time accumulation of PCR products during each amplification cycle. A fluorescence threshold was set within the exponential phase of amplification to determine the cycle threshold (Ct) value based on changes in CyGreen fluorescence intensity. Relative gene expression was subsequently quantified by comparing the Ct values among the different samples.

Analysis of the melting curves revealed a single sharp peak with nearly identical melting temperatures (Tm) across all samples, indicating the specificity of the amplification and the absence of non-specific PCR products or primer-dimer formation. These findings confirm the accuracy and reliability of the designed primers for gene expression analysis (Figures 1A and 1B, and Figures 2A and 2B).



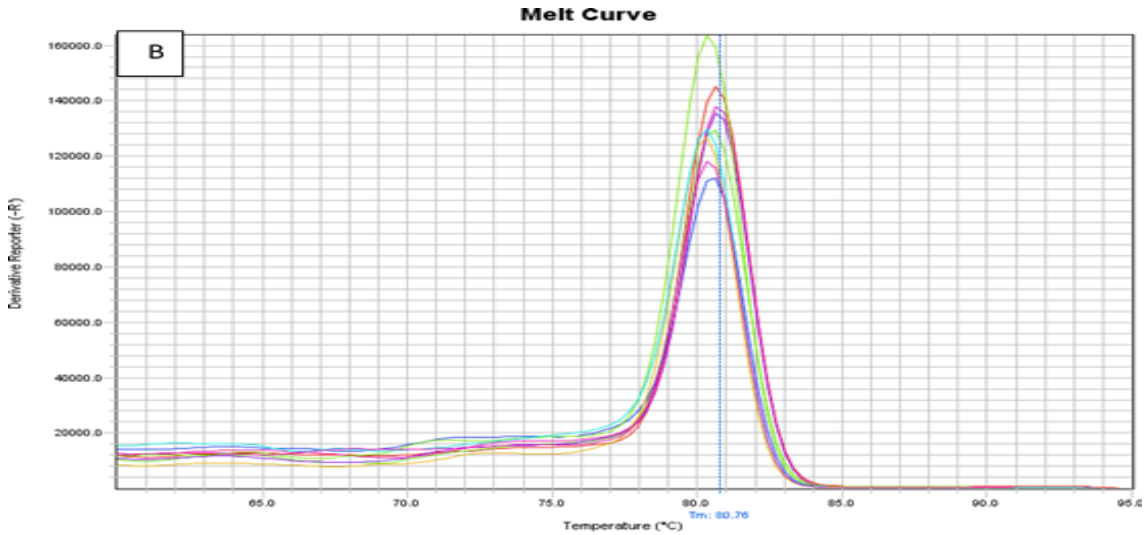


Figure 1: Amplification (A), and Melting (B) curves of the house keeping gene (β-actin)

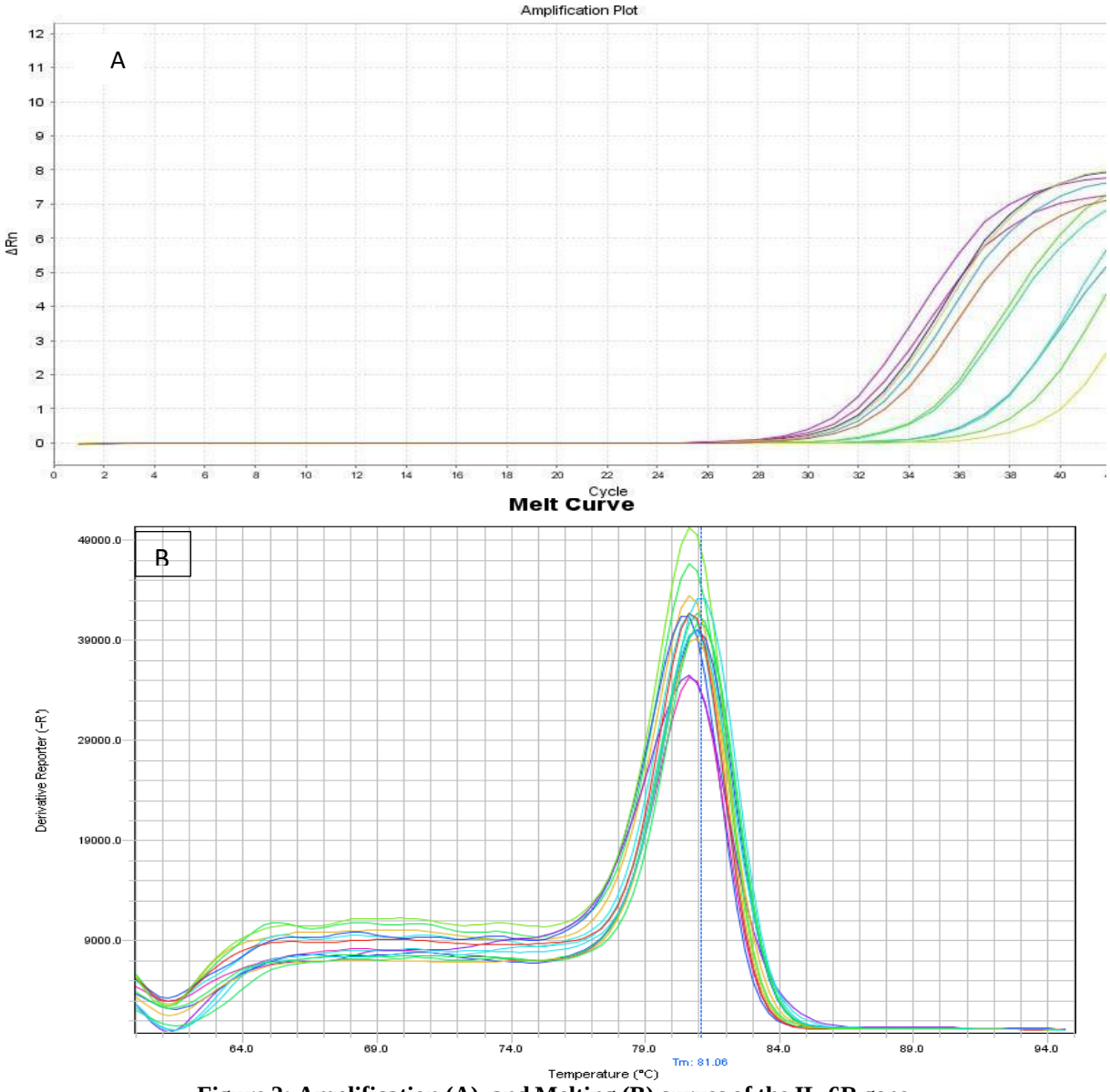


Figure 2: Amplification (A), and Melting (B) curves of the IL-6R gene.

5.2 Expression analysis

According to our results, exposure to IC50 concentrations for 72 hours result in a notable alteration in the expression of the IL-6R gene with resveratrol treatment. However, a significant down regulation in IL-6R ($P < 0.001$) mRNA levels was observed in cells treated with the resveratrol, showing an approximate 1.5 decreased compared to control cells (Figure 3).

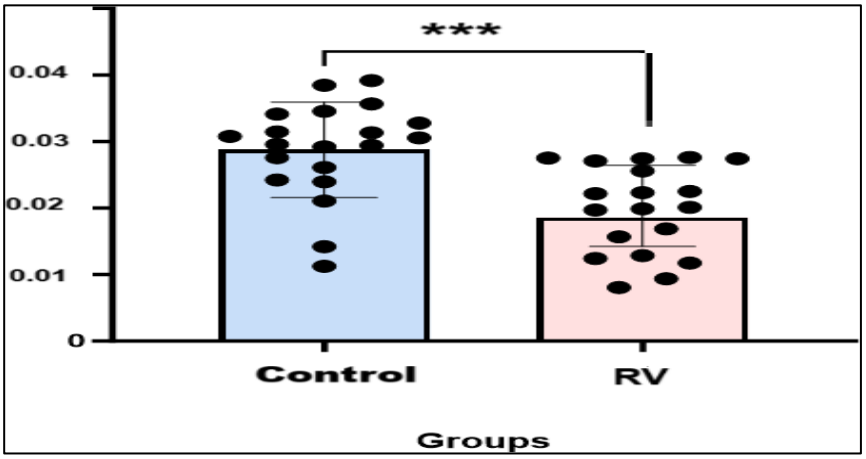


Figure 3. IL-6R gene expression in resveratrol (RV) treatment (40 µM) compared to control (0 µM) was significantly lower with the P-value < 0.001 (***).

6. DISCUSSION

The present study demonstrated that resveratrol exerts significant anti-leukemic activity against KG1-a acute myeloid leukemia (AML) cells. The MTT assay showed a significant reduction in cell viability in both a dose- and time-dependent manner, with IC_{50} values ranging from 30 to 40 µM. These findings indicate that increasing concentrations of resveratrol effectively inhibit the proliferation and survival of AML cells. Moreover, prolonged exposure enhanced the cytotoxic effect, suggesting that extended treatment may improve the therapeutic efficacy of resveratrol.[7]

The quality of the extracted RNA was confirmed prior to gene expression analysis. Agarose gel electrophoresis demonstrated distinct 28S and 18S rRNA bands, indicating that the isolated RNA was intact and of high quality. In addition, the real-time RT-PCR amplification and melting curves exhibited efficient amplification and a single, specific melting peak, confirming the specificity of the designed primers and the absence of non-specific amplification or primer-dimer formation. These findings validate the reliability of the RNA extraction process and the accuracy of the gene expression analyses performed in this study.

A major finding of the present study was the significant downregulation of IL-6R gene expression following treatment of KG1-a cells with the IC_{50} concentration of resveratrol for 72 hours. IL-6R mRNA expression was reduced by approximately 1.5-fold compared with untreated control cells ($P < 0.001$), indicating that resveratrol effectively suppresses IL-6R transcription. Given the critical role of IL-6R in mediating inflammatory signaling, promoting leukemic cell proliferation, and enhancing resistance to apoptosis through activation of downstream pathways such as JAK/STAT3, its reduced expression may contribute to the anti-leukemic effects of resveratrol. These findings suggest that resveratrol interferes with signaling pathways essential for leukemia cell survival and disease progression. The present results are consistent with previous studies demonstrating that resveratrol suppresses IL-6/IL-6R-mediated signaling, inhibits STAT3 activation, reduces inflammatory cytokine production, and promotes apoptosis in various hematological and solid malignancies, thereby limiting tumor cell growth and survival.[8,9]

The observed effects may be explained by several molecular mechanisms. Previous studies have shown that resveratrol induces apoptosis, causes cell-cycle arrest, suppresses inflammatory mediators, and inhibits angiogenesis.[10] In addition, resveratrol has been reported to activate SIRT, while suppressing NF-κB signaling, a pathway frequently associated with chronic inflammation, cancer progression, and resistance to cell death.[7, 11] Activation of SIRT1 may also enhance the activity of tumor suppressor proteins such as p53, further promoting apoptosis and inhibiting cellular proliferation.[12] The downregulation of IL-6R observed in the present study may consequently reduce activation of downstream pathways, including JAK/STAT signaling, which is known to support leukemia cell survival and immune evasion.[9]

These findings are consistent with previous reports demonstrating that resveratrol reduces the expression of pro-inflammatory mediators, particularly IL-6 and TNF-α, while attenuating oxidative stress and modulating inflammatory signaling pathways.[12–14] The pleiotropic nature of resveratrol enables it to target multiple molecular pathways involved in inflammation, cell proliferation, and apoptosis, highlighting its potential as

a multifunctional therapeutic agent with both anti-inflammatory and anti-cancer properties. Furthermore, its immunomodulatory and pro-apoptotic effects may enhance the efficacy of conventional leukemia therapies, supporting its potential use as an adjuvant treatment in AML management.[8, 12]

CONCLUSION

Resveratrol significantly reduced KG1-a cell viability in a dose- and time-dependent manner, demonstrating notable anti-leukemic activity. In addition, treatment with resveratrol markedly downregulated IL-6R gene expression, suggesting its role in modulating inflammatory and survival pathways involved in acute myeloid leukemia. These findings support the potential of resveratrol as a promising therapeutic agent for AML and provide a basis for further studies to elucidate its molecular mechanisms and clinical applications.

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