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# Quercetin sensitizes human myeloid leukemia KG-1 cells against TRAIL-induced apoptosis

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## Abstract

**Introduction:** Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Conventional treatments are associated with cytotoxicity and systemic side effects. Hence, efforts in the field of cancer treatment are focused on finding the strategies which can specifically target the tumor cells without affecting the normal cells. TNF-related apoptosis-inducing ligand (TRAIL) is a biological cytokine which has the mentioned specificity, but the resistance of some cancer cells limits its use as a therapeutic strategy. Recent studies have shown that quercetin (QUR) can be used as a sensitizing agent alongside with TRAIL. The present study showed that QUR can increase the effect of TRAIL-induced cytotoxicity in KG-1 cells.

**Materials and Methods:** In this descriptive study, the IC50 dose for QUR in the KG-1 cell line was first determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide assay. Then, the cells were treated with TRAIL and QUR for 12, 24, and 48 hr. The rate of apoptosis was measured by Annexin V/propidium iodide assay. Also, the molecular evaluation of candidate genes was accomplished before and after the treatment.

**Results:** The results indicated that QUR could sensitize the KG-1 cells against the TRAIL-induced apoptosis. This outcome is achieved by increasing the messenger RNA expression levels of the death receptor genes and reducing the expression of antiapoptotic proteins, as well as decreasing the expression of the NF-κB subunit.

**Conclusion:** Our findings suggest that QUR can sensitize the acute myeloid KG-1 cells against TRAIL. Moreover, the combinational therapy of these agents might promisingly improve the clinical efficacy of TRAIL in patients with AML.

KEYWORDS

acute myeloid leukemia (AML), apoptosis, leukemia, quercetin (QUR), TNF-related apoptosis-inducing ligand (TRAIL)

## 1 | INTRODUCTION

Acute myeloid leukemia (AML) is the most common malignancy that affects the myeloid lineage of the hematopoietic system in adults (Song et al., 2018). Although most patients with AML reach a partial remission after receiving the conventional therapies such as chemotherapies, the risk of recurrence, the resistance of the malignant cells and the side effects of these treatments are the inevitable problems that increasingly reduce the therapeutic value of these treatments (Dohner et al., 2017). The primary goal of cancer treatment research during the recent decades was to develop new strategies which can efficiently kill the tumor cells with fewer side effects and toxicity for the normal cells. The

  

selective induction of apoptosis is one of the main strategies which can specifically eliminate the malignant cells.

TNF-related apoptosis-inducing ligand (TRAIL), a member of TNF superfamily, is scientifically reported to be able to induce the process of apoptosis via binding to its agonist receptors only in the infected or cancer cells and the healthy cells are not affected (Walczak et al., 1999). There have been several studies in the literature reporting that TRAIL is an effective apoptosis inducer in a variety of cancer cell lines such as lung, prostate, skin, pancreas, kidney, colon, thyroid, central nervous system, breast, and the hematological malignancies (Lim et al., 2015; Younes et al., 2010). Although TRAIL can be widely used in the treatment of cancer, there are some kind of cancer cells which can use special strategies to resist against the TRAIL-induced apoptosis (TIA) and thereby, there is still a lot of debate about using TRAIL as an anticancer agent. As a solution, previous studies have stated that the cotreatment of TRAIL alongside with the other agents, such as natural compounds, chemotherapy agents, or radiation can overcome this problem. Quercetin (QUR) is one of the main dietary flavonoids that is plentifully found in a wide range of vegetables and fruits (Lesjak et al., 2018). As yet, several attempts have been reported that this flavonol exerts a broad range of pharmacological effects including the anti-inflammatory and antioxidant activities. Also, the previous studies demonstrated that QUR is correlated with the induction of cell death, mainly via an apoptotic process, and a selective antiproliferative effect in tumor cell lines without affecting normal cells (Calgarotto et al., 2018; Nair et al., 2004; G. L. Russo, Russo, & Spagnuolo, 2014; Spagnuolo et al., 2012).

In this study, we used both QUR and TRAIL to synergistically induce the apoptosis in human myeloid leukemia KG-1 cells for the first time and then we investigated whether QUR can augment the TIA through changing the messenger RNA (mRNA) expression levels of death receptors, apoptosis inhibitors, and the p65 gene or not.

## 2 | MATERIALS AND METHODS

### 2.1 | Cell culture

The AML cell line KG-1 purchased from general cell collection bank of Iran (Pasteur Institute, Tehran, Iran). The cell line maintained in exponential growth in Roswell Park Memorial Institute (RPMI)-1640 (Gibco, Paisley, UK) supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma-Aldrich, Gillingham, UK), 50 units/ml of penicillin (Sigma-Aldrich), and 50 µg/ml of streptomycin (Sigma-Aldrich) at 37°C in a humidified atmosphere containing 5% $CO_2$.

### 2.2 | Reagents

A 0.5 mg/ml stock solution of the recombinant human Apo2L/TRAIL (Merck, Darmstadt, Germany), was prepared in water. Also, a 100 mM stock solution of QUR (>99% pure; Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO), stored at −20°C, and then diluted as needed in RPMI-1640 to its final concentration in each culture dish. RPMI-1640 medium, FBS, penicillin, streptomycin, and phosphate buffered saline were purchased from Gibco (Carlsbad, CA). DMSO and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were obtained from Sigma-Aldrich.

### 2.3 | Cell viability assay

To assess the cytotoxicity of QUR in the KG-1 cells we used colorimetric MTT assay. Briefly, $1.5 \times 10^4$ exponentially growing cells/well in 100 µl of culture medium seeded in a 96-well plate (Sorfa, Zhejiang, China) and treated with increasing concentrations of QUR (0–300 µM). At the indicated time (12, 24, and 48 hr), 20 µl of reagent (5 mg MTT/ml medium) added and were incubated at 37°C for 4 hr. Then 100 µl of 10% SDS in 0.01 M HCl solution added to dissolve crystals. Finally, the optical density of wells was measured at 570 nm with Epoch ELISA reader (BioTek, Winooski, VT). Control wells only treated with DMSO. The viability of treated samples was expressed as a percentage of the control samples viability. The IC50 as a concentration inducing a 50% inhibition of cell growth was determined graphically using GraphPad Prism version 7.04 for Windows (GraphPad Software, La Jolla CA; www.graphpad.com). Values are means ± *S.E.M.* from three independent experiments, each performed in triplicate.

### 2.4 | Detection of apoptosis by flow cytometry

Apoptosis quantification was performed by Annexin V assay using the Apoptotest™-FITC Kit (Dako, Glostrup, Denmark) that quantify the levels of phosphatidylserine (PS) on the outer membrane of apoptotic cells. The mobilization of PS from the inner membrane to the outer membrane occurs during the process of apoptosis. This phenomenon causes PS on the outer membrane was labeled by Annexin V (a cellular protein with a high affinity for PS) conjugated with fluorescein isothiocyanate (FITC). KG-1 cells were treated with the indicated rhTRAIL treatment doses (50, 250 ng/ml), QUR treatment dose (100 µM) and cotreatment of various periods of time (12, 24, and 48 hr). Subsequent to incubation cells were harvested by careful centrifugation and washed twice with binding buffer, then the cell pellets were suspended in binding buffer and stained with Annexin V and propidium iodide (PI). Cells were incubated with 10 µl Annexin V-FITC and 5 µl PI for 15 min at room temperature in the dark, then the apoptosis of the cells was measured by the FACSCalibur (BD Biosciences, Franklin Lakes, NJ). Annexin V-FITC and PI fluorescence were detected in the FL-1 (green) and FL-2 (red) channels, respectively. Flow cytometry data were analyzed using FlowJo software Version 10.4.1 (Treestar, FlowJo).

### 2.5 | Quantitative real-time polymerase chain reaction

We used the primer-blast software on NCBI website (http://www.nchi.nlm.nih.gov) to BLAST our all primer sequences before the experiment. The primers used in this study and target sequences data listed in Table 1. After removing the medium, total RNA was extracted from $10^7$ cells using TRIzol reagent (Invitrogen, Milan, Italy) following the manufacturer's instructions. Five hundred

**TABLE 1** Summary of the primers sequences. All of the primers sequences are presented in 5′ to 3′ orientation

| Name | | Sequence |
|---|---|---|
| XIAP | Forward | AATAGTGCCACGCAGTCTACA |
| | Reverse | CAGATGGCCTGTCTAAGGCAA |
| DR4 | Forward | ACCTTCAAGTTTGTCGTCGTC |
| | Reverse | CCAAAGGGCTATGTTCCCATT |
| DR5 | Forward | GCCCCACAACAAAAGAGGTC |
| | Reverse | AGGTCATTCCAGTGAGTGCTA |
| c-IAP$_1$ | Forward | AGCACGATCTTGTCAGATTGG |
| | Reverse | GGCGGGGAAAGTTGAATATGTA |
| c-IAP$_2$ | Forward | TTTCCGTGGCTCTTATTCAAACT |
| | Reverse | GCACAGTGGTAGGAACTTCTCAT |
| NF-κB | Forward | GGCTACACAGGACCAGGGAC |
| | Reverse | AGAGCTCAGCCTCATAGAAGCC |
| c-FLIP | Forward | AGCTCACCATCCCTGTACCT |
| | Reverse | TGTGCTGCAGCCAGACATAA |

*Note*. c-FLIP: cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; c-IAP: cellular inhibitor of apoptosis protein; DR: death receptor; NF-κB: nuclear factor kappaB; qRT-PCR: quantitative real-time polymerase chain reaction; XIAP: X-linked inhibitor of apoptosis.

nanograms of the extracted RNA was reverse-transcribed into cDNA using, PrimeScript™ reagent Kit (Takara, Japan). Real-time polymerase chain reaction (PCR) was performed in duplicate for all samples using the RealQ Plus 2x Master Mix Green (Ampliqon, Herlev, Denmark). The analysis of melting curves was performed using the real-time PCR system (Rotor-Gene 6000; Corbett Life Science, Sydney, Australia). ACTB was used as an internal control.

### 2.6 | Statistical analysis

Statistical analyses were performed using the GraphPad Prism version 7.04 for Windows (GraphPad Software; www.graphpad.com). Flow cytometry data (reporting percentages) and gene expression data were log base 10 (y = log10 y) transformed before performing a two-way analysis of variance with the post hoc Tukey and Dunnett multiple comparison tests. Data were presented as the mean ± *S.E.M.* and were representative of at least three independent experiments. Statistical tests were two-sided. $P$ values < 0.05 were considered to be statistically significant.

## 3 | RESULTS

### 3.1 | QUR has a cytotoxicity effect against KG-1 cells

According to our results, QUR showed a cytotoxicity effect against the KG-1 cells. To measure the inhibitory effect of QUR on the growth of KG-1 cells, we measured the IC50 value following the MTT assays. As shown in Figure 1, the IC50 value of QUR was measured 105.6 μM for KG-1 cells at the 12 hr of incubation. Previous studies demonstrated that this inhibitory effect of QUR on the growth of cells is due to the induction of apoptosis in hematological cell lines

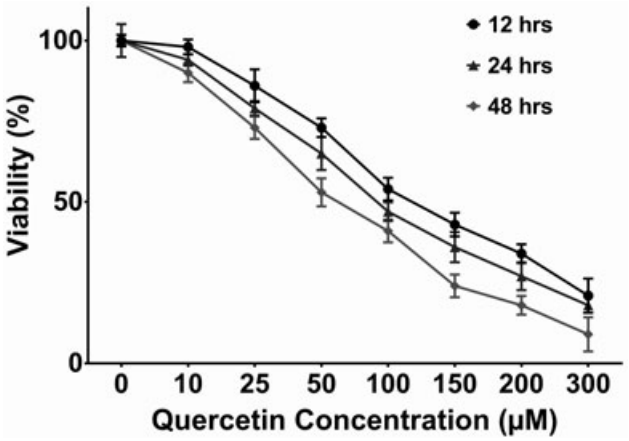


**FIGURE 1** The cytotoxic effect of quercetin on the growth of KG-1 cells. The IC50 values in KG-1 cells were determined by MTT assays in the presence of various concentrations of quercetin (0–300 μM) for 12, 24, and 48 hr of incubation. The data is the mean ± *S.E.M.* of three independent experiments. IC50: The half maximal inhibitory concentration; MTT: methyl thiazolyl tetrazolium assay; *S.E.M.*: Standard error of the mean

such as HL-60 and the other cancer cell lines (Calgarotto et al., 2018; Lu et al., 2017). Annexin V/PI assay showed that the percentage of apoptosis in QUR-treated cells was associated with a statistically significant increase compared with the control group.

### 3.2 | QUR augments the TRAIL-induced cell death in KG-1 cells

The KG-1 cells were prepared to do the apoptosis analysis via Annexin V-FITC following the treatment with QUR (100 μM) in the presence or absence of rhTRAIL (50, 250 ng/mL) for 12, 24, and 48 hr. Early apoptotic cells (PI negative and Annexin V-FITC positive) and late apoptotic cells (PI positive and Annexin V-FITC positive) were shown in the quadrant 4 and 2, respectively. In the QUR-treated group alone, the apoptosis was significantly increased in a time-dependent manner, during the indicated times. The KG-1 cells were also treated with DMSO as the control group.

The results specified that QUR was able to augment the TIA in KG-1 cells. The cotreatment of rhTRAIL with QUR induced a statistically significant increase in a time-dependent manner compared to the untreated group. Yet, it is important to note that, there was no statistically significant change in the rate of apoptosis in the cells treated with rhTRAIL alone compared to the untreated cells. The results of Annexin V/PI assay following the treatment with QUR and rhTRAIL are shown in Figures 2, 3, and Table 2.

### 3.3 | QUR did not induce any change in the c-FLIP gene expression in acute myeloid KG-1 cells (at mRNA level)

The recent studies have shown that the resistance of some cancer cells to the effect of TRAIL is correlated with the c-FLIP gene

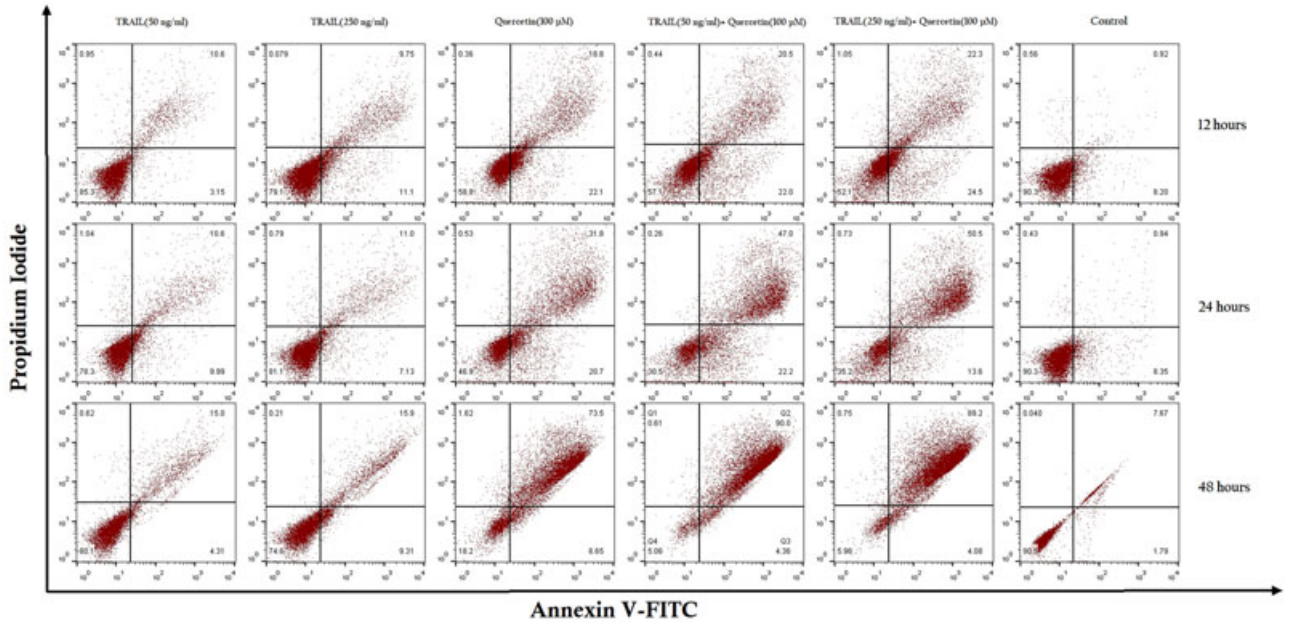


**FIGURE 2** Assessment of quercetin effect on the rhTRAIL–induced apoptosis using Annexin V staining and PI. Annexin V/PI analysis was completed on KG-1 cells treated with quercetin alone (100 µM), TRAIL alone (50, 250 ng/ml), and the combination of quercetin and rhTRAIL (50, 250 ng/ml of rhTRAIL in combination of 100 µM of quercetin) for 12, 24, and 48 hr of incubation. For the representative FACS plots, early apoptotic cells (Annexin V+/PI−) are shown in Q3 (the right lower quadrant), late apoptotic cells (Annexin V+/PI+) are showed in Q2 (the right upper quadrant), necrotic cells Annexin V−/PI+are showed in Q1 (the left upper quadrant), and double negative (healthy) cells Annexin V−/PI− are showed in Q4 (the left lower quadrant). The data present three independent experiments. FACS: fluorescence-activated cell sorter; FITC: fluorescein isothiocyanate; PI: propidium iodide; Q: quadrant; rhTRAIL: recombinant human tumor necrosis factor related apoptosis-inducing ligand [Color figure can be viewed at wileyonlinelibrary.com]

expression (Ricci et al., 2004). Several studies have reported that the downregulation of c-FLIP in some tumor cells could deteriorate the cell sensitivity against TIA (Bleumink et al., 2011; Lagneaux et al., 2007; Roue et al., 2007; Wu et al., 2016). The c-FLIP gene is expressed into the several splicing variants at the mRNA level. However, two of them are translated into the proteins including the c-FLIP long form (c-FLIPL) and the c-FLIP short form (c-FLIPS).

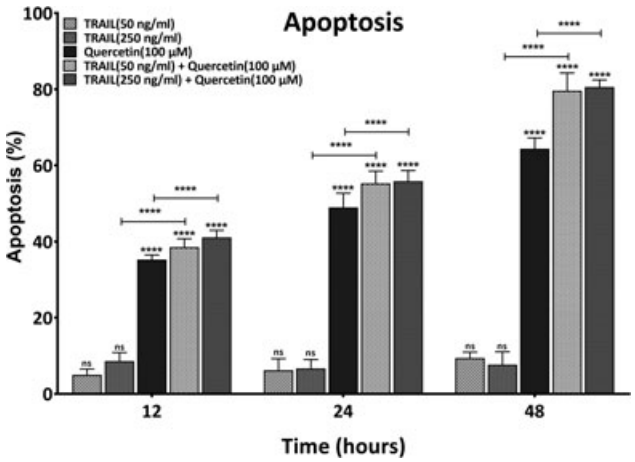


**FIGURE 3** The bar graph displays the average total percent of apoptotic cells (Annexin V+/PI− and Annexin V+/PI+ cells) ± *S.E.M.* The average was calculated from three independent experiments. ns means nonsignificant; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$. PI: propidium iodide; *S.E.M.*: Standard error of

The ability of c-FLIP to affect the TRAIL signaling pathway is one of the important factors which can induce the cellular resistance against TIA. Therefore, we measured the expression level of this gene at the mRNA level to find out its potential role in the QUR-sensitized TIA in KG-1 cells. Then, the primers were designed in such a way to be able to amplify both of the c-FLIPL and c-FLIPS. As shown in Figure 5d, it can be understood that QUR could not make any significant change in the expression levels of the c-FLIP gene in any of the treated groups during the indicated times.

### 3.4 | QUR increased the expression levels of DR genes in acute myeloid KG-1 cells (at mRNA level)

Sheridan et al. (1997) have shown that TRAIL can induce the process of apoptosis through binding to the DR4 and DR5. To examine the possible role of these receptors in sensitizing the KG-1 cells to TIA, we evaluated their mRNA expression levels. Subsequently, we found that QUR could induce a time-dependent increase in the mRNA expression levels of DR4 and DR5 genes in KG-1 cells. The real-time PCR results are shown in Figure 4.

The rhTRAIL-treated cells alone have shown no significant change in the expression of these receptor genes during the indicated times. However, the cotreatment of rhTRAIL with QUR could make a noticeable increase in the expression of DR4 gene in a time-dependent manner.

The DR5 gene expression is roughly similar to DR4 at the transcriptional level. However, the combination of rhTRAIL with

**TABLE 2** Apoptosis rate of the KG-1 cells in the control group and other groups treated with rhTRAIL and quercetin (reporting percentages) during the indicated times. The average was calculated from three independent experiments

| Groups | 12 hr | 24 hr | 48 hr |
|---|---|---|---|
| Control | 7.90 ± 2.96 | 9.23 ± 1.75 | 10.88 ± 3.12 |
| TRAIL (50 ng/ml) | 12.92 ± 1.50 | 15.45 ± 3.02 | 20.30 ± 1.52 |
| TRAIL (250 ng/ml) | 16.50 ± 2.24 | 15.94 ± 2.31 | 18.58 ± 3.34 |
| QUR (100 μM) | 43.12 ± 1.25 | 58.20 ± 3.69 | 75.25 ± 2.86 |
| TRAIL (50 ng/ml) + QUR (100 μM) | 46.38 ± 2.24 | 64.52 ± 3.21 | 90.48 ± 4.64 |
| TRAIL (250 ng/ml) + QUR (100 μM) | 48.99 ± 1.84 | 65.09 ± 2.78 | 91.44 ± 1.89 |

*Note.* QUR: quercetin; rhTRAIL: recombinant human tumor necrosis factor–related apoptosis-inducing ligand.

QUR had a greater effect on the expression of the DR5 gene compared with the DR4.

### 3.5 | QUR decreased the expression of apoptosis inhibitor genes in the acute myeloid KG-1 cells (at mRNA level)

The XIAP, c-IAP1, and c-IAP2 genes were evaluated to determine the expression levels of the mRNAs in both of the treated groups and the untreated group. On the basis of the results shown in Figure 5c, it can be concluded that the XIAP mRNA expression level did not differ significantly in any of the groups at 12 hr after the treatment. In addition, the expression of XIAP gene in the rhTRAIL-treated group was not associated with a statistically significant change at 24 and 48 hr after the treatment compared to the control group. Meanwhile, we found that QUR treatment of KG-1 cells had been able to significantly downregulate the mRNA expression levels of XIAP either in QUR alone or in combination with rhTRAIL-treated cells at 24 and 48 hr compared with the control group.

The cotreatment of rhTRAIL and QUR first made a minor increase in the c-IAP1 gene expression at 12 hr after the treatment. Then it resulted in a statistically significant reduction in the c-IAP1 gene expression at 24 and 48 hr in KG-1 cells. It should be noted that the simultaneous treatment of rhTRAIL (50 ng/ml) with QUR, as well as treating with QUR alone, could dramatically affect the expression of this gene just by 48 hr after the treatment in KG-1 cells.

The observed changes in c-IAP2 gene expression were associated with a slight statistically significant increase at 12 hr after the treatment only in the rhTRAIL-treated groups. But surprisingly the expression changes continued with a statistically significant decrease in the rhTRAIL-treated groups compared with the untreated group at 24 and 48 hr. The treatment with QUR alone did not change the c-IAP2 gene expression within 12 hr after the treatment, while the expression of this gene was significantly reduced during the 24 and 48 hr after the treatment. The cotreatment of rhTRAIL with QUR caused no statistically significant changes in the expression of the c-IAP2 gene compared with the rhTRAIL-treated group alone at 12 hr after the treatment. But noticeably the expression of this gene was associated with a significant decrease at 24 and 48 hr. The real-time PCR results for c-IAP1 and c-IAP2 are shown in Figure 5a,b, respectively.

### 3.6 | QUR decreased the expression of NF-κB (p65 subunit) gene in the acute myeloid KG-1 cells (at mRNA level)

A number of studies have shown that TRAIL signaling pathway can result in cell survival and proliferation under certain conditions (Jeremias & Debatin, 1998; L. Zhang & Fang, 2005). Hence, this pathway can play an inverted role in response to the TRAIL. In addition, it has been shown that the expression of some apoptotic

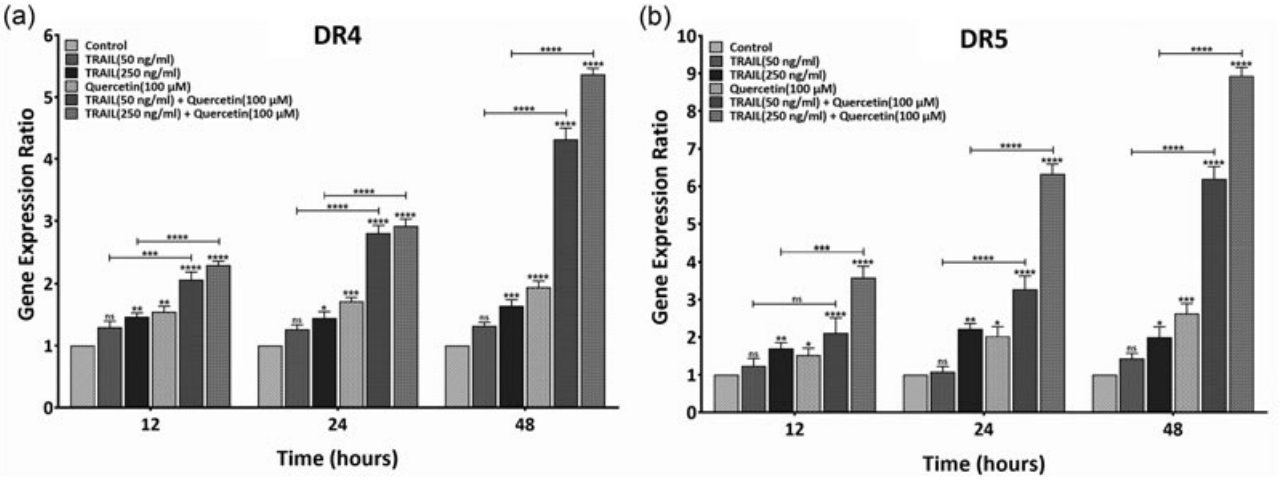


**FIGURE 4** The real time-PCR data for (a) DR4 and (b) DR5 genes expression in KG-1 cells after 12, 24, and 48 hr of the cotreatment of rhTRAIL (50, 250 ng/ml) with quercetin (100 μM). The data is presented as means ± *S.E.M.* of three independent experiments. ns means nonsignificant; *$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$; ****$p \le 0.0001$. DR: death receptor; PCR: polymerase chain reaction; rhTRAIL: recombinant human tumor necrosis factor related apoptosis-inducing ligand; *S.E.M.*: standard error of the mean

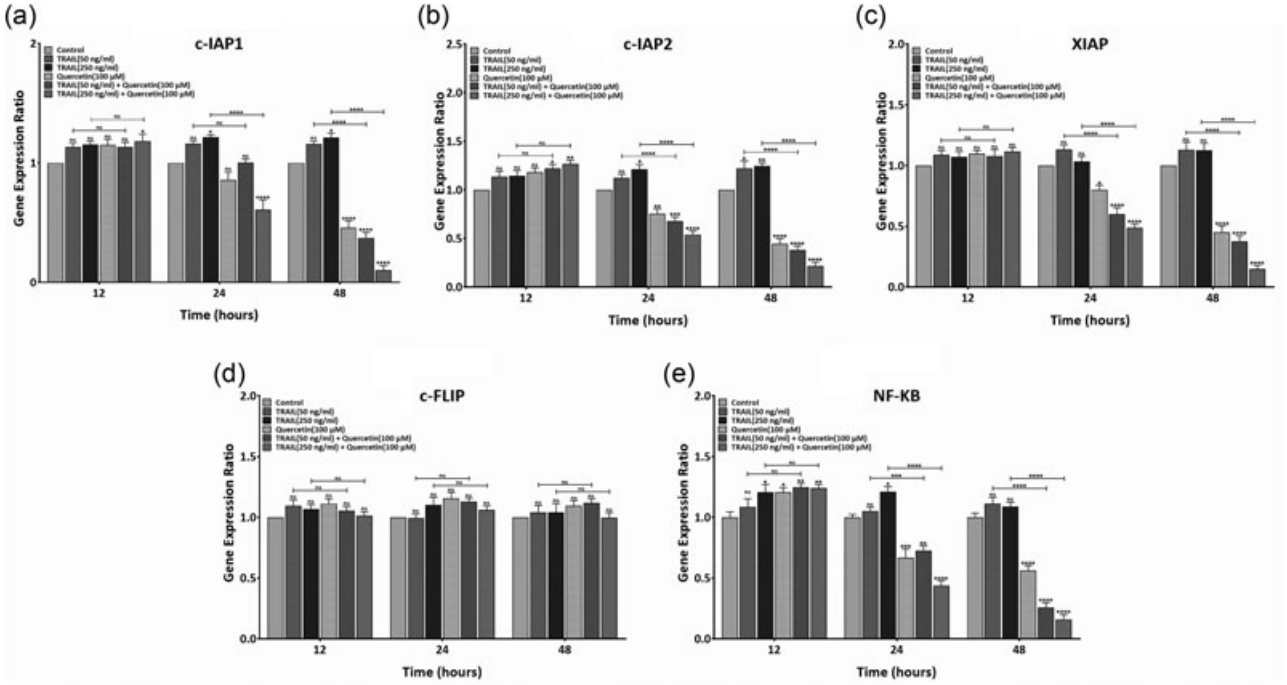


**FIGURE 5** The real time-PCR data for (a) c-IAP1, (b) c-IAP2, (c) XIAP, (d) c-FLIP, and (e) NF-KB expression in KG-1 cells after 12, 24, and 48 hr of the cotreatment of rhTRAIL (50, 250 ng/ml) with quercetin (100 μM). The data are presented as means ± *S.E.M.* of three independent experiments. ns means nonsignificant; *$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$; ****$p \le 0.0001$. c-FLIP: cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; c-IAP: cellular inhibitor of apoptosis protein; PCR: polymerase chain reaction; NF-κB: nuclear factor kappaB; rhTRAIL: recombinant human tumor necrosis factor related apoptosis-inducing ligand; *S.E.M.*: Standard error of the mean; XIAP: X-linked inhibitor of apoptosis protein

inhibitors such as XIAP and c-FLIP is controlled by this signaling pathway (Micheau, Lens, Gaide, Alevizopoulos, & Tschopp, 2001; Pahl, 1999). Therefore, we decided to investigate the expression of the p65 subunit of NF-κB complex at the mRNA levels in KG-1 cells.

On the basis of the results shown in Figure 5e, it can be concluded that the treatment with 50 ng/ml of rhTRAIL alone had no statistically significant effect on the expression of p65 gene in KG-1 cells during the indicated times. However, the treatment with 250 ng/ml of rhTRAIL alone led to a slight increase in the expression of this subunit in KG-1 cells. While the treatment with QUR alone caused a slight increase in the expression of p65 at 12 hr, QUR was able to reduce the expression of this subunit at 24 and 48 hr. This reduction was recorded statistically significant.

The cotreatment of rhTRAIL with QUR caused no significant changes in the expression of p65 gene compared with the rhTRAIL-treated group alone at 12 hr. But the expression of this gene was associated with a significant decrease at 24 and 48 hr after the treatment.

## 4 | DISCUSSION

In the present report, we demonstrated that QUR, a herbal flavonol, alone and alongside with the use of TRAIL can significantly increase the apoptotic effects of TRAIL against the acute myeloid KG-1 cells. This finding is consistent with the results of previous studies exploring the effect of QUR on TIA in other tumor cells (Jacquemin et al., 2012; J. Y. Kim et al., 2008; Y. H. Kim, Lee, Jeong, Guo, & Lee, 2008; M. Russo, Nigro, Rosiello, D'Arienzo, & Russo, 2007). As yet, many efforts have been made to identify the pathways which QUR can cause sensitivity against TRAIL. According to the past studies, the most important mechanism of QUR-mediated sensitization is correlated with the regulating of numerous genes involved in the apoptosis and cell cycle (Chen, Wang, Zhuang, Zhang, & Lin, 2007; Jung, Heo, Lee, Kwon, & Kim, 2010; J. Y. Kim et al., 2008; Youn, Jeong, Jeong, Kim, & Um, 2013). According to the results shown in Figure 3, as well as the Dijk et al. (2011) study, KG-1 cells display a high-level resistance to rhTRAIL showing a cell viability of >85% at the rhTRAIL alone treatment. We found that the mechanism by which QUR leads to overcoming the cell resistance against TRAIL is probably due to the induction of DRs expression and the downregulation of p65, XIAP, c-IAP1, and c-IAP2. In the present research, the increased expression of DR genes at the mRNA level is consistent with the findings of past studies which have indicated that how the rate of upregulation of DRs influences the intensity of TRAIL-induced death receptor-mediated apoptosis (Chen et al., 2007; Jung et al., 2010; Siegelin, Reuss, Habel, Rami, & von Deimling, 2009; Yi et al., 2014).

Previous research have shown that the upregulation of CCAAT enhancer-binding protein homologous protein (CHOP) is one of the important cellular responses under the condition of exposure to QUR, which might be considered as a major key factor to upregulate the expression of DR genes (Sung, Prasad, Ravindran, Yadav, & Aggarwal, 2012; Yi et al., 2014). The results of Yi et al. (2014) showed

that under using quercetin, the reactive oxygen species (ROS) are generated in response to the ER-stress condition. The ROS play an important role in sensitizing the tumor cells against TIA (Yi et al., 2014). They also indicated that the ROS can promote the expression of CHOP by affecting its downstream factors such as IRE1a-JNK signaling, PERK, and ATF6. Finally, they concluded that CHOP was able to enhance the expression level of the DR5 gene via binding to the promoter region. So far, all efforts have been made in this regard to determine the role of DR genes upregulation in increasing the rate of TIA. As mentioned above, this upregulation is a result of the treatment of cancer cells with quercetin. Of course, only measuring the expression of DR genes at the mRNA level is not enough to enable us to make a definite opinion about the role of these genes in eliminating or reducing the resistance of KG-1 cells against TIA. Therefore, it is necessary to examine the expression of these receptors at the protein level and on the cell surface.

In addition to the induction of DRs, the downregulation of c-FLIP by QUR can probably be one of the other reasons for the enhancement of TIA. The results of our study have shown that QUR could induce no significant changes in the mRNA expression levels of the c-FLIP gene. The possible explanation for this finding might be that the expression of c-FLIP is hardly controlled at the posttranslational level of regulation by the ubiquitin-proteasome system (J. Y. Kim et al., 2008). This finding is consistent with the results of the previous studies exploring the effect of QUR on the expression of the c-FLIP gene. For example, Manouchehri, Turner, and Kalafatis (2018) showed that QUR had no increasing or decreasing effect on the level of c-FLIP mRNA, but it was able to reduce the protein level of the c-FLIP gene. More comprehensive data about the c-FLIP expression at the protein level alongside with its possible role on the TRAIL signaling pathways may help us to establish more accurate information about the function of QUR in KG-1 cells. Dijk et al. (2011) studied the effect of bortezomib as a proteasome inhibitor in sensitizing KG-1 cells against TIA. They showed that bortezomib could dramatically increase the amount of TIA. This finding suggests that the ubiquitin-proteasome system can also play an important function in inducing a resistance against TRAIL in KG-1 cells by controlling the expression of a c-FLIP gene at the protein level.

Recent studies have reported that the signaling pathways such as NF-κB and Akt/PKB, which play an important role in the cell growth and survival can also be considered more effective in inducing resistance against TIA by interfering with the TRAIL signaling pathways. Previous studies have revealed that QUR can affect the activity of these pathways. Yi et al. have shown that QUR can decrease the activity of NF-κB signaling pathway by reducing the expression level of p50 subunit and IKK gene in the cancer cells (X. Zhang et al., 2004). In the present study, QUR has been able to reduce the expression of p65 subunit at the mRNA level. This subunit forms the most common NF-κB heterodimer in normal cells (Hayden & Ghosh, 2004). This finding suggests that the reduction of p65 gene expression may lead to a reduction in the formation of the NF-κB complex, which consequently leads to a decrease in the activity of this signaling pathway in KG-1 cells. To confirm this finding, further investigations about the activity of this pathway in KG-1 cells are highly required. Moreover, it has been shown that the activity of the NF-κB pathway can also affect the expression of the inhibitors of apoptosis (IAPs) such as XIAP, c-IAP1, and c-IAP2. The present study's results indicated that QUR can also reduce the expression of IAP genes. Although a comprehensive analysis of the apoptosis process was so further than the scope of this study, we suggest that the downregulation of the NF-κB pathway in KG-1 cells may probably be one of the possible explanations for the reduction of IAP genes expression. Therefore, it is recommended that supplementary research be conducted to determine the role of this signaling pathway on the expression of IAP genes.

The Akt/PKB signaling pathway is one of the other pathways that has been discussed so far and it has been shown that its elevated activity can protect the cancer cells against TIA (Nesterov et al., 2001). Thakkar et al. (2001) have demonstrated that the pharmacological inhibition of this signaling pathway in tumor cells can lead to a significant increase in the rate of TIA (Nesterov et al., 2001). Through another research study, Y. H. Kim and Lee (2007), also found that the Akt/PKB pathway is one of the other important targets of QUR in tumor cells. Because the AKT/PKB pathways can be considered as an enhancer of TIA, this finding provides a future insight for a broader range of research about the role of this signaling pathway in KG-1 cells.

The present study was designed to determine the effect of QUR as a sensitizing agent alongside with the use of TRAIL in KG-1 cells. The most obvious finding of this project is that QUR was able to increase the sensitivity of the acute myeloid KG-1 cells against TIA. In this way, QUR could upregulate the expression of DR genes including DR4 and DR5 and reduce the expression of the p65 subunit and antiapoptotic proteins such as XIAP, c-IAP1, and c-IAP2 at the mRNA level. Also, our results have confirmed the fact that QUR can induce no significant changes in the expression of a c-FLIP gene at the mRNA level. Our findings suggest that QUR can be considered as an effective and useful sensitizing agent for the treatment of cancer cells and this may offer a reasoning for the use of QUR to improve the clinical efficacy of TRAIL as a potential therapeutic target in acute myeloid KG-1 cells.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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