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# Aloe-Emodin Induces Endoplasmic Reticulum Stress-Dependent Apoptosis in Colorectal Cancer Cells

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
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Manuscript Preparation E
Literature Search F
Funds Collection G

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**Background:** Recently, colorectal cancer has become a more common type of tumor in the world. Research has reported that several kinds of single compounds of Chinese herbs have shown anti-tumor activity in colorectal cancer. Aloe-emodin (AE), a natural compound extract from Aloe Vera, has been discovered to suppress cell proliferation and accelerate apoptosis in a variety of tumor cells. Whether AE exerts an effect on colorectal cancer cells has not yet been investigated.

**Material/Methods:** In this study, we examined the impact of AE on SW620 and HT29 colorectal cancer cell lines. After treatment with various concentrations of AE (10, 20, and 40 μM), cell proliferation, cell apoptosis, reactive oxygen species (ROS) generation, cytosolic calcium level, and related gene expression were analyzed.

**Results:** Our results indicated that AE suppressed cell viability and induced cell apoptosis in SW620 and HT29 cell lines. Furthermore, both cell lines when exposed to AE generated ROS, which induces endoplasmic reticulum (ER) stress. We then detected the expression of ER stress-related proteins and cytosolic calcium levels. We found that cells exposure to AE had upregulation of unfolded protein response (UPR) proteins like glucose-related protein 78 (GRP78), phosphorylated protein kinase R (PKR)-like ER kinase (p-PERK), phosphorylated eukaryotic initiation factor-2α (p-eIF2α), and transcription factor C/EBP homologous protein (CHOP). Meanwhile, we detected an increased cytosolic calcium content followed by the upregulation of the calpain-1, calpain-2 and caspase-12. CHOP and caspase-12 are important regulatory factors leading to cell apoptosis.

**Conclusions:** AE might serve as a candidate in the treatment of colorectal cancer through inducing ER stress-dependent apoptosis.

**MeSH Keywords:** Aloe • Apoptosis • Colorectal Neoplasms • Endoplasmic Reticulum

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## Background

Colorectal cancer is one of the most prevalent and aggressive malignancies worldwide [1]. Most colorectal cancers are due to aging and lifestyle factors, which include diet, obesity, smoking, and lack of physical activity [2]. Surgery and chemotherapy are the main clinical therapies for the treatment of colorectal cancer [3]. However, current chemotherapeutic drugs still have some deficiencies, such as severe side effects. Development of novel therapeutic drugs is urgently needed. Nowadays, traditional Chinese herbs have drawn broad attention from the scientific community; in the past 70 years, about half of small molecular compounds that achieved SFDA approval for cancer treatment were natural medicines or direct derivatives of natural products.

Aloe-emodin (1, 3, 8-trihydroxyanthraquinone) (AE) is an anthraquinone and a kind of emodin extracted from the aloe latex, and it has been reported to exert a variety of pharmacological activities [4], especially noted for its anti-neoplastic effect on various kinds of tumors [5]. Research has shown that AE can enhance tamoxifen cytotoxicity in breast cancer cells, exert photodynamic activity on the apoptosis of human gastric cancer cells [6], as well as suppress proliferation and induce apoptosis in human lung squamous carcinoma via reactive oxygen species (ROS) generation [7]. However, we have not yet seen any detailed reports involving the effects of AE on colorectal cancer.

Cell apoptosis is an important approach in fighting cancer [8]. Research has reported that cells suffering from apoptosis involve endoplasmic reticulum (ER) stress, mitochondrial dysfunction, and death receptors [9,10]. Furthermore, oxidative stress plays a crucial role in regulating cancer cell behavior, especially associated with cell apoptosis [11]. High ROS levels were reported to have damaged ER function, resulting in unfolded proteins accumulation and ER stress [12].

The ER is the primary compartment for protein synthesis, modification, and delivery to appropriate sites in eukaryotic cells [13]. A variety of pathological stimuli can induce accumulation of unfolded proteins in the ER lumen, which contributes to ER stress [14]. Usually, an integrated signal transduction pathway, also called the unfolded protein response (UPR) is activated. The UPR function is to establish ER homeostasis by halting protein synthesis and bolstering protein folding capacity [15]. However, excessive ER stress can cause cellular homeostasis breakdown, thus triggering the apoptosis pathway. GRP-78 is an important protein in the ER and is broadly regarded as a bio-marker for ER stress. Once the UPR is triggered, glucose-related protein 78 (GRP78) dissociates from ER trans-membrane receptors; PERK (pancreatic eIF2α kinase or protein kinase R (PKR)-like ER kinase) is one of the vital receptors. The dissociation of GRP78 from PERK initiates the phosphorylation of PERK and activates the downstream protein p-eIF2α [16], which finally induces upregulation of CHOP (C/EBP homologous protein), which plays a vital role in promoting cell apoptosis.

In addition, ER stress is usually accompanied by intracellular calcium overload. Calpain is a kind of thiol proteases that participates in the change of calcium content [17]. The major isoforms of calpain are calpain-1 and calpain-2. Binding of calcium to calpain-1 and calpain-2 can induce the release of a regulatory subunit, sequentially activating a catalytic subunit. An elevated calcium content can lead to obvious calpain activation [18]. The activated calpain can cleave procaspase-12 to caspase-12, thus triggering ER stress mediate apoptosis.

In this study, we aimed to investigate the impact of AE treatment on colorectal cancer cells. We noticed a suppressed cell proliferation and increased cell apoptosis in SW620 and HT29 colorectal cancer cell lines, which represented a potential application of AE in colorectal cancer treatment. Further, an increased ROS was detected in SW620 and HT29 cells after AE treatment. Considering that cell apoptosis and ROS were important inducement of ER stress, we examined the ER stress-related proteins and the cytosolic calcium content to find out whether ER stress was involved in the process. The results showed that ER stress-related proteins were activated, and that the cytosolic calcium levels showed an obvious elevation, which contributed to our conclusion that the cell apoptosis induced by AE was mediated by ER stress.

## Material and Methods

### Cell culture

SW620 and HT29 colorectal cancer cell lines were obtained from American Type Culture Collection (ATCC, USA). Cells were cultured in the RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 μg/mL penicillin (Cellgro) and 100 μg/mL streptomycin (Cellgro) at 37°C in an incubator containing 95% $O_2$ and 5% $CO_2$. Cells were treated with various concentration of AE (10, 20, and 40 μM) [19] (Sigma-Aldrich, St Louis, MO, USA) for 24 hours, then the following experiments were applied.

### Cell viability assay

We seeded $5\times10^3$ cells per well on 96-well plates and incubated for 48 hours. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) was added to wells at a final concentration of 0.5 mg/mL and incubated for additional 4 hours at 37°C. Dimethyl sulfoxide (DMSO; Sigma-Aldrich) was added





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to dissolve the formazan product. After incubating for 10 min at 37°C, absorbance was measured at a wavelength of 570 nm.

### Clone formation assay

The effects of AE on the proliferation of SW620 and HT29 cells were evaluated by clone formation assay. Cells were cultured in 12-well plates with complete culture medium for 24 h. Then, the cells were treated with several concentrations of AE for 7 days. Cells were stained with crystal violet for 20 min. Representative pictures of the colonies were captured by a digital camera.

### Fluorescence microscopy assay

The cell apoptosis was assessed by Hoechst 33342 staining. Cultured SW620 and HT29 cells were treated with several concentrations of AE and incubated for 24 hours at 37°C. Then we added the DNA-specific fluorescent dye Hoechst 33342 to each well and incubate for 10 min at 37°C. Stained cells were examined through fluorescence microscope (DFC480; Leica Microsystems, Wetzlar, Germany).

### Apoptosis detection by flow cytometry

SW620 and HT29 cells were incubated in 6-well plates for 48 hours with indicated concentrations of AE. After that, the cells were harvested, washed with PBS and fixed in 70% ethanol at 4°C for 30 min. Then the cells were exposed to 20 μg/mL RNase I and 50 μg/mL PI (Cell Signaling Technology, Danvers, MA, USA) for 30 min at 37°C. Flow cytometry was performed according to the manufacturer's protocol. Apoptotic cells were assessed by flow cytometry on a FACScan (Beckman Instruments, Fullerton, CA, USA).

### ROS detection by flow cytometry

After treatment with the indicated concentrations of AE to the cells for 48 hours, cells were incubated with 10 μM 2', 7'-dichlorofluorescin diacetate (DCFH-DA) for 20 min at 37°C, then washed with serum-free medium 3 times. Cells were resuspended in ice-cold PBS avoiding light. The intracellular peroxide levels, which represent the levels of ROS, were measured by flow cytometry at wavelength of 525 nm.

### Calcium measurement by flow cytometry

Cells were loaded with 2 μM Fluo4-AM (Invitrogen) in buffer (145 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5 mM $NaHCO_3$, 10 mM glucose, and 10 mM Na-HEPES pH 7.25) with 0.02% Pluronic-F27 (Invitrogen) for 15 min at 37°C in the dark. After that, we washed the cells with the same buffer without fluo-4. Fluo4 fluorescence was recorded at 505 nm.

### Western blot analysis

Total proteins were harvested through RIPA lysis buffer (Thermo Scientific, Rockford, IL, USA) which containing Complete Protease Inhibitor Cocktail (Roche, Indianapolis, IN, USA). The quantitation of protein was examined by BCA protein quantification kit. Equal amounts of proteins were subjected to SDS/PAGE electrophoresis, and the separated proteins were transferred onto a PVDF membrane. Blocking the membranes with 5% non-fat milk in 1 x TBST (10 mM Tris-HCl, 150 mM NaCl, pH 8.0, and 0.1% Tween 20) for 30 min at room temperature, then incubated in primary antibody overnight at 4°C. The primary antibodies included: Bcl-2 (1: 2000, Epitmics, USA), Bax (1: 1000, CST, USA), GRP78 (1: 1000, CST, USA), p-PERK (1: 1000, CST, USA), p-eIF2α (1: 1000, CST, USA), CHOP (1: 1000, CST, USA), calpain-1 (1: 1000, CST, USA), calpain-2 (1: 1000, CST, USA) and caspase-12 (1: 1000, CST, USA). Next, the PVDF membrane was washed with TBST solution for 10 min and repeated 3 times. Then, the membranes were incubated with secondary antibodies (Santa Cruz Biotechnology) for 1 hour at room temperature. The membranes were washed and developed by the ECL chemiluminescent kit (Amersham Biosciences, Piscataway, NJ, USA).

### Statistical analysis

The data were presented as the mean ± SEM. Student's *t*-test was conducted between 2 groups. Statistical differences among multiple groups were analyzed by one-way ANOVA analysis of variance. A $P$ value of $<0.05$ was considered statistically significant.

## Results

### Aloe-emodin inhibited cell proliferation in SW620 and HT29 colorectal cancer cell lines

In order to study the effects of AE on cell proliferation, MTT assay was used to detect the cell viability of SW620 and HT29 colorectal cancer cells treated with different concentrations of AE at different time points. According to the MTT assay, AE significantly reduced cell viability in SW620 cells (Figure 1A) and HT-29 cells (Figure 1B) at the concentration of 10, 20, and 40 μM. At the dose of 10 μM AE, the rate of cell viability relative to normal control cells was approximately 88.71% in SW620 cells and 82.84% in HT-29 cells after treatment for 24 hours. And the intuitive observation showed a further reduction in cell viability as the dose increased over time. Subsequently, we assessed the effect of AE on the proliferation of SW620 cells and HT29 cells through a clone formation assay. As Figure 1C shows, AE observably inhibited SW620 and HT-29 cell colony forming ability in a dose-dependent manner after treatment with the aforementioned concentrations for 7 days.





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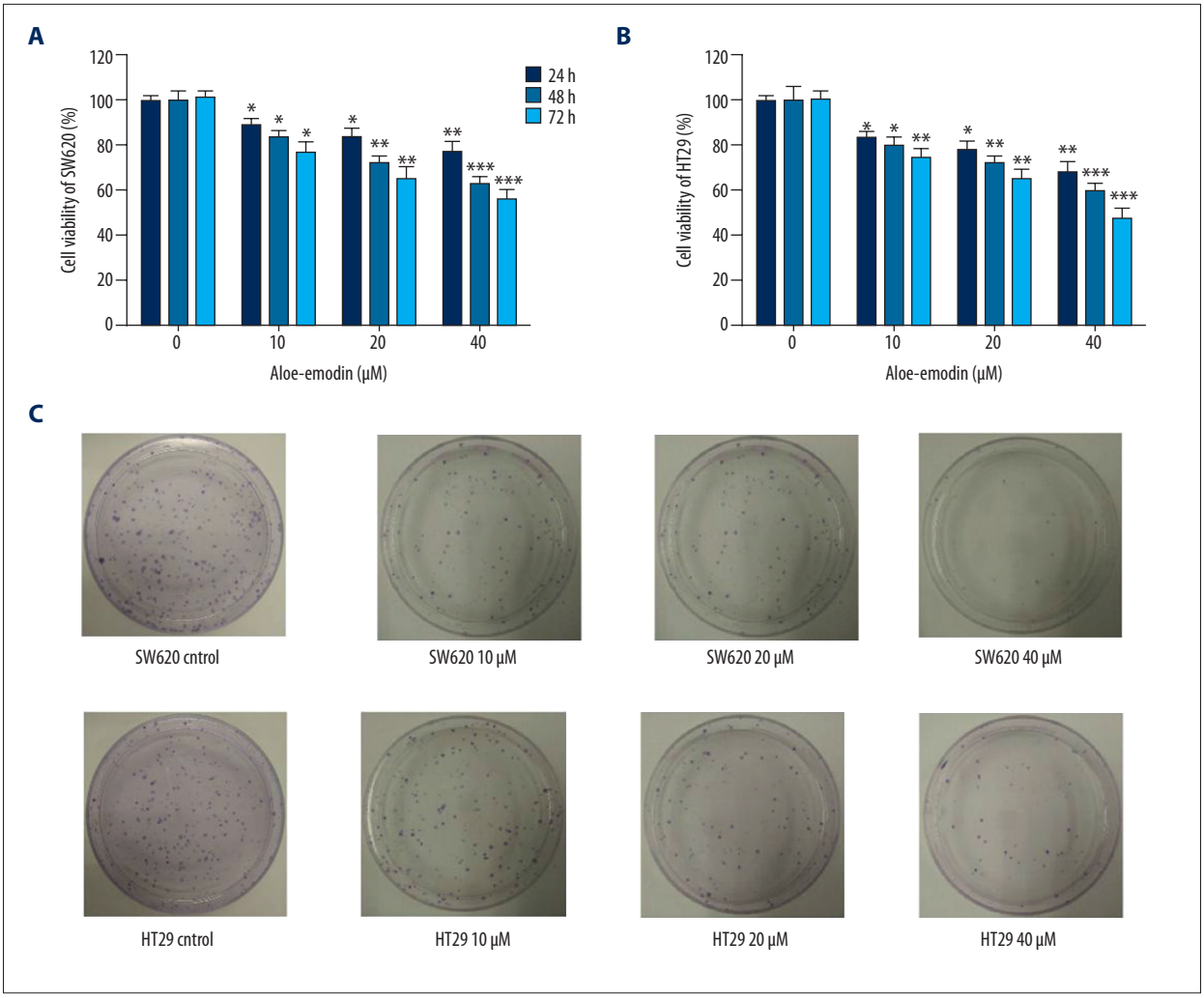


**Figure 1.** Aloe-emodin reduced cell viability in SW620 and HT-29 cells in a dose-dependent manner at different time points. (**A**) SW620 cells and (**B**) HT-29 cells were treated with indicated concentrations of Aloe-emodin, and incubated for 24, 48, and 72 hours. Cell viability was detected via MTT assay. (**C**) Clone formation assay was used to assess the effects of Aloe-emodin on the proliferation of SW620 and HT-29 cells. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, as compared with the control group; n=4.

### Aloe-emodin induced apoptosis in SW620 and HT29 cells

As the cell viability was detected to be decreased after treatment with AE, we further examined the proapoptotic effect of AE on SW620 and HT29 cells. As shown in Figure 2A, we observed the apoptotic nuclear morphology through fluorescence microscopy after Hoechst staining. In the control group, the cells were regular in morphology, grew fully in patches and were confluent, while AE treatment apparently induced the formation of apoptotic bodies in both SW620 and HT29 colorectal cancer cell lines. Meanwhile, apoptosis was detected by Annexin V-FITC/PI staining in combination with flow cytometry to distinguish and quantitatively determine the percentage of apoptotic cells. As we see in Figure 2B, the fraction of apoptotic cells was increased in a dose-dependent manner in SW620 and HT29 cells. The proportions of Annexin V-FITC and PI doubly stained SW620 cells were 7.32%, 13.3%, 18.1%, and 21.23% in the control and AE (10 μM, 20 μM, and 40 μM) groups, respectively. And the same detection was applied in HT29 cells: the percentages of apoptotic cells were 8.39%, 13.52%, 16.86%, and 19.08% after exposure to AE at 0, 10, 20, and 40 μM for 48 hours. Furthermore, we examined the expression of apoptosis-related protein Bcl-2 and Bax by western blot analysis; the results showed that Bcl-2 expression was decreased while Bax expression was increased as a result of treatment with AE in both SW620 and HT29 cells, indicating that AE might induce cell apoptosis (Figure 2C–2H).





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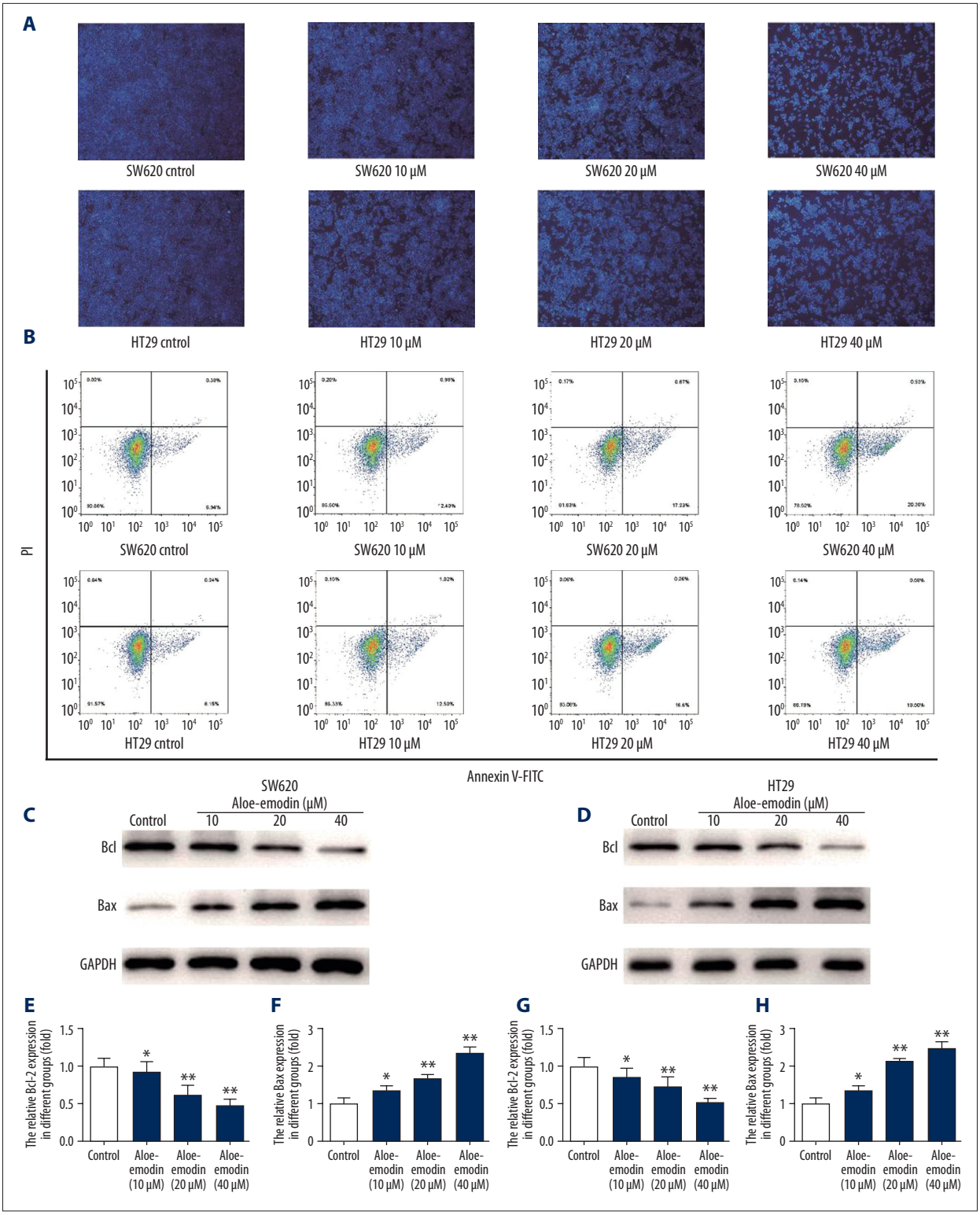


**Figure 2.** Aloe-emodin induced SW620 and HT29 cell apoptosis in a dose-dependent manner. (**A**) Hoechst staining was used to evaluate cell apoptosis. Cells were treated with indicated concentrations of Aloe-emodin for 24 hours and the morphology was revealed using a fluorescence microscope. (**B**) Flow cytometry was used to quantitatively determine the apoptosis rate. (**C**) The expression of apoptosis-associated proteins Bcl-2 and Bax were detected by western blot analysis. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, as compared with the control group; n=4.





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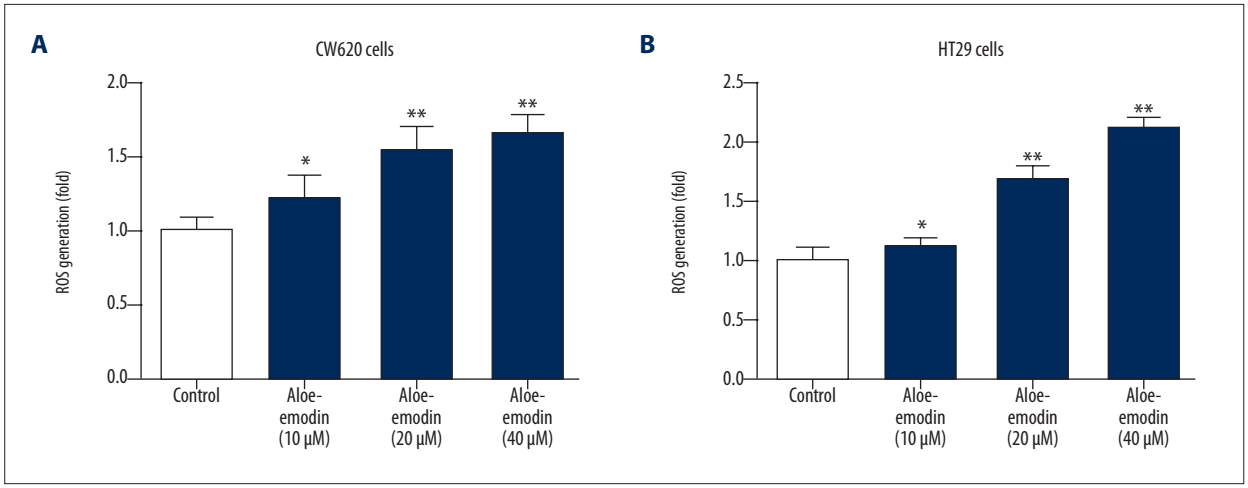


**Figure 3.** Aloe-emodin induced reactive oxygen species (ROS) in SW620 and HT29 cells. (**A**) SW620 cells and (**B**) HT29 cells were treated with indicated concentrations of Aloe-emodin for 24 hours, then stained with DCFH-DA (10 μM); the ROS were quantitatively analyzed through flow cytometry. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, as compared with the control group; n=4.

### Aloe-emodin induced reactive oxygen species in SW620 and HT29 cells

ROS generation is a crucial modulator in controlling cancer cell behavior, which is associated with apoptosis. Reports have shown that AE could induce ROS in human lung squamous cells and human osteosarcoma cells. Here we examined whether increased ROS was relevant to cell apoptosis after exposing SW620 and HT29 cells to AE. The intracellular hydrogen peroxide and superoxide levels were detected by flow cytometric assay. The results shown in Figure 3 demonstrate that, AE upregulated ROS in both cell lines at the doses of 10, 20, and 40 μM, which contributed to evidence that ROS was involved in AE induced cell apoptosis in SW620 and HT29 cells.

### Aloe-emodin induced endoplasmic reticulum (ER) stress in SW620 and HT29 cells

There has been some evidence showing that overwhelming ROS levels could cause endoplasmic reticulum (ER) stress. Thus, we investigated whether AE treatment could induce ER stress. PERK is one of the crucial ER-resident transmembrane receptors, usually maintained in inactive forms by association with the ER chaperone GRP78, which dissociates upon ER stress. We mainly detected the expression of GRP78, phosphorylated PERK, and downstream proteins, and our results are shown in Figure 4. The results suggest that the expression of GRP78 and PERK significantly increased following AE treatment, and PERK facilitated the phosphorylation of eIF2α. The expression of CHOP, which is reported to reduce the anti-apoptotic mitochondrial protein Bcl-2 and foster a pro-apoptotic environment, was also increased observably. All the results revealed that AE could induce ER stress thereby accelerating the apoptosis in SW620 and HT29 cells.

### Cytosolic calcium overload and caspase-12 were implicated in Aloe-emodin mediated ER stress in SW620 and HT29 cells

ER stress is characteristic by cytosolic and mitochondrial calcium overload. Especially, the cytosolic calcium overload, followed by upregulation of the calpain-1, calpain-2 and caspase-12, is a classical pathway to induce apoptosis. Considering that, we detected the cytosolic calcium level in SW620 and HT29 cells that were suffered from AE through flow cytometry. As Figure 5A and 5B show, an observably enhancement of cytosolic calcium level was noticed in AE treated cells as compared to the controls. Afterwards, we examined the levels of calpain-1 and calpain-2, which are cytoplasmic cysteine proteases involved in a variety of calcium-regulated cellular processes; we also examined the level of downstream caspase-12. In Figure 5C–5J, the western blot analysis shows that the protein expression of calpain-1, calpain-2 and caspase-12 were upregulated in both cell lines after exposed to AE. All the results convinced us that there was a cytosolic calcium overload resulting from ER stress, and its downstream protein caspase-12 was involved in the mechanism of cell apoptosis.

## Discussion

Colorectal cancer is one of the most prevalent cancers worldwide, especially in western countries. Nowadays, the incidence in Asian population is growing rapidly, as a result of the popularity of the western diet. Although a series of targeted therapies have been developed to resist cancer [20–22], the severe side effects and drug resistance limit their application, and the curative effect on patients with colorectal cancer remains poor [23], thus, novel therapeutic agents are urgently





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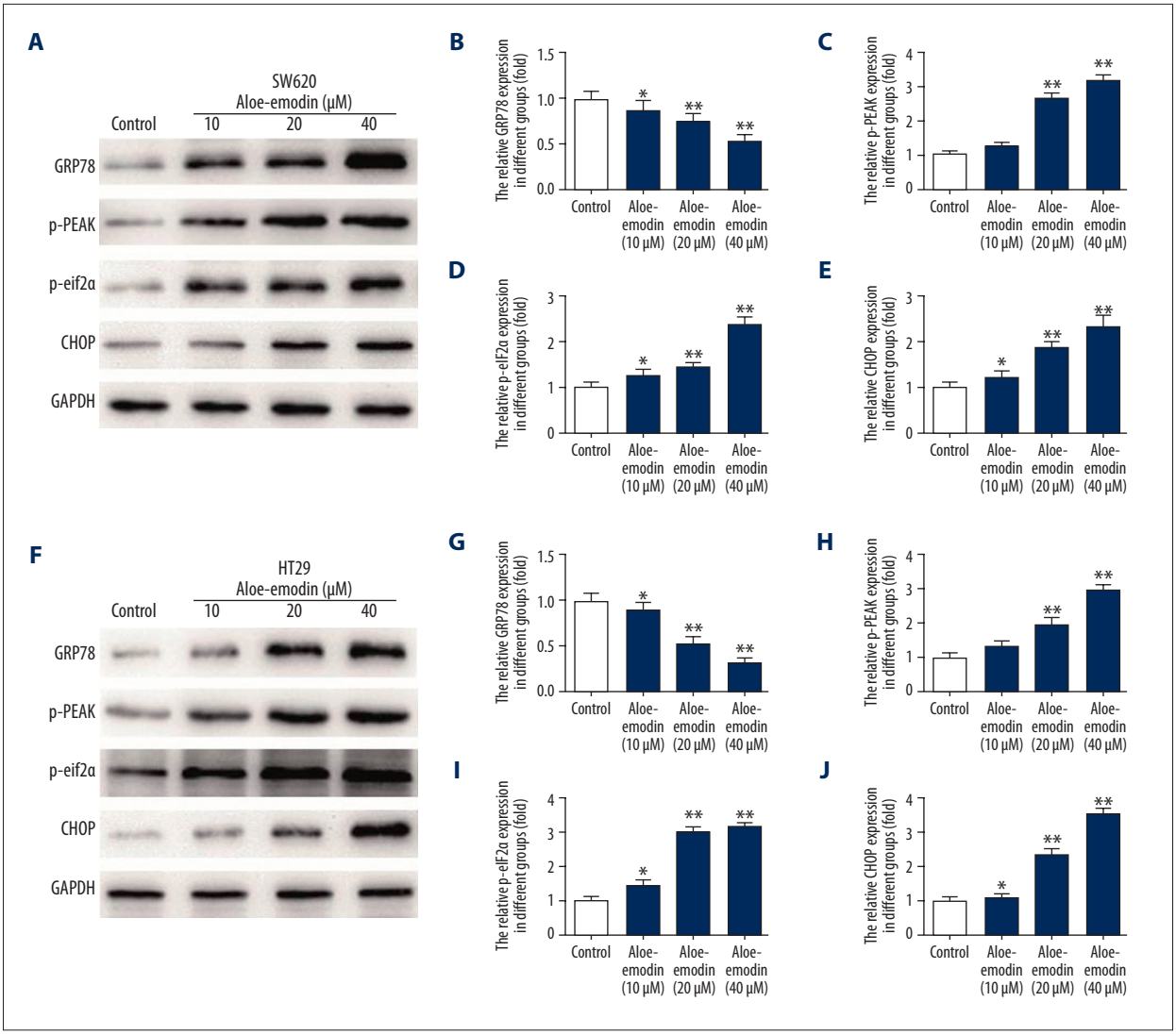


**Figure 4.** Aloe-emodin increased endoplasmic reticulum stress related proteins in SW620 and HT29 cells. Both the cell lines were treated with different concentrations of Aloe-emodin and harvested at 24 hours. The GRP78, p-PERK, p-eIF2α, and CHOP proteins expression were analyzed by western blot. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, as compared with the control group; n=4.

needed. AE is a kind of Chinese herbal monomer, which has been proven to have anticancer effects, while its pharmacological effects have not been investigated in colorectal cancer.

In this study, we showed that AE inhibited cell proliferation in human colorectal cancer cells *in vitro,* using MTT detection and clone formation assay. At 24 hours, 48 hours, and 72 hours, AE showed significant suppression on both SW620 and HT-29 cells in a dose-dependent way. As the cell viability was decreased, we further examined the apoptosis rate through flow cytometry to confirm the anti-cancer effect of AE. The results showed that AE induced apoptosis in SW620 and HT29 cells. We further detected apoptosis related proteins, the downregulation of Bcl-2 and upregulation of Bax are consistent with the flow cytometry results.

ROS play an important role in tumor progression, excessive ROS production can induce intrinsic apoptosis via DNA damage [24,25]. Several studies have indicated that a number of therapeutics can induce ROS-mediated apoptosis [26,27]. Therefore, we further detected ROS through flow cytometric assay. Our results showed that AE observably upregulated ROS production, suggesting that ROS is an inducement of cell apoptosis upon treatment of AE. ROS are mainly produced in the mitochondria via metabolic reactions, in the ER via protein oxidation, and in peroxisomes via β-oxidation of fatty acids. In reverse, high level of ROS can lead to ER stress, as well as ER stress mediated apoptosis.

In recent years, ER stress has aroused general concern because of its potential in induction of cancer cell apoptosis.





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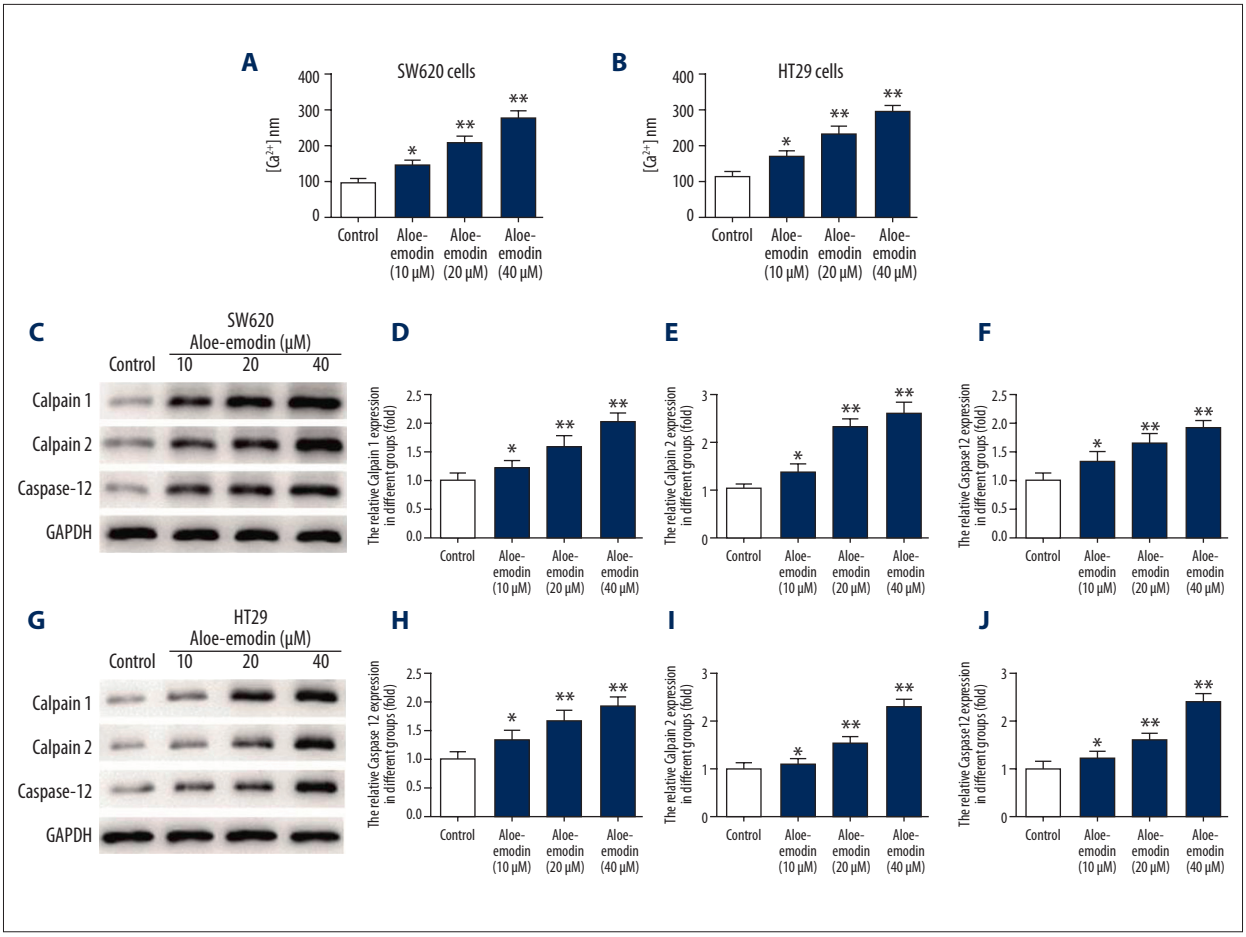


**Figure 5.** Aloe-emodin modulated ER stress involving in cytosolic calcium overload and calpain/caspase-12 pathway in SW620 and HT29 cells. (**A**) SW620 cells and (**B**) HT29 cells were treated with indicated concentrations of Aloe-emodin for 24 hours, then stained with Fluo4-AM (2 μM); the cytosolic calcium levels were quantitatively analyzed through flow cytometry; (**C–F**) The calpain-1, calpain-2, and caspase-12 proteins expression in SW620 cells were analyzed by western blot; (**G–J**) The calpain-1, calpain-2, and caspase-12 proteins expression in HT29 cells were analyzed by western blot. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, as compared with the control group; n=4.

Accumulating evidence has indicated that considerable therapeutic drugs can lead to ER stress. And some drugs targeting ER stress, such as arsenic trioxide, have achieved FDA approval. Therefore, we aim to investigate the role of ER in AE induced apoptosis.

ER stress is responsible for regulating protein synthesis, modification, and trafficking. Accumulation of unfolded or misfolded proteins can activate the unfolded protein response (UPR) signaling to restore cellular homeostasis. Once the UPR cannot maintain ER balance, cell apoptosis follows. PERK, ATF6, and IRE1 are 3 central ER transmembrane receptors. Among these, PERK and the downstream mechanism occupy an important position. When ER stress is triggered, a dissociation of GRP78 from PERK will take place, followed by an activation of PERK. Activated PERK can phosphorylate eIF2α, and this phosphorylation contributes to translation of ATF4, finally resulting in expression of CHOP. CHOP is well known for its proapoptotic role in ER stress [28]. In this study, we investigated that whether ER stress was involved in the apoptosis caused by AE treatment in colorectal cancer cells. The results showed that the GRP78/PERK pathway was activated, which is characterized by elevation of GRP78, p-PEAK, p-eIF2α, and CHOP protein expression, which contributed to the conjecture that AE induced apoptosis was partly mediated by ER stress in colorectal cancer cells.

As a result of ER stress with increased CHOP, calcium leak from the ER usually causes an increased level of cytosolic and mitochondrial calcium content. Calpain can be activated in response to the enhanced cytosolic calcium, and transfer from the cytosol to the membrane to cleave procaspase-12 [29]. The activation of caspase-12 plays a vital role in ER stress mediated apoptosis [30]. Hence, we examined the cytosolic calcium





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content through flow cytometry analysis. The results showed an increased cytosolic calcium in SW620 and HT-29 cells after exposure to AE. Subsequently, we further detected the protein expression of calpain-1, calpain-2 and caspase-12, which showed a significant upregulation upon AE treatment. The results demonstrated that calpain-1/calpain-2/caspase-12 pathway was a branch of ER stress mediated apoptosis.

## Conclusions

Overall, in the study, we confirmed that AE could suppress cell proliferation and induce apoptosis in SW620 and HT29 colorectal cancer cell lines in a dose-dependent manner. ROS production was induced by AE. We further found that ER stress was involved in the mechanism of cell apoptosis, as the results showed that AE significantly elevated GRP78, p-PEAK, p-eIF2α, and CHOP expression, as well as increasing cytosolic calcium accompanied by enhanced caspase-12 expression. These results contributed to the conclusion that AE could induce ER stress mediated apoptosis via upregulation of CHOP and caspase-12 expression in colorectal cancer cells.

### Conflict of interests

None.

## References:

1. Ju YT, Kwag SJ, Park HJ et al: Decreased expression of heat shock protein 20 in colorectal cancer and its implication in tumorigenesis. J Cell Biochem, 2015; 116: 277–86
2. Haggar FA, Boushey RP: Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg, 2009; 22: 191–97
3. Matsushita N, Aruga A, Inoue Y et al: Phase I clinical trial of a peptide vaccine combined with tegafur-uracil plus leucovorin for treatment of advanced or recurrent colorectal cancer. Oncol Rep, 2013; 29: 951–59
4. Divya G, Panonnummal R, Gupta S et al: Acitretin and aloe-emodin loaded chitin nanogel for the treatment of psoriasis. Eur J Pharm Biopharm, 2016; 107: 97–109
5. Yang M, Li L, Heo SM, Soh Y: Aloe-emodin induces chondrogenic differentiation of ATDC5 cells via MAP kinases and BMP-2 signaling pathways. Biomol Ther (Seoul), 2016; 24: 395–401
6. Tseng HS, Wang YF, Tzeng YM et al: Aloe-emodin enhances tamoxifen cytotoxicity by suppressing Ras/ERK and PI3K/mTOR in breast cancer cells. Am J Chin Med, 2017; 45: 337–50
7. Wu YY, Zhang JH, Gao JH, Li YS: Aloe-emodin (AE) nanoparticles suppresses proliferation and induces apoptosis in human lung squamous carcinoma via ROS generation *in vitro* and *in vivo*. Biochem Biophys Res Commun, 2017; 490: 601–7
8. Kim SH, Choi KC: Anti-cancer effect and underlying mechanism(s) of kaempferol, a phytoestrogen, on the regulation of apoptosis in diverse cancer cell models. Toxicol Res, 2013; 29: 229–34
9. Seo K, Ki SH, Shin SM: Methylglyoxal induces mitochondrial dysfunction and cell death in liver. Toxicol Res, 2014; 30: 193–98
10. Park J, Bae EK, Lee C et al: Establishment and characterization of bortezomib-resistant U266 cell line: constitutive activation of NF-kappaB-mediated cell signals and/or alterations of ubiquitylation-related genes reduce bortezomib-induced apoptosis. BMB Rep, 2014; 47: 274–79
11. Park SY, Jeong AJ, Kim GY: Lactoferrin protects human mesenchymal stem cells from oxidative stress-induced senescence and apoptosis. J Microbiol Biotechnol, 2017; 27(10): 1877–84
12. Ruwan Kumara MH, Piao MJ, Kang KA et al: Non-thermal gas plasma-induced endoplasmic reticulum stress mediates apoptosis in human colon cancer cells. Oncol Rep, 2016; 36: 2268–74
13. Todd DJ, Lee AH, Glimcher LH: The endoplasmic reticulum stress response in immunity and autoimmunity. Nat Rev Immunol, 2008; 8: 663–74
14. Rutishauser J, Spiess M: Endoplasmic reticulum storage diseases. Swiss Med Wkly, 2002; 132: 211–22
15. Szegezdi E, Logue SE, Gorman AM, Samali A: Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep, 2006; 7: 880–85
16. Lee AS: Glucose-regulated proteins in cancer: Molecular mechanisms and therapeutic potential. Nat Rev Cancer, 2014; 14: 263–76
17. Molinari M, Carafoli E: Calpain: A cytosolic proteinase active at the membranes. J Membr Biol, 1997; 156: 1–8
18. Hoffmann DB, Williams SK, Bojcevski J et al: Calcium influx and calpain activation mediate preclinical retinal neurodegeneration in autoimmune optic neuritis. J Neuropathol Exp Neurol, 2013; 72: 745–57
19. Huang PH, Huang CY, Chen MC et al: Emodin and aloe-emodin suppress breast cancer cell proliferation through ER α inhibition. Evid Based Complement Alternat Med, 2013; 2013: 376123
20. Zheng X, Liu G, Cui G et al: Angiotensin-converting enzyme gene deletion polymorphism is associated with lymph nodemetastasis in colorectal cancer patients in a Chinese population. Med Sci Monit, 2017; 23: 4926–31
21. Sun X, Yuan W, Hao F, Zhuang W: Promoter methylation of RASSF1A indicates prognosis for patients with stage II and III colorectal cancer treated with oxaliplatin-based chemotherapy. Med Sci Monit, 2017; 23: 5389–95
22. Wang T, Xu H, Liu X et al: Identification of key genes in colorectal cancer regulated by miR-34a. Med Sci Monit, 2017; 23: 5735–43
23. Day LW, Velayos F: Colorectal cancer screening and surveillance in the elderly: Updates and controversies. Gut Liver, 2015; 9: 143–51
24. Zhang M, Harashima N, Moritani T et al: The Roles of ROS and caspases in TRAIL-induced apoptosis and necroptosis in human pancreatic cancer cells. PLoS One, 2015; 10: e0127386
25. Yang Y, Zhang Y, Wang L, Lee S: Levistolide A induces apoptosis via ROS-mediated ER stress pathway in colon cancer cells. Cell Physiol Biochem, 2017; 42: 929–38
26. Liu T, Wu L, Wang D et al: Role of reactive oxygen species-mediated MAPK and NF-kappaB activation in polygonatum cyrtonema lectin-induced apoptosis and autophagy in human lung adenocarcinoma A549 cells. J Biochem, 2016; 160: 315–24
27. Qiu M, Chen L, Tan G et al: A reactive oxygen species activation mechanism contributes to JS-K-induced apoptosis in human bladder cancer cells. Sci Rep, 2015; 5: 15104
28. Woo KJ, Lee TJ, Lee SH et al: Elevated gadd153/chop expression during resveratrol-induced apoptosis in human colon cancer cells. Biochem Pharmacol, 2007; 73: 68–76
29. Wang GJ, Wang Y, Teng YS: Protective effects of emodin-induced neutrophil apoptosis via the Ca2+-caspase 12 pathway against SIRS in rats with severe acute pancreatitis. Biomed Res Int, 2016; 2016: 1736024
30. Tan Y, Dourdin N, Wu C et al: Ubiquitous calpains promote caspase-12 and JNK activation during endoplasmic reticulum stress-induced apoptosis. J Biol Chem, 2006; 281: 16016–24





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