

Received: 2019.02.20  
Accepted: 2019.03.19  
Published: 2019.04.07

# The Mechanism of Bufalin-Induced Apoptosis of K562/A02

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**Source of support:** Departmental sources

**Background:** In clinical practice, many patients become multidrug resistant during chemotherapy, resulting in reduced or no healing effect. Therefore, the present study focused on bufalin, which is extracted from a traditional Chinese medicine named Chan Su (*Venenum bufonis*). We assessed the effect of bufalin in reversing K562/A02 cell drug resistance and inducing apoptosis, and explored the possible mechanism by which bufalin induces K562/A02 cell apoptosis.





**Material/Methods:** We used flow cytometry to evaluate intracellular ADM concentration, and RT-PCR and Western blot analysis were used to assess the effect of nuclear factor erythroid-2-related factor 2 (Nrf2) bufalin-related resistance gene expression. We used MTT and flow cytometry to detect apoptosis, and RT-PCR and Western blot were used to detect endoplasmic reticulum stress and apoptosis gene action.

**Results:** We found that bufalin can increase the concentration of Adriamycin (ADM) in K562/A02 cells by inhibiting the expression of Nrf2 and related drug resistance factors. The results showed that bufalin induced apoptosis of K562/A02 cells by the IRE1 $\alpha$ /TRAF2/JNK/caspase-12 pathway.

**Conclusions:** These results suggest bufalin can reverse drug resistance in K562/A02 cells and that it induces apoptosis of K562/A02 cells by the IRE1 $\alpha$ /TRAF2/JNK/caspase-12 pathway.

**MeSH Keywords:** **Apoptosis • Drug Resistance, Multiple • Endoplasmic Reticulum Stress • K562 Cells • Medicine, Chinese Traditional**

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/915802>

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## Background

Leukemia is a malignant disease [1]. With the increasing incidence and mortality of leukemia, the problem of successful treatment has become a focus of attention [2]. At present, in clinical practice, chemotherapy is the first choice for the treatment of various types of leukemia [2,3]. While more chemotherapy treatment options for leukemia patients are now available and the average survival time of patients is significantly longer than before, there are still many patients undergoing chemotherapy who develop tumor drug resistance and multidrug resistance (MDR) leading to chemotherapy failure [4].

At present, common drug resistance reversal agents have single-chemical effects and obvious adverse effects, which greatly limit their application [5,6]. There are many kinds of traditional Chinese medicines with a large spectrum of effects and few adverse effects [6]. Compared with normal cells, tumor cells are more likely to be in an anoxic state, affecting the correct expression of folded proteins in the endoplasmic reticulum (ER) [3,7,8], which in turn causes a large accumulation of erroneous protein, resulting in endoplasmic reticulum stress (ERS), which increases unfolded protein response (UPR) [9,10]. When ERS is not severe, activation of UPR can prevent apoptosis, so as the degree of UPR is increased, ERS becomes more and more severe [11]. When ERS time is too long or if strength is too great, exceeding the capacity of the endoplasmic reticulum, the endoplasmic reticulum will phosphorylate JNK under the induction of IRE1, initiating programmed apoptosis. We speculated that UPR is likely to be a cause of drug resistance in tumor cells [9,11].

Researchers are attempting to find efficient drug resistance reversal agents in traditional Chinese medicines. This study found that bufalin, extracted from a Chinese herbal medicine, inhibits tumor cell proliferation and drug resistance [12,13]. We assessed the apoptosis-inducing effect of bufalin on K562/A02 cells and analyzed the possible mechanisms involved.

We studied the induction of tumor cell apoptosis and multidrug resistance by traditional Chinese medicine, which is expected to promote further research on traditional Chinese medicine preparations as anti-tumor and anti-MDR agents [14], so that they can be applied to clinical medicine as soon as possible to improve the treatment of drug-resistant leukemia.

## Material and Methods

Cell lines K562 and K562/A02 cells were purchased from the Shanghai Institute of Cell Sciences, Chinese Academy of Sciences (Shanghai, PRC). ADM and bufalin were purchased from Sigma-Aldrich (St. Louis, MO). Nrf2, HO-1, P-gp, Bax,

Bcl-2, cytc, PAPR, IRE1 $\alpha$ , TRAF2, JNK, p-JNK, and Caspase-12 antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX). The apoptosis detection kit was purchased from KeyGen Biotech (Nanjing, PR China). RT-PCR and HiPerfect transfection kits were purchased from Qiagen (Hilden, Germany).

### Cell culture

The cells were cultured in an incubator containing 5% CO<sub>2</sub> at 37°C. When the cell coverage in the dish was above 85%, the cells were digested using 0.25% trypsin. The growth of the cells was observed daily. Generally, after 48–72 h, the growth density of the cells was 85–90%, and the cells were again treated with trypsin or frozen.

### MTT method

When the cells entered logarithmic growth phase, cell digestion was begun, cell concentration was adjusted, cells were inoculated into 96-well plates, and treatment was administered according to group. After 24 h of culturing, 20  $\mu$ l of MTT (5 mg/ml) was added to each well. After 4 h, the supernatant was discarded and 150  $\mu$ l of DMSO was added to each well. After 10 min, a microplate reader was used to assess OD at 570 nm.

### Determination of the safe concentration of bufalin

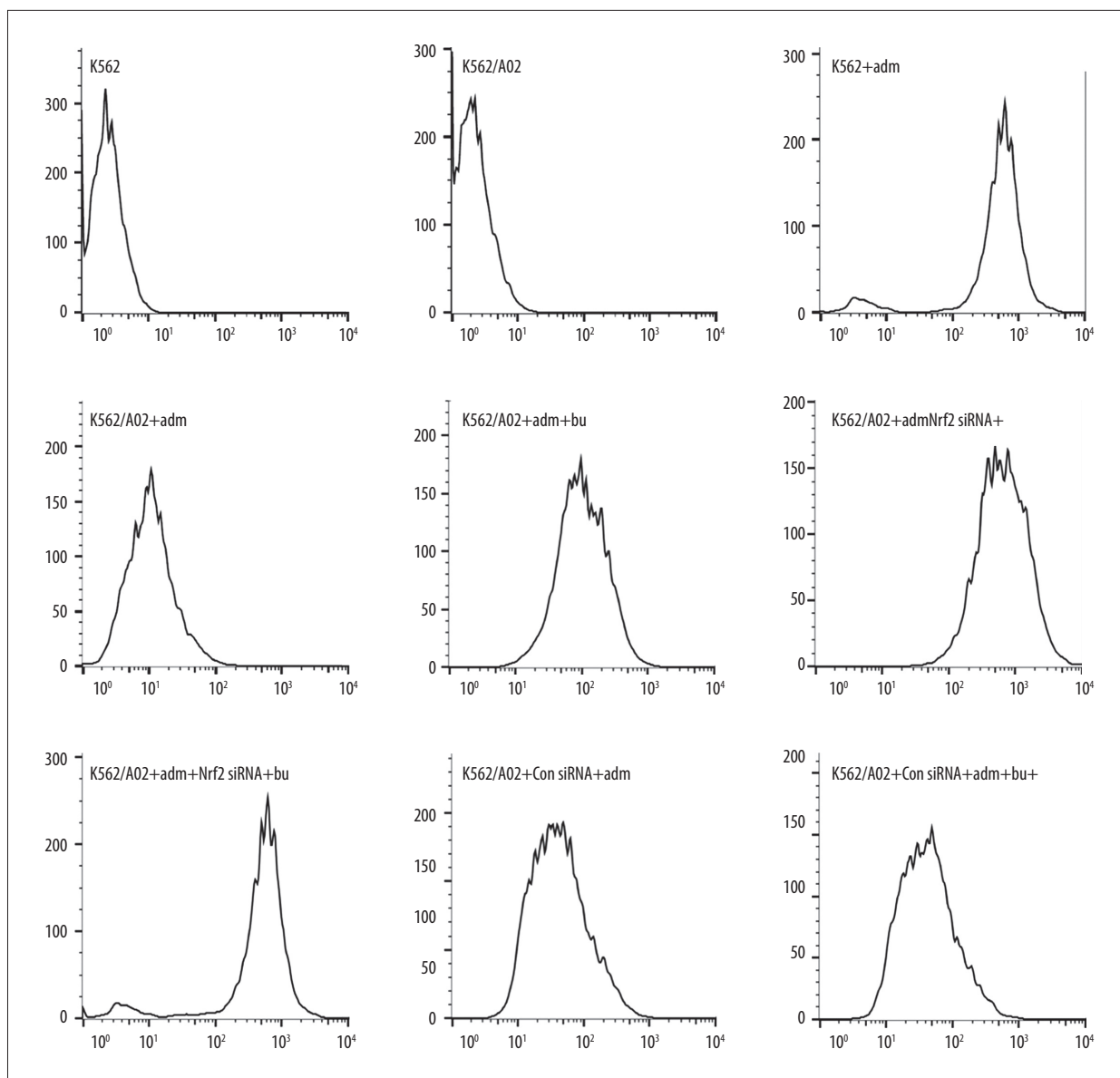
K562/A02 cells were added to a final concentration of 12.5 nM, 25 nM, 50 nM, and 100 nM of bufalin. OD value was measured on a microplate reader after 48 h of incubation. We assessed the effects of various concentrations of bufalin on K562/A02 cells, and we selected a concentration less than the IC<sub>10</sub> as a non-cytotoxic dose for use in the following experiments.

### Flowcytometry to detect intracellular ADM concentration

K562 and K562/A02 cells in logarithmic growth phase were divided into 4 groups: 1) K562 cells; 2) K562/A02; 3) K562/A02+ADM; and 4) K562/A02+bufalin+ADM. The final concentration of bufalin was the concentration required for IC<sub>10</sub>. The cell concentration was adjusted in each group to 1 $\times$ 10<sup>5</sup> cells/mL. Bufalin was added to the bufalin group and groups were cultured for 48 h, centrifuged, double-washed with PBS, and resuspended. Using a characteristic autofluorescence ADM, ADM cells were detected by flow cytometry according to fluorescence intensity.

### Cell interference

K562/A02 cells (3 $\times$ 10<sup>5</sup>) in the logarithmic growth phase were seeded on culture plates. At the time of transfection, a cell culture plate with a fusion rate of 40–50% was selected. Transfection was performed in strict accordance with the



**Figure 1.** Flow cytometry to detect ADM concentration in each group of cells. The relative fluorescence intensity of each relative group showed that the results were statistically significant with mean  $\pm$ SEM,  $n=4$ , between experimental groups ( $P<0.05$ ,  $P<0.01$ ).

lipofectamine 2000 transfection instructions. After transfection, the cells were cultured at 37°C for 6 h, then the transfection solution was removed and fresh medium was added for another 48 h.

#### RT-PCR testing method

RNA extraction was accomplished using TRIzol RNA extraction kit according to the operating instructions. Fragments were amplified using cDNA as a template. The amplification conditions were pre-denaturation at 95°C for 2 min, denaturation at 95°C for 20 s, annealing at 56°C for 20 s, and extension at 72°C for 20 s. After 30 cycles, there was a further extension at 72°C for 7 min.

#### Western blot method

K562/A02 cells in the log phase of growth were inoculated onto a 6-well plate, and the drug was administered according to the above-described groups. The protein sample was extracted after 48 h of culturing. Protein concentration was measured using a BCA kit. After the gel was prepared, the protein was loaded, SDS gel electrophoresis was performed, followed by wet film transfer, then skim milk powder was blocked, and the primary antibody was added at 4°C overnight and cells were washed with PBS 3 times. The next day, the secondary antibody was incubated for 2 h at room temperature, washed 3 times with PBS, and analyzed using a BioRad system and Quantity One image analysis.

## Statistical analysis

The experimental data are expressed as means and standard deviations. One-way ANOVA was used for comparison between groups. SPSS 21.0 software was used to analyze the data. Charts were produced using the Prism 6.0 software operating system.

## Results

### Flow cytometry to detect intracellular ADM concentration:

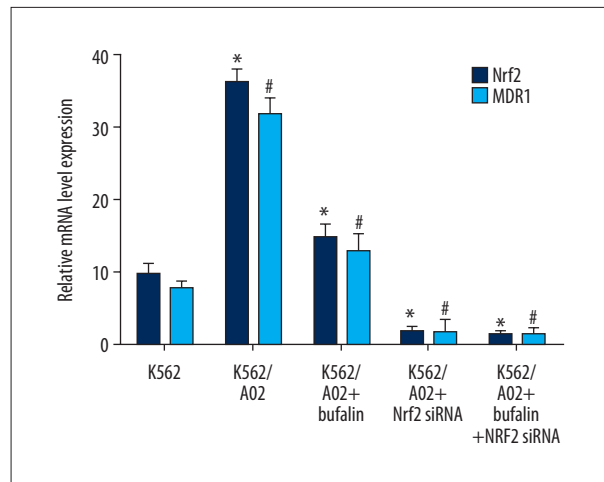
K562+ADM cell fluorescence intensity was significantly higher than K562/A02+ADM intensity ( $P<0.05$ ); K562/A02+ADM+bufalin fluorescence intensity was significantly higher than K562/A02+ADM intensity ( $P<0.01$ ); and Nrf2 siRNA group fluorescence intensity was significantly higher than K562/A02+ADM + bufalin intensity ( $P<0.01$ ), as shown in Figure 1.

### Bufalin downregulates the expression of Nrf2 and its downstream target genes

The expression of Nrf2 and MDR1 mRNA in each group was found to be: (1) The expression of Nrf2 and MDR1 mRNA in the K562 group was significantly weaker than that in the K562/A02 group and K562/A02+bufalin group ( $P<0.01$ ); and (2) The expression of Nrf2 and MDR1 mRNA was the highest in the K562/A02 group ( $P<0.01$ ). The expression of Nrf2 and MDR1 mRNA in the K562/A02+bufalin group was lower than that in the K562/A02 group ( $P<0.01$ ). The expression of Nrf2 and MDR1 mRNA in the K562 group was lower than that in the K562/A02 group ( $P<0.01$ ). (3) The expression of Nrf2 and MDR1 mRNA in the Nrf2 siRNA group was significantly lower than that in the other groups ( $P<0.01$ ), as shown in Figure 2.

### The effect of bufalin on the resistance of K562/A02 cells induced by Nrf2

RT-PCR detection revealed a significant decrease in MDR1 mRNA in the Nrf2 siRNA group, as shown in Figure 2. Western blot analysis showed that bufalin also reduced the expression levels of Nrf2 and its target genes heme oxygenase-1 (HO-1) and P-glycoprotein (P-gp) protein (Figure 3A). Correlation with protein detection in K562/A02 cells in Nrf2 siRNA groups indicated that P-gp expression in the Nrf2 siRNA group was significantly weaker, and was consistent with the RT-PCR test results. The expression of Nrf2 and HO-1 in Nrf2 siRNA K562/A02+ADM+bufalin cells was also significantly lower than that in the non-bufalin groups (Figure 3B).



**Figure 2.** Detection of Nrf2 and MDR1 mRNA expression by RT-PCR. Results  $x\pm s$ ,  $n=4$ , compared with K562 group, \*  $P<0.05$ , the difference was statistically significant; compared with K562 group, #  $P<0.01$ , the difference was statistically significant.

### Bufalin inhibited the growth of K562/A02 cells

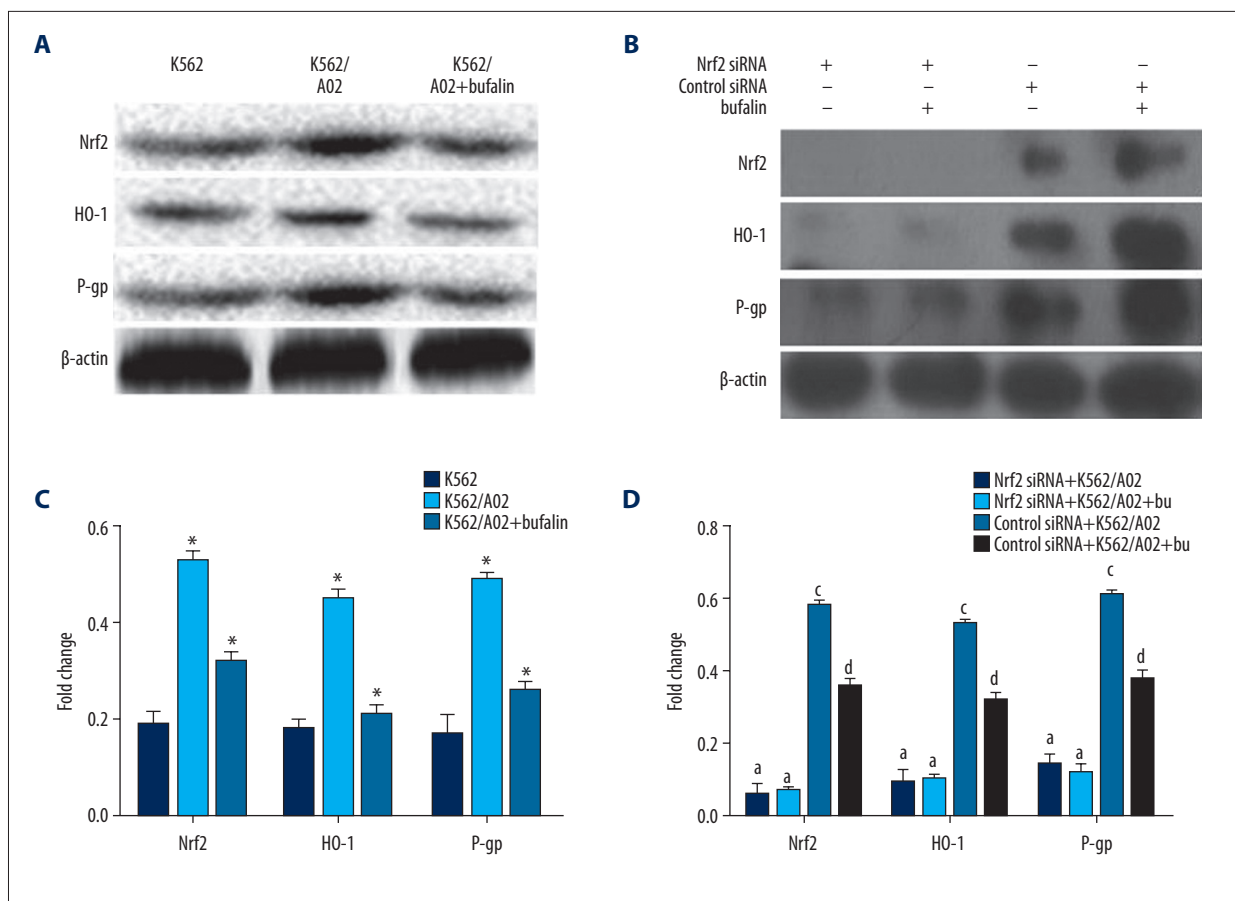
Bufalin was added to K562/A02 cells to give a final concentration of 0, 12.5, 25, 50, 100, and 200 nM. Each group was incubated for 48 and 72h. MTT assay showed that the OD value of each group of cells decreased with the increase of bufalin concentration in each group, indicating that the inhibition was concentration-dependent. The OD value of each group of cells also decreased significantly with time of incubation, indicating that the inhibition of bufalin in cells was time-dependent (Figure 4).

### Effect of bufalin on apoptosis of K562/A02 cells

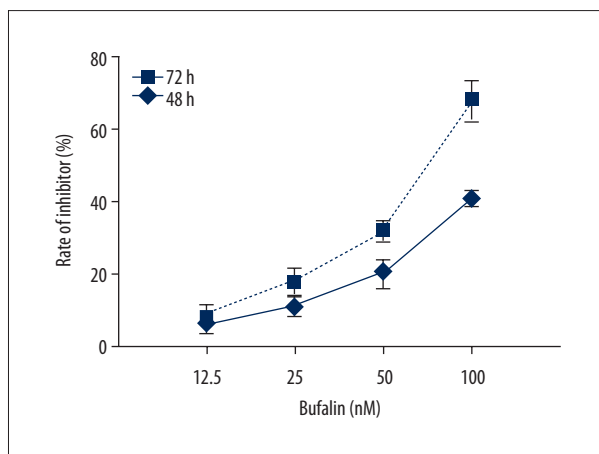
Flow cytometry was used to detect apoptosis in each group. The results are shown in the Figure 5: (a) K562/A02+ADM+bufalin group, (b) K562+ADM group, (c) K562/A02+ADM group, and (d) K562/A02 group. The apoptosis rate of group A was significantly higher than that of group C and group D ( $P<0.05$ ). The difference between groups C and D was not statistically significant ( $p>0.05$ ). The results showed that the apoptotic rate of group B was higher than that of the other 3 groups, as shown in Figure 5.

### Detection of related genes in each group of cells by RT-PCR

We assessed the expression of mRNA of endoplasmic reticulum marker protein GRP78,94 in the cells, showing that the expression of GRP78,94 in the bufalin group was significantly increased. The detection of apoptosis-related genes Bax and Bcl-2 mRNA showed that the ratio of Bax/Bcl-2 in the bufalin group



**Figure 3.** Detection of drug resistance-associated proteins by Western blot. Figure (A) shows the expression of drug-resistance-related proteins, and Figure (B) shows the expression of related proteins after interference with Nrf2 siRNA. The figure below shows the statistical analysis: As shown in Figure (C), \* P<0.01. As shown in the Figure (D), <sup>ac</sup> P<0.0, <sup>ad</sup> P<0.01, <sup>cd</sup> P<0.01.



**Figure 4.** Dose-response curve of inhibition of K562/A02 by bufalin. Figure 4 shows that the inhibition of bufalin on cells was concentration-dependent and time-dependent.

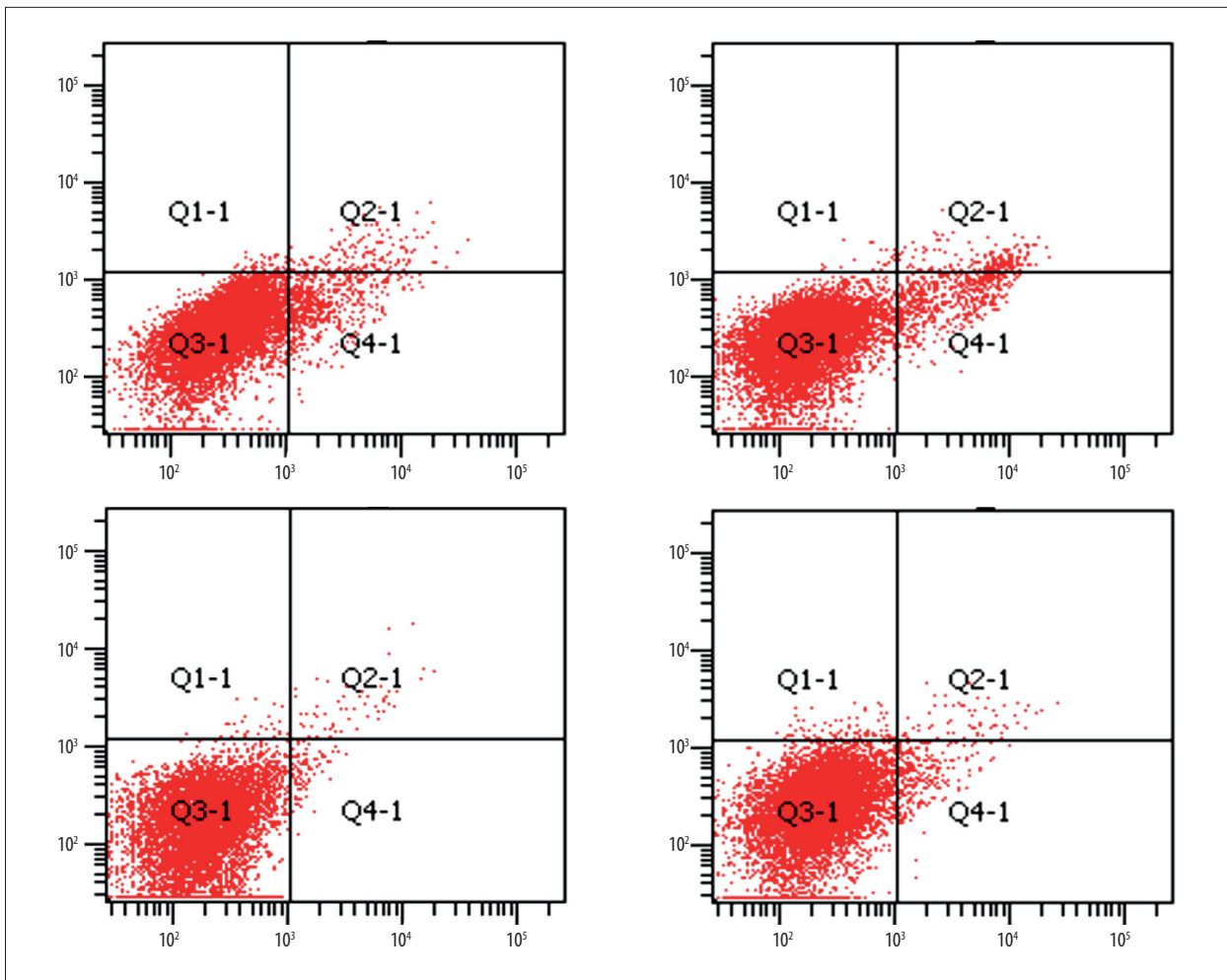
that the ratio of Bax/Bcl-2 in the K562/A02+ADM+bufalin group was higher than that in other groups.

**Western blot analysis of apoptosis-related protein expression**

Application of Western blot analysis of apoptosis-related proteins showed Bcl-2 protein expression in the bufalin group was significantly weaker than in the K562/A02 group, while expression of Bax in the K562/A02 group was significantly enhanced. The cytoplasmic cyt-c was detected by Western blot. The expression of cyt-c in cytoplasm was significantly increased after bufalin treatment of K562/A02 cells for 72 h. When cyt-c is released into the cytosol, it can induce caspase-3 cleavage. The results show the bufalin group had significantly increased cleaved caspase-3 protein expression. Furthermore, PAPP was cleaved to induce apoptosis (Figure 7).

was increased. To investigate how bufalin induces apoptosis in K562/A02 cells, we conducted further studies. Figure 6 shows





**Figure 5.** Flow cytometry for detection of apoptosis in K562/A02 cells. As shown in the figure, there was no statistically significant difference between groups C and D ( $P>0.05$ ). When group A is compared with groups C and D, there is a significant difference ( $p<0.05$ ). Apoptosis in group B was higher than in groups A, C, and D.

#### **Effect of bufalin on K562/A02 endoplasmic reticulum-associated proteins**

Detection of K562/A02 in the endoplasmic reticulum-associated protein showed that bufalin can induce ERS, increase IRE1 $\alpha$  expression, and activate JNK (Figure 8).

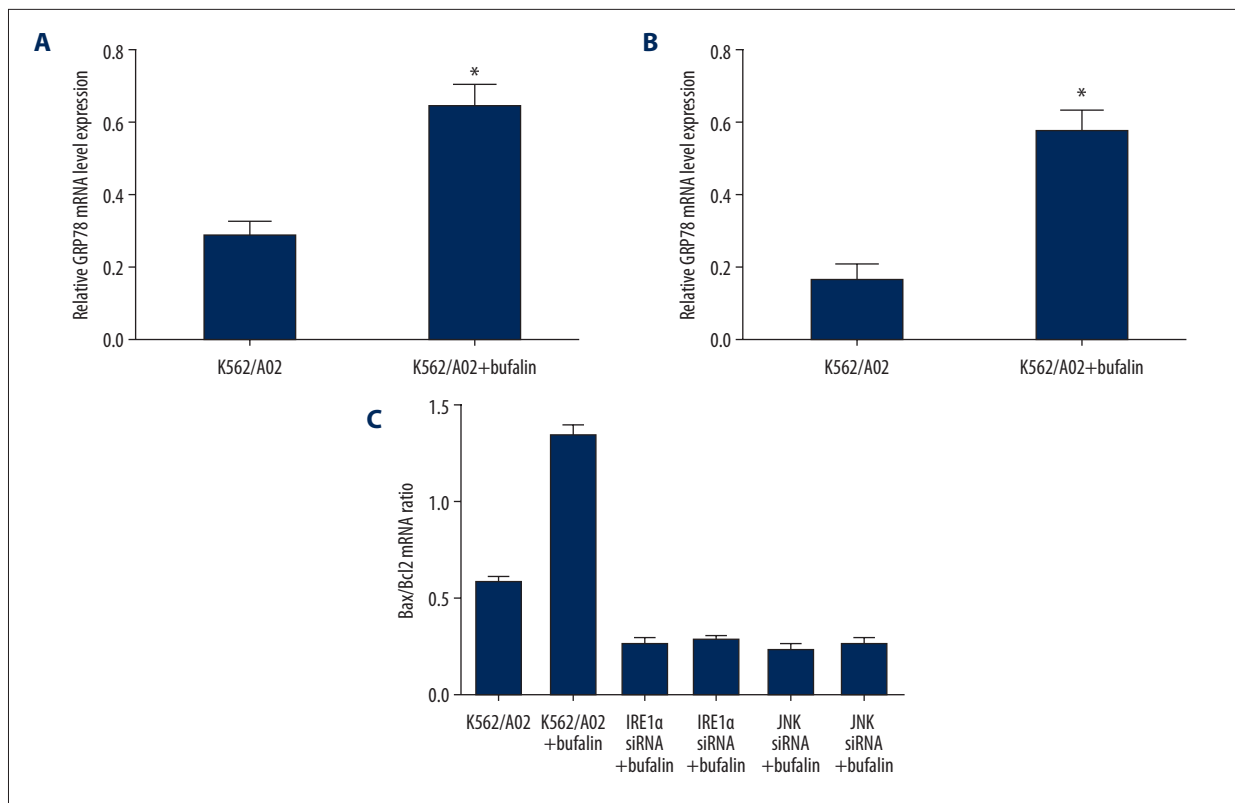
#### **Bufalin induces apoptosis of K562/A02 cells by IRE1 $\alpha$ /TRAF2/JNK/Caspase-12**

##### *Detection of apoptosis in IRE1siRNA and JNK siRNA cells*

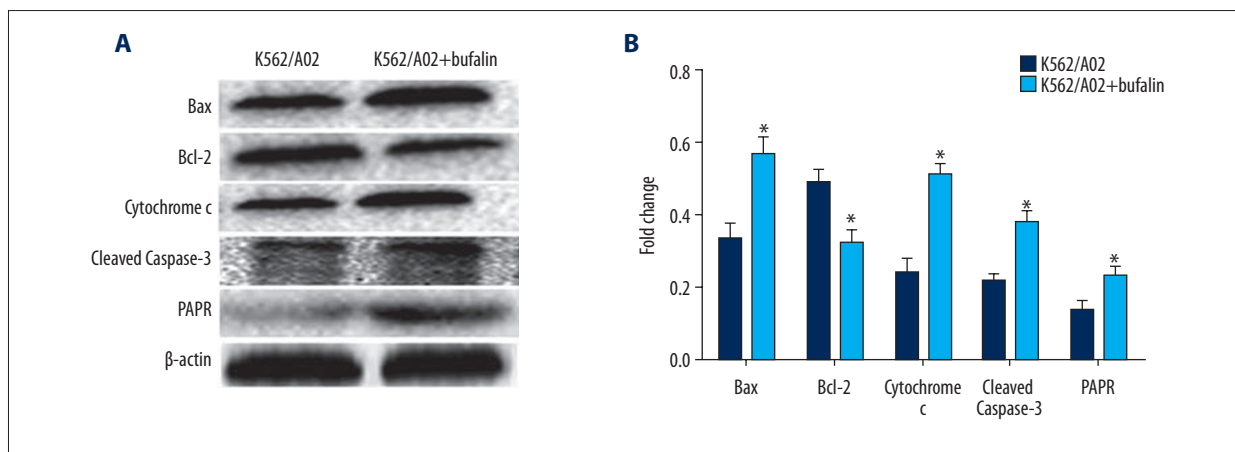
Flow cytometry was used to detect apoptosis in each group. The apoptotic rates of the cells in the IRE1siRNA group (Figure 9A) and JNK siRNA group (Figure 9B) were significantly lower than in the groups in Figure 5A and B ( $P<0.05$ ). The difference between groups Figure 9A, 9B and Figure 5C, 5D was not statistically significant ( $p>0.05$ ) (Figure 9).

#### *Bufalin induces apoptosis of K562/A02 cells by IRE1 $\alpha$ /TRAF2/JNK/Caspase-12*

We used siRNA technology to reduce IRE1 $\alpha$  and JNK expression for 48 h, then added bufalin to treat the cells. We then collected protein and RNA from each group, and used Western blot and RT-PCR analyses to detect related proteins and genes. Our results showed that the expression of IRE1 $\alpha$ , JNK, and Caspase-12 was significantly reduced in the transfected bufalin group compared with the untransfected bufalin group (Figure 10). RT-PCR was used to detect the ratio of Bax/Bcl-2 in bufalin-transfected cells, which was found to be significantly higher than that in the non-bufalin group (Figure 6C). Flow cytometry was used to detect apoptosis of cells in bufalin-transfected cells. The rate was significantly lower than that of the untransfected bufalin cell group (Figure 5).



**Figure 6.** RT-PCR detection of related genes in each group of cells. As shown in Figure (A) (\*  $P < 0.01$ ), the difference was statistically significant. Figure (B) also shows a statistically significant difference (\*  $P < 0.01$ ). Figure (C) shows that the K562/A02+ADM+bufalin group had higher levels than in the other groups.

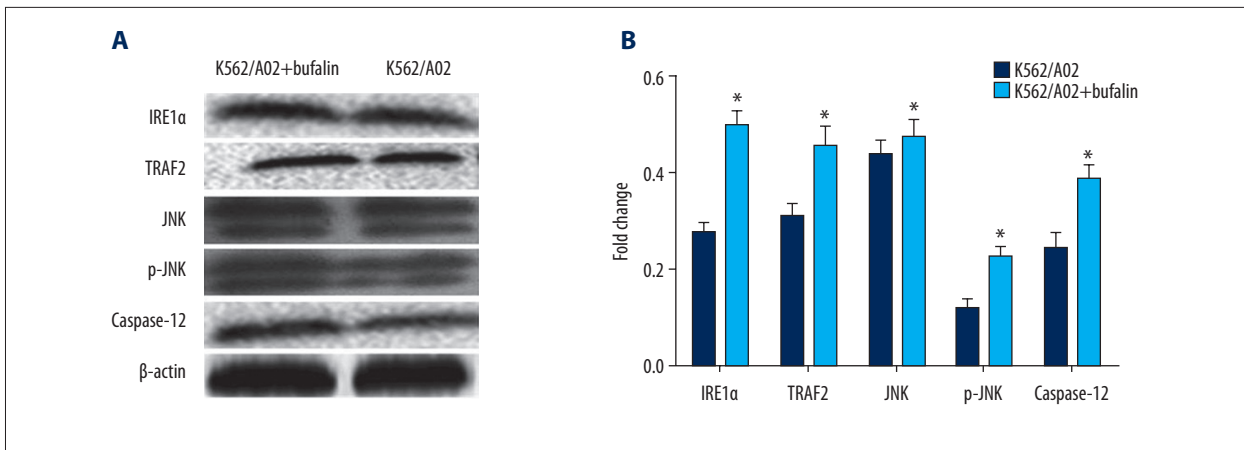


**Figure 7. (A, B)** K562/A02 apoptosis-related protein detection. A typical picture of each group of proteins as expressed by Western blot. Statistical analysis of each group of proteins, as shown in the graph on the right. The comparison of individual groups of proteins shows a statistically significant difference between the groups (\*  $P < 0.01$ ).

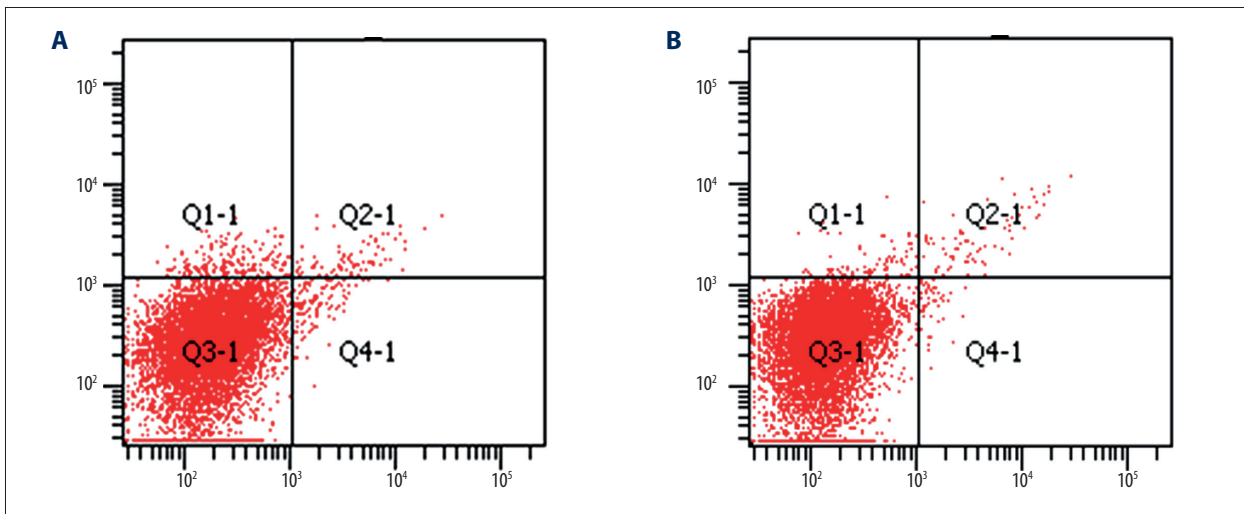
## Discussion

The emergence of drug resistance in cells during leukemia treatment increases the difficulty of treatment, and has become a difficult problem in leukemia management [1,12,15]. Therefore, reversing or avoiding tumor MDR has become a focus

of research [15]. Some toxic substances in Chinese medicine also have certain therapeutic effects on leukemia cells [16,17]. For example, arsenic and snake venom can inhibit the proliferation of tumor cells [6,18]. We have found that many ancient medical masterpieces have described the effect of toad venom on the treatment of tumors [19], but toad venom has certain



**Figure 8. (A, B)** K562/A02 endoplasmic reticulum stress-related protein detection. Western blot was used to detect the expression of each group of proteins. Statistical analysis is shown in the graph on the right, and the difference between individual protein groups is significant (\* P<0.01).



**Figure 9.** Detection of apoptosis in IRE1siRNA and JNK siRNA cells. As shown in the figure, there was no statistically significant difference between the groups in (A) and (B) vs. the groups in Figure 6C and 6D.

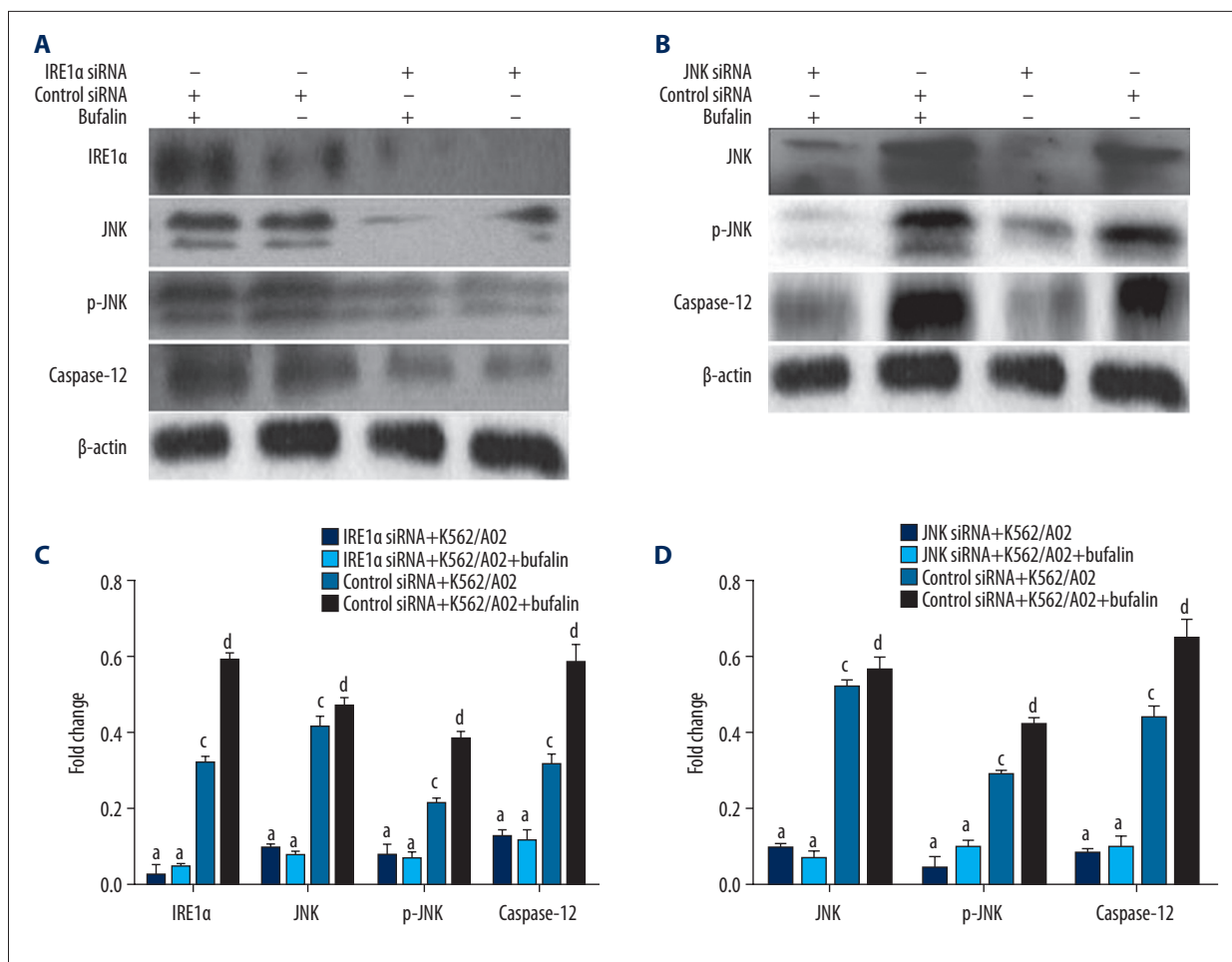
toxic adverse effects, and it is not suitable for use by itself or for long-term use. It has been found that the active ingredient with effect on tumor cells is bufalin [14,19]. This was an *in vitro* study of the mechanism of bufalin in reversing tumor cell resistance in K562/A02 cell apoptosis.

Multiple studies have confirmed that bufalin has high anti-cancer activity against liver cancer, prostate cancer, and leukemia, and indicated that this activity is realized by inducing apoptosis and differentiation [13,18,20]. Bufalin acts on endometrial cancer and ovarian cancer by regulating the expressions of Bcl-2, Bax and caspase-9, while in human lung cancer, it induces apoptosis by Bax translocation mediated by oxygen free radicals and caspase-3 activation [21,22]. Studies have shown that bufalin can reduce the activity of Mg<sup>2+</sup>-ATPase and endoplasmic reticulum marker G-6-Pase in hepatoma cells of

H22-bearing mice [23]. Bufalin can inhibit the proliferation of glioma cells and induce apoptosis through endoplasmic reticulum stress [24]. Bufalin also shows efficacy in drug-resistant cell lines. It has been reported that bufalin can reverse the multidrug resistance of the multidrug-resistant human leukocyte cell line HL60/Adriamycin, enhance the sensitivity of HL60/Adriamycin cells, and thereby inducing apoptosis of tumors [25]. The mechanism by which bufalin reverses drug resistance of K562/A02 for anti-tumor effects has been unclear. To elucidate this issue, it would be helpful to better understand the potential mechanism of bufalin's anti-tumor effect and lay an experimental foundation for research and development of novel anti-cancer drugs.

In recent years, the role of Nrf2 in tumor MDR has received much attention. Studies have shown that the Nrf2-ARE signaling





**Figure 10.** (A–D) IRE1α siRNA K562/A02 and JNK siRNA K562/A02 cell-related protein expression levels. A typical picture of each group of proteins expressed by Western blot and statistical analysis of each group of proteins. As shown in Figures: <sup>a</sup>c P>0.05, <sup>a</sup>d P<0.01, <sup>c</sup>d P<0.01.

pathway [3,26], an important antioxidant regulatory pathway in the cell, not only participates in tumor resistance by regulating the transcriptional expression of phase II enzyme, but also cascades multiple drug-resistance mechanisms to produce tumor multiple-drug resistance [4,5,27]. Several laboratories have confirmed that siRNA can interfere with the expression of Nrf2, which can inhibit tumor growth and increase the sensitivity of various tumor cells to chemotherapeutic drugs [3,28,29]. Currently, research into tumor resistance from Nrf2 is focused on liver, lung, breast, colon, prostate, ovarian, and other tumors [17,28–30]. It has not been reported whether this mechanism also mediates resistance for leukemia cells. Excessive P-gp content in cells may combine with more drug molecules, resulting in the release of energy by ATP hydrolysis, keeping the intracellular drug levels at a lower concentration and interfering with the therapeutic effect, a common mechanism for drug resistance in tumor cells [11,12,17]. We also confirmed that after interference with Nrf2, the expression of the drug-related protein P-gp was significantly decreased and the concentration of ADM in the cells was increased.

The UPR signaling pathway mediated by the IRE1 pathway plays a very important role in the process of ERS protection and apoptosis [7,9,31]. In the ERS state, under the induction of IRE1, JNK and apoptotic signaling enzymes can activate the apoptotic signaling pathway [9,32,33]. TNF receptor-associated factor 2 (TRAF2) acts as a regulatory molecule activating caspase-12 [10]. Activated caspase-12 further activates caspase-9, and caspase-9 activates the apoptotic effector molecule caspase-3, leading to apoptosis. In the ERS state, if caspase-12 is deleted from the cell, TRAF2 cannot function, and the apoptotic signaling pathway cannot be normally turned on [8,34,35]. However, if caspase-12 is also deleted in cells under other stress conditions, the apoptotic signaling pathway can be normally turned on, indicating that caspase-12 only affects apoptosis in the ERS state, and ERS can specifically activate caspase-12 [7,9,11].

Apoptosis includes both endogenous and exogenous pathways, both of which ultimately activate caspase-3 [6,12,36].

Cyt-c causes apoptosis through the caspase and non-caspase pathways. In the caspase pathway, caspase-3 plays an important role [12,35,37]; in the non-caspase pathway, it is found that Bcl-2 and Bax can participate in the release of cyt-c [19,36]. When studying the mechanism of action between Bcl-2, Bax, and caspase, it was found that Bcl-2 and Bax can both affect the activity of caspase-3 as an upstream signal and also participate in the reaction of caspase-3 as a downstream substrate [36,37]. Bcl-2, Bax, and caspase-3 bind to each other and interact with each other to participate in the cell apoptosis process [14,18].

Research on bufalin is mainly focused on its therapeutic effect on hepatoma cells [30], and few studies have described the effect of bufalin on K562/A02 cells. In this paper, the apoptosis rate of K562/A02 in each group was detected by flow cytometry, and bufalin was found to be able to induce apoptosis of K562/A02 cells. Western blot results also showed that the ratio of Bax/Bcl-2 was increased, caspase-3 was cleaved, and caspase was activated, which stimulated apoptosis of K562/A02 cells.

Whether IRE1 $\alpha$  is activated after bufalin acts on K562/A02 has not been reported previously. We have detected GRP78,94 endoplasmic reticulum marker protein gene mRNA expression in the course of the experiment, which was significantly increased in the bufalin group explaining, bufalin induction of ERS in K562/A02 cells. To further validate the mechanism by which K562/A02 bufalin induced apoptosis, we used Western blot analysis to detect ERS-associated proteins, finding that the expression of IRE1 $\alpha$  and JNK and the expression of bufalin and caspase-12 were significantly increased.

To determine if JNK and caspase-12 are mediated by IRE1 $\alpha$ , we analyzed the correlation between IRE1 $\alpha$ , p-JNK, and caspase-12 expression, and found that IRE1 $\alpha$  was positively correlated with the expression of p-JNK and caspase-12. We also found that the expression of p-JNK and other apoptosis-related proteins was significantly decreased after siIRE1 $\alpha$  was detected in K562/A02. Flow cytometry detected apoptosis and found a significant decrease in apoptotic rate compared with the other groups. Based on this result, we can speculate that IRE1 $\alpha$  activates the expression of the downstream apoptotic molecules p-JNK and caspase-12 and then induces apoptosis. The results indicate that bufalin can stimulate JNK phosphorylation and induce cell death.

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We conducted a related study on JNK, which is important in the process of inducing apoptosis. After siJNK was detected in K562/A02 cells, we observed that cell apoptosis was significantly inhibited and was correlated with the siJNK group. After detection, by assessing the expression of the ERS apoptosis-related protein caspase-12 and the downstream genes of apoptosis, we found that the expression was significantly reduced. Taken together, we our results suggest that bufalin induces excessive stress in the endoplasmic reticulum, activates the IRE1 $\alpha$ -mediated IRE1 $\alpha$ /JNK/caspase-12 endoplasmic reticulum signaling pathway, and participates in the K562/A02 cell apoptosis pathway.

This study aimed to reverse the resistance to bufalin of K562/A02 and induce apoptosis of K562/A02 and provide a theoretical basis for the treatment of leukemia with traditional Chinese medicines combined with chemotherapy. We plan to further investigate the mechanism of bufalin in drug resistance of leukemia based on the current research results, and will carry out animal experiments to verify and supplement results of cell experiments. It is hoped that, in the future, more effective active ingredients of Chinese herbal medicines will be used in clinical anti-tumor treatment, providing new ideas for cancer treatment by developing new drugs, and bringing new hope to cancer patients.

## Conclusions

Our results show that bufalin can suppress ADM efflux to reverse drug resistance of K562/A02 cells by inhibiting Nrf2 and weakening the downstream drug-resistant gene expression. We also found that bufalin can induce ERS in K562/A02 cells and promote apoptosis of this cell line through the IRE1 $\alpha$ /TRAF2/JNK/caspase-12 signaling pathway. These results suggest that bufalin is a potential drug-resistance reversal agent and can promote apoptosis of tumor cells, which provides a new idea for the treatment of leukemia in clinical practice.

## Conflict of interest

None.

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