Available online at www.sciencedirect.com
ScienceDirect
*Chinese Journal of Natural Medicines* 2021, **19**(8): 608-620
doi: 10.1016/S1875-5364(21)60060-2
Chinese Journal of Natural Medicines

•Research article•

# Seed oil of *Brucea javanica* induces apoptosis through the PI3K/Akt signaling pathway in acute lymphocytic leukemia Jurkat cells

ZHANG Hong[1], YIN Shi-Liang[2], WANG Li-Hui[1], JIA Li-Na[1], SU Guang-Yue[3], LIU Xiao-Qing[4], ZHOU Fan[5], BRESLIN Peter[6], MENG Ran[7], LI Qi-Yi[7], YANG Jing-Yu[1*], WU Chun-Fu[1*]

[1] *Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, China;*
[2] *Department of Pharmacology, Shenyang Medical College, Shenyang 110034, China;*
[3] *Faculty of Functional Food And Wine, Shenyang Pharmaceutical University, Shenyang 110016, China;*
[4] *Department of Technology, Industry of Shenyang Pharmaceutical University of Lei Yun Shang Pharmaceutical Co., Ltd., Benxi 117004, China;*
[5] *Department of Hematology, General Hospital of Northern Theater Command, Shenyang 11016, China;*
[6] *Department of Molecular/Cellular Physiology, Loyola University Chicago, Chicago 60153, USA;*
[7] *School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China*

Available online 20 Aug., 2021

**[ABSTRACT]** *Brucea javanica* oil emulsion (BJOE) has been used to treat tumor in China for more than 40 years. However, its components and effectiveness in the treatment of acute lymphocytic leukemia (ALL) and its mechanism of anti-cancer activity remain unknown. In the current study, high-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) was used to analyze the components of BJOE. Then, the anti-leukemia effects of BJOE were examined both *in vitro* and *in vivo* using ALL Jurkat cells and the p388 mouse leukemia transplant model, respectively. The primary ALL leukemia cells were also used to confirm the anti-leukemia effects of BJOE. The apoptotic-related results indicated that BJOE induced apoptosis in Jurkat cells and were suggestive of intrinsic apoptotic induction. Moreover, BJOE inhibited Akt (protein kinase B) activation and upregulated its downstream targets p53 and FoxO1 (forkhead box gene, group O-1) to initiate apoptosis. The activation of GSK3$\beta$ was also involved. Our findings demonstrate that BJOE has anti-leukemia effects on ALL cells and can induce apoptosis in Jurkat cells through the phosphoinositide3-kinase (PI3K) /Akt signaling pathway.

**[KEY WORDS]** Apoptosis; Cancer; Leukemia; *Brucea javanica* oil emulsion
**[CLC Number]** R965 **[Document code]** A **[Article ID]** 2095-6975(2021)08-0608-13

## Introduction

Acute lymphocytic leukemia (ALL) is an aggressive malignant disease of the bone marrow affecting human hematopoietic function [1]. In recent years, it becomes the most common childhood cancer and a major cause of death in children and adolescents [2-4]. Though ALL is responsive to chemotherapy, its relapse rate remains high [5]. Previous studies showed that the phosphoinositide-3-kinase (PI3K)/Akt signaling pathway is frequently activated in ALL cells, which plays a key role in leukemogenesis and is an indicator for prognosis and relapse after therapeutic intervention [6-8].

The PI3K/Akt signaling pathway is essential to cell growth and survival. This pathway is often dysregulated by various toxins or cellular stimuli prior to the development of a variety of cancers [9]. In cancer cells, PI3K can activate Akt through phosphorylation to regulate various downstream signaling molecules and diverse cell processes such as survival, and proliferation [10]. Its anti-apoptotic effects are related to the transcriptional activity of FoxO1 and p53 to regulate the apoptosis-related Bcl-2 (B-cell lymphoma-2) family proteins. PI3K/Akt negatively regulates the activity of FoxO1 through phosphorylation at multiple sites and causes the translocation of FoxO1 into the cytoplasm, with weak capacity to induce transcriptional activation [11]. Bim (Bcl-2 interacting mediator of cell death), as a pro-apoptotic BH3 domain-only member of the Bcl-2 family is a downstream target of the Akt/FoxO1 signaling pathway. p53 is one of the most important tumor in-

**[Received on]** 01-Mar.-2021
**[Research funding]** This work was supported by the Young Scientific and Technological Talents Seeding Program of Liaoning Education Office (No. 2019LQN10), and Shenyang Young and Middle-Aged Science and Technology Innovation Program (No. RC180308).
**[*Corresponding author]** E-mails: yangjingyu2006@gmail.com (YANG Jing-Yu); wucf@syphu.edu.cn (WU Chun-Fu)
These authors have no conflict of interest to declare.

hibitory factors in cells that targets the anti-apoptotic protein Bcl-2. PI3K/Akt can also directly weaken the activity of Bax (Bcl-2 associated X protein) and enhance XIAP (X-linked inhibitor of apoptosis protein) activity to inhibit apoptosis [12, 13]. GSK3*β* is also thought to facilitate the mitochondrial intrinsic apoptotic pathway [14]. Its activity can be inhibited by Akt mediated phosphorylation at Ser9 site, while inhibition of GSK3*β* can prevent mitochondrial cytochrome c release [14]. Therapeutic agents that can target Akt activation and upregulate apoptotic signaling pathways show clinical potential for the treatment of ALL.

Natural products derived from herbs and nutritional supplements have long been used for the treatment of malignant diseases [15]. *Brucea javanica* (L.) Merr. (Simaroubaceae) is an evergreen shrub that originated in the southeast of Asia and Northern Australia [16, 17]. In China, it was first recorded in *Bencao Gangmu Shiyi* from 1765 A.D.. *B. javanica* decoction was orally taken to treat malarial hemorrhoids, whilst the oil from its seed was externally used to treat skin and mucosal neoplasms. It has been reported that tropical use of the seed oil of *B. javanica* (BJO) can treat hemorrhoids and clavus [18]. It also inhibit the growth and recurrence of papillomas in the acoustic duct, vocal cords, gums and nasal polyps [19]. Furthermore, the emulsion formulation for BJO, *Brucea javanica* oil emulsion (BJOE) plays a role in anti-cancer treatment [20]. BJOE can inhibit DNA synthesis in mouse Ehrlich's ascites carcinoma cells [21, 22]. In a 96-case clinical trial, BJOE treatment reduced tumor size and improved the quality of life for patients with gastrointestinal cancer and cervical cancer [18]. As shown in pharmacokinetic studies, BJOE crossed the blood-brain barrier [23]. In another 100-case clinical trial, BJOE prolonged the survival of patients with brain metastases from lung cancer [24]. Currently, BJOE is intravenously administered for the clinical treatment of lung cancer [25-28] and gastric cancer [29-31]. It also exhibits cytotoxic activity against human hepatocellular cancer (HepG2) [32], human bladder cancer (T24) [33], human ovarian cancer [34], and human esophageal cancer [35]. In consideration of the existing uses for BJOE, our laboratory have recently focused on the anti-leukemic effects of BJOE. Our previous study showed that BJOE can induce apoptosis in acute myeloid leukemia (AML) cell lines HL-60 and U937 [36]. However, whether BJOE has the potential to treat ALL remains unknown and the underlying mechanisms for the anti-leukemia activity of BJOE have not been determined.

The present study was designed to determine the anti-leukemic effects of BJOE on ALL cells and assessed its underlying mechanism of action and related signaling targets. Our results demonstrated that BJOE can induce apoptosis in Jurkat ALL cells. Then, MMP collapse and ROS production in Jurkat cells were also observed following BJOE treatment. Furthermore, we found that BJOE targeted Akt to stimulate FoxO1 and XIAP to induce apoptosis. In primary isolated leukemia cells, BJOE induced significant apoptotic response and showed anti-leukemic effects *in vivo* in the p388 mouse leukemia transplant model. These findings highlight BJOE as a promising anti-ALL agent.

## Materials and Methods

### *Reagents*

*Brucea javanica* oil emulsion (Lot No. 150320) and soybean phospholipids (Lot No. 150320, an emulsifying agent) were generously donated by the Industry of Shenyang Pharmaceutical University of Lei Yun Shang Pharmaceutical Co., Ltd. (Benxi, China). Professor TANG Xing from Department of Pharmaceutics, Shenyang Pharmaceutical University donated etoposide emulsion. MTT reagent (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), DCFA-DA reagent (6-carboxy-2′, 7′-dichloro dihydrofluorescein diacetate), rhodamine 123, Gimesa reagent and H&E stain were purchased from Sigma-Aldrich (St. Louis, MO, USA). RNAase and propidium iodide were purchased from Thermo Fisher Scientific (USA). Fetal bovine serum (FBS), Roswell Park Memorial Institute 1640 medium (RPMI-1640) and lympholyte-human 1077 were purchased from Gibco ThermoFisher Scientific (Grand Island, NY, USA). Antibodies against caspase-3, caspase-9, caspase-8, PARP, p-GSK3*β*, *β*-catenin, phospho-*β*-catenin, Bcl-2, Bax, Bad, GAPDH and *β*-actin were purchased from Cell Signaling Technologies (Danvers, MA, USA).

### *Cells and animals*

Acute lymphocytic leukemia Jurkat cells were purchased from American Type Culture Collection (Manassas, VA, USA). Adult male KM mice, aged between 6 and 7 weeks and weighing 18−22 g, were supplied by the Experimental Animal Centre of Shenyang Pharmaceutical University (Shenyang, China). The mice were housed in groups of five per cage under standardized environmental conditions (22 ± 2 °C, 12 h light/dark cycle with light on at 8 : 00 a.m.) with free access to food and water. All experiments were performed in accordance with relevant guidelines and regulations approved by the Experimental Animal Research Committee of Shenyang Pharmaceutical University (SYPU-IACUC-C2019-9-20-107). All efforts were made to minimize suffering and reduce the number of animals used and all procedures were adhered to the Declaration of Helsinki.

### *BJOE preparation*

Seeds of *Brucea javanica* were purchased from Suzhou Lei Yun Shang Pharmaceutical Co., Ltd. (Suzhou, China). Voucher specimens were deposited in the Industry of Shenyang Pharmaceutical University of Lei Yun Shang Pharmaceutical Co., Ltd. (Analytical laboratory, herbarium code: 1501231). BJOE was prepared as previously described [36]. Briefly, BJO was extracted from the seeds of *Brucea javanica* using petroleum ether at a boiling range of 60−90 °C. The residual petroleum ether was evaporated through heating to obtain crude BJO. Furthermore, the crude product was mixed with active carbon and heated through a stainless steel Bucher's funnel to obtain refined BJO. The refined product was emulsified using soybean phospholipids to obtain the final BJOE product [maintained by the Industry of Shenyang Pharmaceutical University of Lei Yun Shang Pharmaceutical

Co., Ltd. (Benxi, China)].

*HPLC-ELSD assay*

The components of BJO were examined through HPLC-ELSD analysis (Waters H-Class UPLC, Waters Corporation, MA, USA). Briefly, 10 mg of BJO was placed into a 10 mL flask and mixed with actonitrile−dichloromethane mixture (1 : 1). The resultant solution was then diluted to 10 mL prior to filtering through a 0.22 μm membrane. The flasks were shaken until a homogeneous solution was obtained and HPLC-ELSD analysis was performed using a Aglient poroshell EC-$C_{18}$ column (4.6 mm × 150 mm, 2.7 μm). Actonitrile−dichloromethane mixture (70 : 30) was used as the mobile phase. The flow rate was set at 0.5 mL·min$^{-1}$. The column temperature was 35 °C and the sample size was 10 mL. The parameters of the ELSD were as follows: drift tube 40 °C, carrier gas 40 psi.

*Cell culture*

Jurkat cells (human T lymphocytic leukemia cells) were cultured in RPMI 1640 supplemented with 100 units·mL$^{-1}$ penicillin, 100 μg·mL$^{-1}$ streptomycin, 1 mmol·L$^{-1}$ L-glutamine and 10% heat-inactivated FBS. The mouse leukemia cell line p388 was cultured in MEM supplemented with 100 units·mL$^{-1}$ of penicillin, 100 μg·mL$^{-1}$ streptomycin, 1 mmol·L$^{-1}$ L-glutamine and 10% heat-inactivated FBS. The cells were grown in 75 cm$^2$ flasks in a humidified incubator at 37 °C, 5% $CO_2$.

*Cell viability assay*

Modified MTT assay was used to measure cell viability [37]. The cells were seeded into 96-well plates at a density of 1.5 × 10$^5$. After 24 h, the cells were incubated with an emulsifying agent (soybean phospholipids, 1 : 200, *V/V*), BJOE (62.5, 125, 250, 500 and 1000 μg·mL$^{-1}$) or etoposide (positive control, 10 μmol·L$^{-1}$) for 24 h. MTT reagent (10 μL) was then added to 2.5 mg·mL$^{-1}$ in PBS and the cells were incubated at 37 °C for an additional 3−4 h. The plates were centrifuged at 2500 r·min$^{-1}$ for 10 min and the resultant supernatants were replaced with MTT and 100 μL of DMSO. Absorbance was read at 492 nm on a Biotek Synergy TM HT plate reader.

*Cell cycle analysis*

The cells were seeded onto 6-well plates at a density of 1.5 × 10$^5$ and treated with BJOE or emulsifying agent for 6 h. Samples were collected and fixed in 70% ethanol at a temperature of 0−4 °C for 12 h. The cells were washed in PBS, pelleted, and treated with 200 μg·mL$^{-1}$ RNase A for 30 min. The cells were stained with propidium iodide (1 mg·mL$^{-1}$) before measurement of DNA content by flow cytometry (Becton Dickinson, San Jose, CA). Data were analyzed using CELLQuest (Becton Dickinson) software [38].

*Apoptosis analysis*

Jurkat cells were cultured in 10 cm culture dishes and exposed to BJOE at the indicated concentrations. After incubation for 24 h, the cells were harvested by centrifugation and washed twice in PBS. The staining method was used according to the FITC Annexin V Apoptosis Detection Kit II (BD Biosciences, USA). Control cells stained with Annexin-V or PI alone were used to compensate for flow cytometry analysis. Viable cells were those with both Annexin-V and PI-double negative staining. Annexin-V-positive and PI-negative cells were defined as early apoptotic cells, and Annexin-V and PI-double-positive cells were defined as late-arising apoptotic cells.

*Intracellular ROS production*

Intracellular $H_2O_2$ levels were assessed by flow cytometry after staining with the oxidant-sensing probe 6-carboxy-2′, 7′-dichloro dihydrofluorescein diacetate (DCFH-DA) [39]. Briefly, the cells were treated with different concentrations of BJOE before being labeled with 5 μmol·L$^{-1}$ of DCFH-DA for 1 h. After washing twice in PBS, the cells were analyzed by flow cytometry at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Those stimulated with 100 μm $H_2O_2$ for 1 h were set as a positive control.

*Mitochondrial membrane potential assay*

ALL cells were treated with BJOE and washed in PBS [40]. Then, the cells were incubated with rhodamine 123 (Rh123, Ex/Em = 507/529 nm). Mitochondria can selectively uptake Rh123 *via* the mitochondrial membrane potential (MMP). After 30 min, the cells were washed and analyzed by flow cytometry.

*Confocal fluorescence imaging*

The cytochrome c release was examined by confocal fluorescence imaging. After treatment with different concentrations of BJOE for 24 h, the cells were incubated with Mito Tracker Red CMXRos (200 nmol·L$^{-1}$, for mitochondria staining) for 30 min. Then, the cells were fixed and permeabilized. After blockage, the cells were incubated with cytochrome c antibody (D18C7, Cell signaling Co.) at 4 °C overnight. Then, the cells were incubated with the fluorescent second antibody (Alexa Fluro™ 488 goat anti-rabbit IgG) for 1 h. In confocal fluorescence imaging of cytochrome c release, a FITC (488 nm) and a 2-dodecylresorufin (561 nm) filter channel were utilized for the acquisition of cytochrome c and mitochondrial images (NIS-Elements Viewer 4.0). These experiments were repeated three times.

*Isolation of human lymphocytic leukemia cells from the blood samples of ALL patients*

From September 2018 to March 2019, blood samples were collected from five newly diagnosed AML patients. All procedures were approved by the Medical Ethical Committee, General Hospital of Northern Theater Command (Shenyang, China), and informed consent was obatined from each patient. To isolate primary leukemia cells, the samples were diluted with PBS at a ratio of 1 : 1 and gently layered on top of a density gradient of lympholyte-human 1077. After centrifugation at 300 *g* for 15 min, the lymphocytes were washed twice in PBS. The cells were resuspended in RPMI 1640 and subject to Trypan blue exclusion assay. The viability of the isolated lymphocytes were about 99% [41].

*Western blot*

After treatment with BJOE for 24 h, the cells were washed twice in cold PBS and harvested in radioimmune pre-

cipitation lysis buffer to prepare protein extracts. After determination of protein concentrations using BCA assays (Beyotime Biotechnology, Beijing, China), the protein extracts were resolved on 8% and 12% SDS-polyacrylamide gels and transferred to nitrocellulose membrane (Merk Millipore, USA). Equal protein loading was confirmed by 0.2% Poceau-S red staining and the membrane was blocked in 5% skimmed milk (Wonderson Dairy Co., China). Then, the membrane was probed with primary antibodies at 4 °C for 12 h and labeled with HRP conjugated secondary antibodies. Protein bands were visualized using the ECL system (Sigma-Aldrich, MO, USA) [42].

*In vivo anti-leukemic effects on the p388 mouse leukemia transplant model*

In the current study, the p388 mouse leukemia transplant model was adopted to examine the anti-leukemic effects of BJOE *in vivo* [43]. Trypan blue staining was used to determine the viability of p388 cells, and the cells were then subcutaneously injected into the right flank of 18−22 g male KM mice at a density of $5 \times 10^7$/200 μL PBS per mouse. After inoculation, 50 mice were randomly divided into five groups ($n$ = 10): (1) control (saline solution), (2) etoposide injection at 2.5 mg·kg$^{-1}$, (3) BJOE at 5.5 mg·kg$^{-1}$, (4) BJOE at 16.5 mg·kg$^{-1}$, and (5) BJOE at 49.5 mg·kg$^{-1}$. All treatments were intravenously administered each day for a total of 14 days. Body weight, food consumption and tumor sizes of the mice were recorded every other day. Mice were euthanized on Day 14 and the tumors were excised and weighed. Tumor tissues were fixed in formalin and embedded in paraffin. The obtained sections (4 μm in thickness) were stained with hematoxylin-eosin (H&E) and observed under a DM2500 microscope and a fitted CCD camera (Germany).

*Immunohistochemistry staining*

For immunohistochemistry, tissue specimens from the tumors were embedded in paraffin, sectioned (6 μm) and deparaffinized. Immunohistochemical staining was performed with antibodies to Ki67, Akt and phosphor Akt (Ser 473). Antigen retrieval was performed through microwave pretreatment for 10 min and nonspecific binding inhibited by incubation with normal serum (Dako, corresponding to the species in which the secondary antibody was produced) 1 ∶ 5 in Tris buffered saline (TBS). Each primary antibody was applied at 4 °C overnight. The secondary antibodies were incubated at room temperature for 2 h. Then, visualization was achieved with peroxidase-labeled streptavidin-biotin and diaminobenzidine (DAB) for at least 5 min. The slides were examined under a Leica DM2500 microscope and a fitted CCD camera (Germany).

*H-Score analysis*

The H-Score were anaylzed by the software ImageScope (Weztlar, Germany). Three different fields of vision (400 times manification) were selected from each section. Enter the analysis module of image scope software, and set all dark brown on the tissue section as strong positive, brown-yellow as moderate positive, light yellow as weak positive, and blue cell nuclei as negative. Then, each tissue point was identified to analyze the area of strong positive, moderate positive, weak positive and negative. The positive percentage and H-score were then scored.

*Data analysis*

Data are presented as mean ± SEM. A one-way ANOVA followed by Dunnett's $t$-test was used for statistical analysis (SPSS 19.0 software, IBM, NY, USA).

## Results

*BJO component analysis*

HPLC-ELSD was used to examine the components of BJO (Fig. 1). Eight components were identified in total, namely trilinolein (0.007 mg·g$^{-1}$), 1, 2-linolein-3-olein (0.078 mg·g$^{-1}$), 1, 2-linolein-3-palmitin (0.005 mg·g$^{-1}$), 1, 2-olein-3-linolein (0.325 mg·g$^{-1}$), 1-palmitin-2-linolein-3-olein (0.035 mg·g$^{-1}$), triolein (0.622 mg·g$^{-1}$), 1, 2-olein-3-palmitin (0.025 mg·g$^{-1}$), and 1, 2-olein-3-stearin (0.112 mg·g$^{-1}$). The name, content and retention time of each component are shown in Table 1.

*BJOE is cytotoxic to Jurkat cells*

MTT assay was used to examine the cytotoxic effects of BJOE on Jurkat cells. The cells were treated with BJOE (62.5, 125, 250 and 500 μg·mL$^{-1}$) or a saline solution of etoposide emulsion. As shown in Fig. 2A, after treatment for

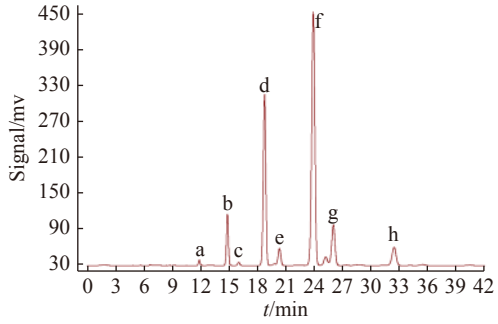



**Fig. 1 Total components of BJO. The contents of BJO were analyzed by HPLC-ELSD as described in Materials and Methods. a: trilinolein; b: 1, 2-linolein-3-olein; c: 1, 2-linolein-3-palmitin; d: 1, 2-olein-3-linolein; e: 1-palmitin-2-linolein-3-olein; f: triolein; g: 1, 2-olein-3-palmitin; h: 1, 2-olein-3-stearin**

**Table 1 The components of BJO (mean, $n$ = 3)**

| No. | Name | Contents (mg·g$^{-1}$) | $t_R$/min |
|---|---|---|---|
| a | Trilinolein | 0.007 | 11.868 |
| b | 1, 2-Linolein-3-olein | 0.078 | 14.848 |
| c | 1, 2-Linolein-3-palmitin | 0.005 | 16.033 |
| d | 1, 2-Olein-3-linolein | 0.325 | 18.760 |
| e | 1-Palmitin-2-linolein-3-olein | 0.035 | 20.338 |
| f | Triolein | 0.62 | 23.893 |
| g | 1, 2-Olein-3-palmitin | 0.025 | 26.027 |
| h | 1, 2-Olein-3-stearin | 0.118 | 32.422 |

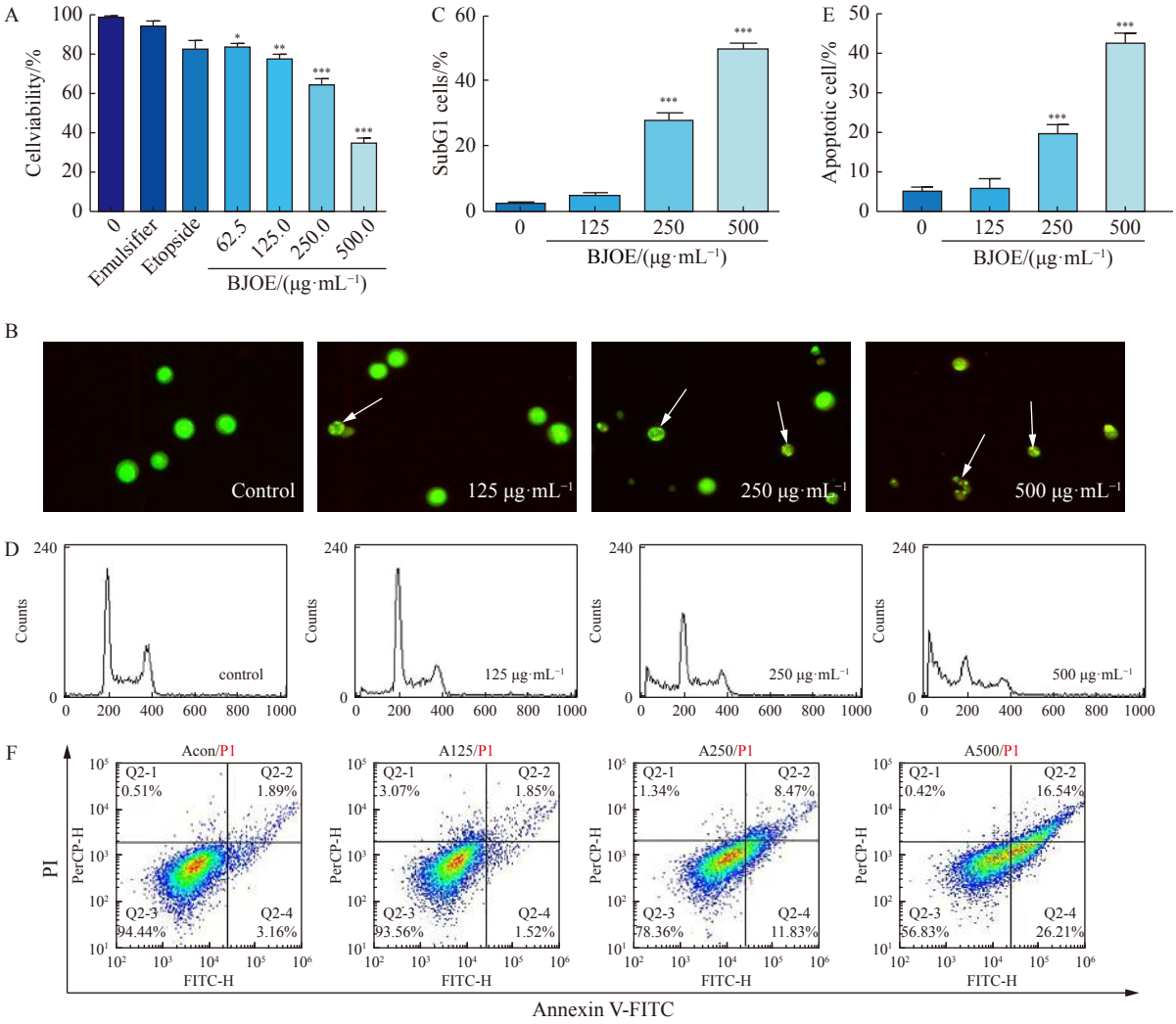



**Fig. 2 BJOE-induced apoptosis of Jurkat cells. (A) Cell viability was determined by MTT assay. (B) Representative images of AO/EB dual-stain cells by fluorescence microscopy. (C) Percentages of subG$_1$ cells were determined by flow cytometry. (D) Representative images of cell cycle analysis. (E) Percentages of apoptotic cells by flow cytometry. (F) Representative images of PI/Annexin-V staining. Data are expressed as mean ± SEM. Results were obtained from three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* control (one-way ANOVA followed by Dunnett's *t*-test)**

24 h, BJOE significantly reduced Jurkat cell viability. The degree of reduction varied according to the concentrations of BJOE, with an $IC_{50}$ values of 329.9 μg·mL$^{-1}$. Negative control treatment and soybean phospholipids showed no effect on the viability of the cells.

*BJOE induces apoptosis in Jurkat cells*

Morphological changes in Jurkat cells were observed after BJOE treatment. After staining with AO/EB (10 μg·mL$^{-1}$), Jurkat cells were observed under a fluorescent microscope. As shown in Fig. 2B, after treatment with different concentrations of BJOE for 6 h, Jurkat cells presented apoptotic characteristics, including apoptotic bodies and chromatin condensation. Cell cycle dysregulation is a hallmark of cancer cells [44]. To further understand the mechanisms of BJOE-induced cancer cell death, the cell cycle of Jurkat cells was analyzed after BJOE treatment. As shown in Figs. 2C–2D, following BJOE treatment for 6 h, the number of apoptotic cells in the sub-$G_1$ phase significantly increased in a concentration-dependent manner. Neither $G_0/G_1$ nor $G_2/S$ phase arrest was observed. BJOE treatment at different concentrations led to a significant increase in the apoptotic cell population in a concentration-dependent manner (Figs. 2E–2F), which suggests that BJOE induces apoptosis in Jurkat cells.

The caspases are a family of cysteine protease enzymes that play an essential role in apoptosis [45]. For example, caspase-8, caspase-9 and caspase-3 are critical effector caspases for apoptosis [46]. Upon caspase-3 activation by caspase-8 and caspase-9, the cleavage of PARP is induced. PARPs are a family of proteins involved in cell survival and repair responses, with PARP cleavage an indicator of apoptosis [47]. As shown in Fig. 3, BJOE promoted the activation of caspase-9, caspase-8 and caspase-3. The cleavage of PARP proteins was also observed. These results suggest that BJOE does in-

duce apoptosis in Jurkat cells.

*BJOE-induces apoptosis through intrinsic mitochondrial pathways*

To further investigate the mechanisms of BJOE-induced apoptosis, the levels of MMP and ROS in Jurkat cells were examied after BJOE treatment. MMP collapse and accompanying ROS release are hallmarks of mitochondrial-related apoptosis [48, 49]. After BJOE treatment for 6 h, a significant reduction in MMP was observed (Fig. 4A), whilst increases in intracellular ROS levels occurred (Fig. 4C) in a manner dependent on the BJOE concentrations(Figs. 4B and 4D). We also observed cytochrome c diffused from mitochondria into the cytoplasm of Jurkat cells after BJOE treatment (Fig. 4E). These results suggest that BJOE mediated apoptosis is related to intrinsic mitochondrial activity.

*BJOE inhibits the PI3K/Akt axis*

The role of PI3K/Akt signaling in BJOE-induced apoptosis in Jurkat cells was examined. We found that the expression of PI3K (110 and 85 kDa) was not affected by BJOE treatment (Fig. 5A), whilst p-Akt was down-regulated. Furthermore, the p-Akt/Akt ratio decreased following BJOE treatment (Fig. 5B) , which indicated the inhibition of the

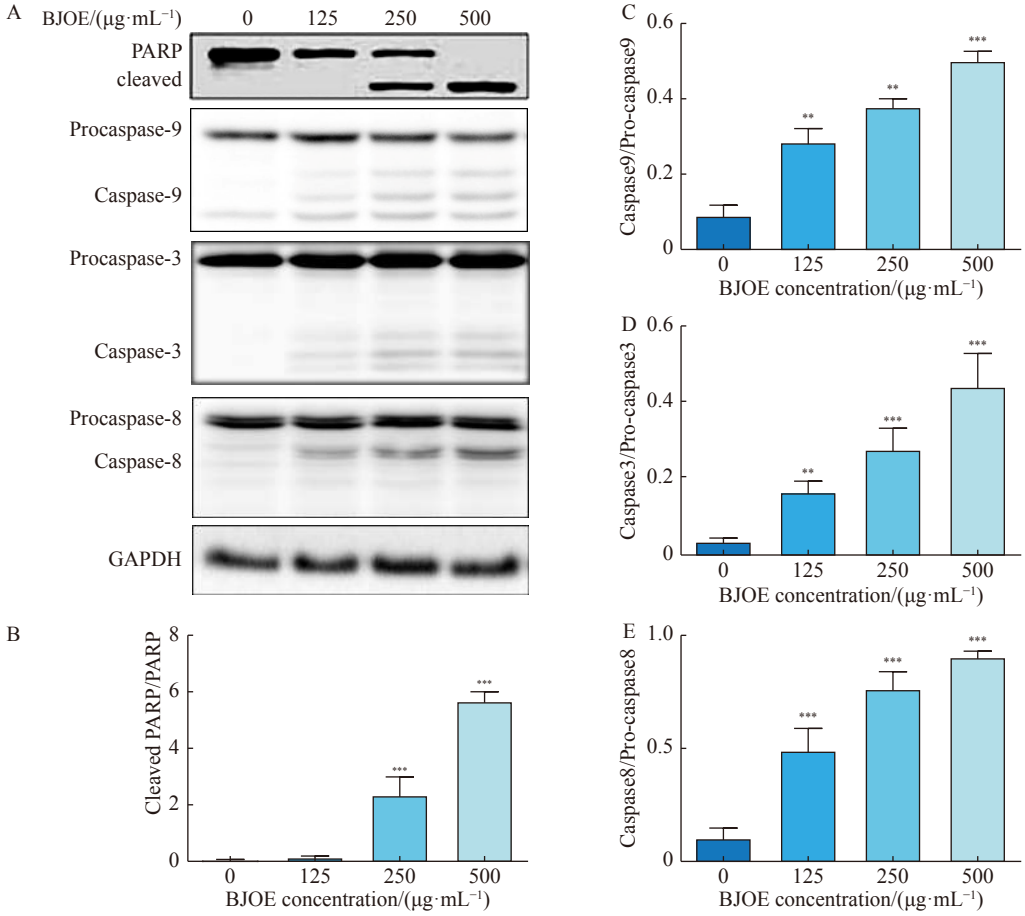
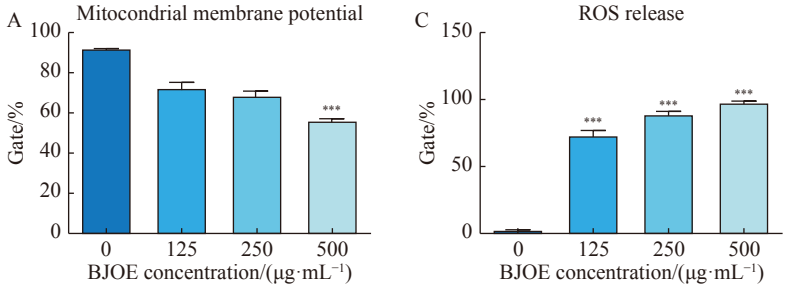



**Fig. 3 Effects of BJOE on apoptosis-related proteins. (A) Representative images of Western blot analysis of caspase-9, capase-8, caspase-3 and PARP. *β*-catenin was used as a loading control. (B−E) The ratios of cleaved PARP/PARP, caspase9/pro-caspase9, caspase3/procaspase3 and caspase8/pro-caspase8 were calculated with Image J. Data are expressed as mean ± SEM. Results were obtained from three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* control (one-way ANOVA followed by Dunnett's *t*-test)**



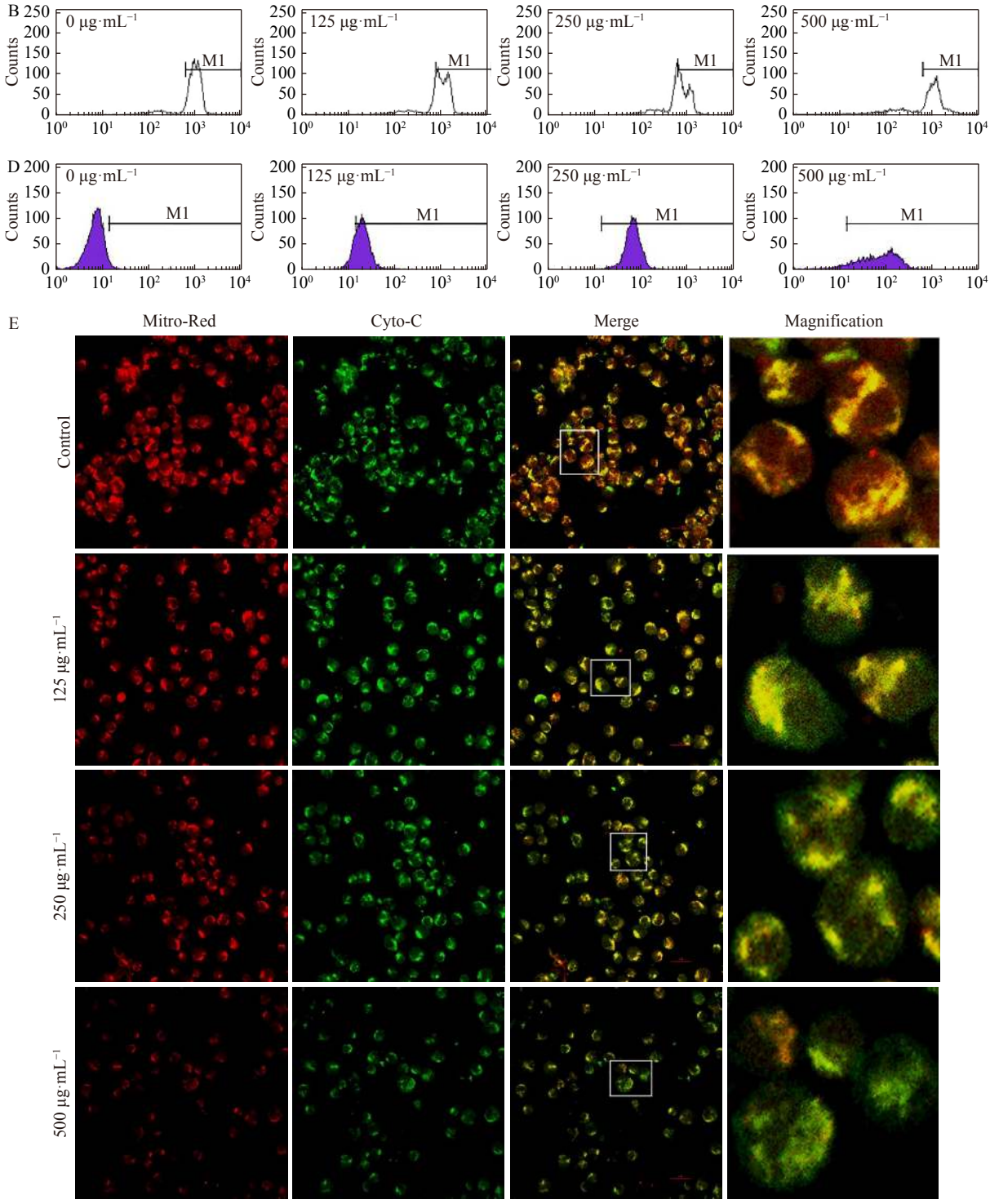



**Fig. 4 BJOE induces apoptosis through mitochondrial pathway in Jurkat cells. (A) The percentages of cells with MMP collapse. (B) Representative images of MMP collapse. (C) The percentages of cells showing ROS release. (D) Representative images of ROS release. Data are expressed as mean ± SEM. Results were obtained from three separate experiments. $^{***}P < 0.001$ *vs* control (one-way ANOVA followed by Dunnett's *t*-test). (E) Representative images of cytochrome c release by confocal microscopy**

PI3K/Akt signaling pathway by BJOE. Then, the downstream Akt/FoxO1 signaling pathway was examined. As shown in Fig. 5A, the levels of FoxO1 protein and its transcriptional target Bim were affected upon Akt inactivation. Akt inactivation can also result in increased levels of p53 and decreased levels of Bcl-2. Both Bax and XIAP are substrates for Akt. Following BJOE treatment, the levels of Bax increased (Fig. 5A) with a concomitant increase in the Bax/Bcl-

2 ratio (Fig. 5C). BJOE also decreased the levels of XIAP to induce apoptosis. Meanwhile, the levels of p-GSK3*β* (Ser 9) protein were reduced after BJOE treatment, but the levels of mTOR and p-mTOR (Ser 2448) protein did not show significant change after BJOE treatment (Figs. 6A−6B). These data suggest that BJOE induces apoptosis through the

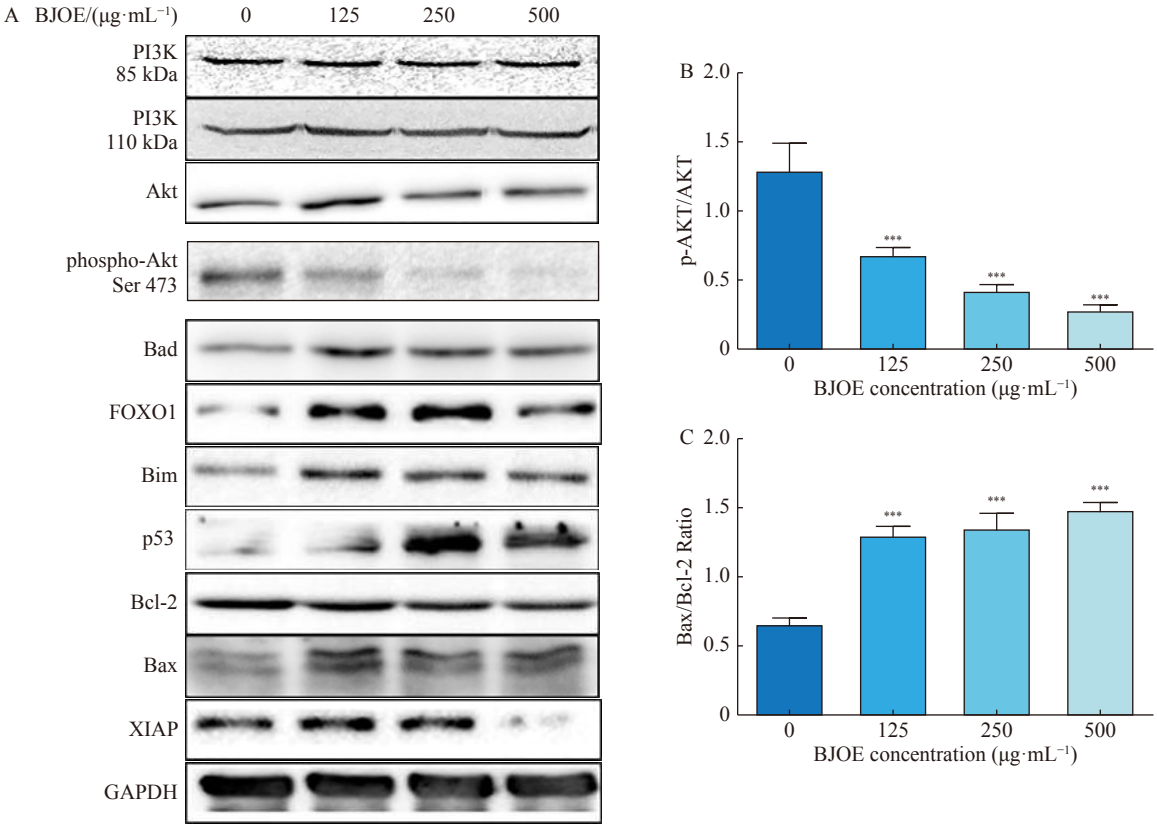



**Fig. 5 The PI3K/Akt signaling pathway is involved in BJOE-induced apoptosis of Jurkat cells. (A) Representative Western blot analysis of proteins. GAPDH is used as a loading control. (B−C) The ratios of pAkt/Akt and Bax/Bcl-2 were calculated using Image J. Data are expressed as mean ± SEM. Results were obtained from three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* control (one-way ANOVA followed by Dunnett's *t*-test)**

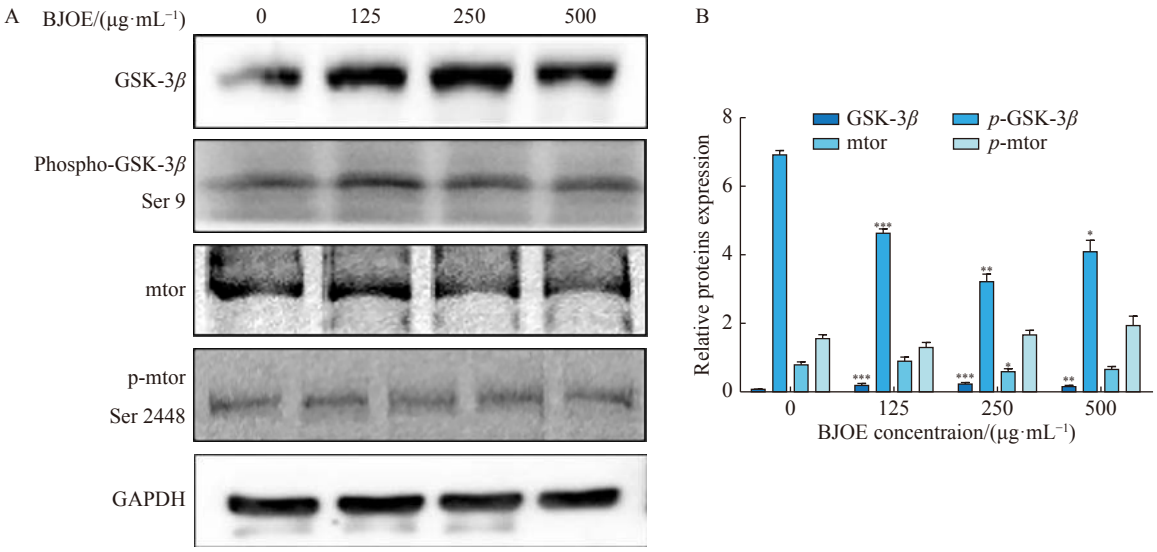



**Fig. 6 GSK3*β* is involved in BJOE-induced apoptosis of Jurkat cells. (A) Representative Western blot analysis of proteins. GAPDH is used as a loading control. (B) The relative levels of GSK3*β*, phosphGSK3*β* (Ser9), m-tor, phospho-mtor (Ser2448) protein were calculated using Image J. Data are expressed as mean ± SEM. Results were obtained from three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* control (one-way ANOVA followed by Dunnett's *t*-test)**

PI3K/Akt signaling pathway.

*BJOE-induced apoptosis in primary human leukemic cells*

To evaluate its use in clinical practice, we assessed the ability of BJOE to induce apoptosis in primary isolated leukemic cells of ALL patients. Blood samples were collected from five newly-diagnosed patients in General Hospital of Northern Theater Command (Shenyang, China). Then, clinical diagnosis and Wright-Giemsa-stained bone marrow smears were performed according to the French-America-British (FAB) classification and karyotype identification. Patient data at the time of diagnosis are summarized in Table 2. In the Giemsa-stained bone marrow smears of four representative patients (Fig. 7A), large numbers of lymphoblasts were observed. The shapes of pro-lymphocytes were irregular with either small or absent nucleoli. Some pro-lymphocytes also had granules. After BJOE treatment for 6 h, the cell cycle of primary isolated ALL cells were examined. As shown Table 1, the sub-$G_1$ phase cell percentages increased in all five samples in a BJOE concentration-dependent manner. As an indicator of apoptosis, cleaved PARP was observed in all five patient samples through Western blot analysis (Fig. 7B, four representative patients). These results suggest that BJOE induces apoptosis in the primary isolated leukemic cells of ALL patients.

**Table 2 Clinical data for ALL patients and apoptotic rate induced *in vitro* by BJOE**

| Patient No. | Sex/Age | HB ($g \cdot L^{-1}$) | WBCs ($\times 10^9$) | Platelets ($\times 10^{12}$/L) | APL Cell (%) in BM | Leukemia type | Apoptotic Rate (%, 500 $\mu g \cdot mL^{-1}$) |
|---|---|---|---|---|---|---|---|
| 1 | M/20 | 164 | 51.8 | 5.40 | 97.5 | ALL | 54.44 |
| 2 | F/37 | 64 | 74.8 | 2.31 | 92.5 | ALL | 91.71 |
| 3 | M/48 | 67 | 4.1 | 2.58 | 35.0 | ALL | 86.66 |
| 4 | F/21 | 87 | 12.7 | 2.93 | 81.0 | ALL | 43.61 |
| 5 | M/13 | 75 | 272.8 | 47.00 | 94.5 | ALL | 55.27 |

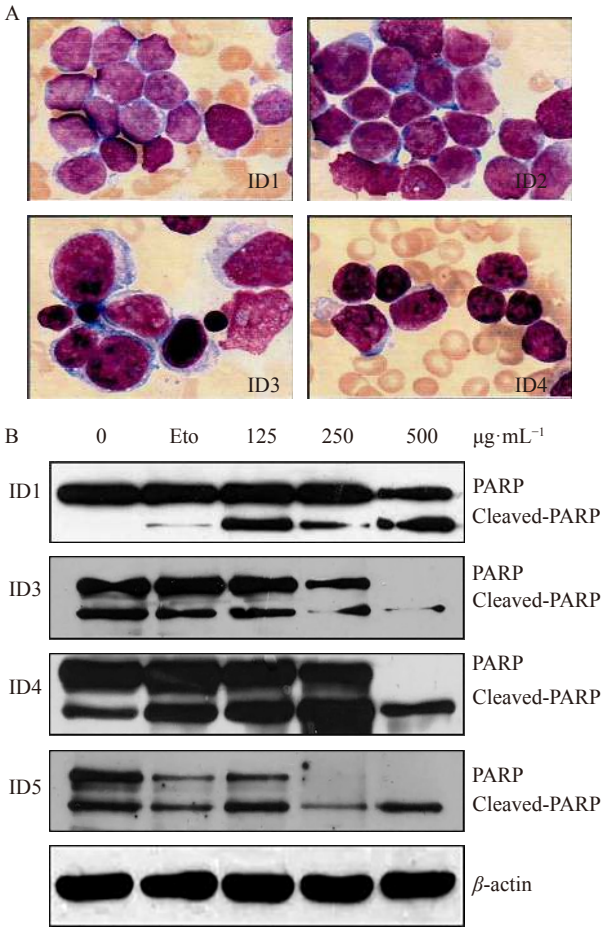



**Fig. 7 The anti-leukemia effect of BJOE in primary human ALL cells. (A) Representative images of ALL leukemia patients' cells stained with Wright Giemsa. (B) Representative Western blot analysis of PARP in primary leukemic cells from ALL patients. *β*-Actin was used as the loading control**

*Anti-leukemic effects of BJOE in the mouse p388 leukemia transplant model*

The *in vivo* anti-leukemia effects of BJOE in the p388 mouse leukemia transplant model were evaluated. BJOE has been used as an anti-cancer agent in China for many years. Based on the recommended clinical dose, we selected conversion coefficients for humans to mice to calculate the optimal doses of BJOE. The three dosages used were 5.5, 16.5 and 49.5 $mg \cdot kg^{-1}$ of BJOE. Following subcutaneous injection of p388 cells into the flanks of the mice, the same method of intravenous injection was used for the administration of BJOE. The results showed that after 14 days, the tumor weights of the BJOE treatment group were lighter than those of the saline-treated group (Fig. 8A). After H&E staining, morphological changes were observed in the tumor sections. As shown in Fig. 8B, both 16.5 and 49.5 $mg \cdot kg^{-1}$ groups showed necrosis and nuclear condensation. The nuclei were hyperchromatic and showed pyknosis, fragmentation, and dissolution. There were also pink staining amorphous material displayed in the sections of the tumor, which is the residue of cell necrosis, as indicated by the arrows in Fig. 8B. These results confirm the significant anti-leukemic effect of BJOE *in vivo*.

We also examined the immunohistochemistry (IHC) staining for ki67, Akt and phospho-Akt (Ser 473) in tumor tissues of the p388 mouse leukemia transplant model. As the results showed, BJOE treatment did not affect the expression of Ki67 and Akt protein in the p388 mouse leukemia transplant model, but decreased the levels of p-Akt protein. These results confirm that BJOE induces apoptosis through the PI3K/Akt signaling pathway *in vivo*.

## Discussion

According to the published cancer statistics for 2017,

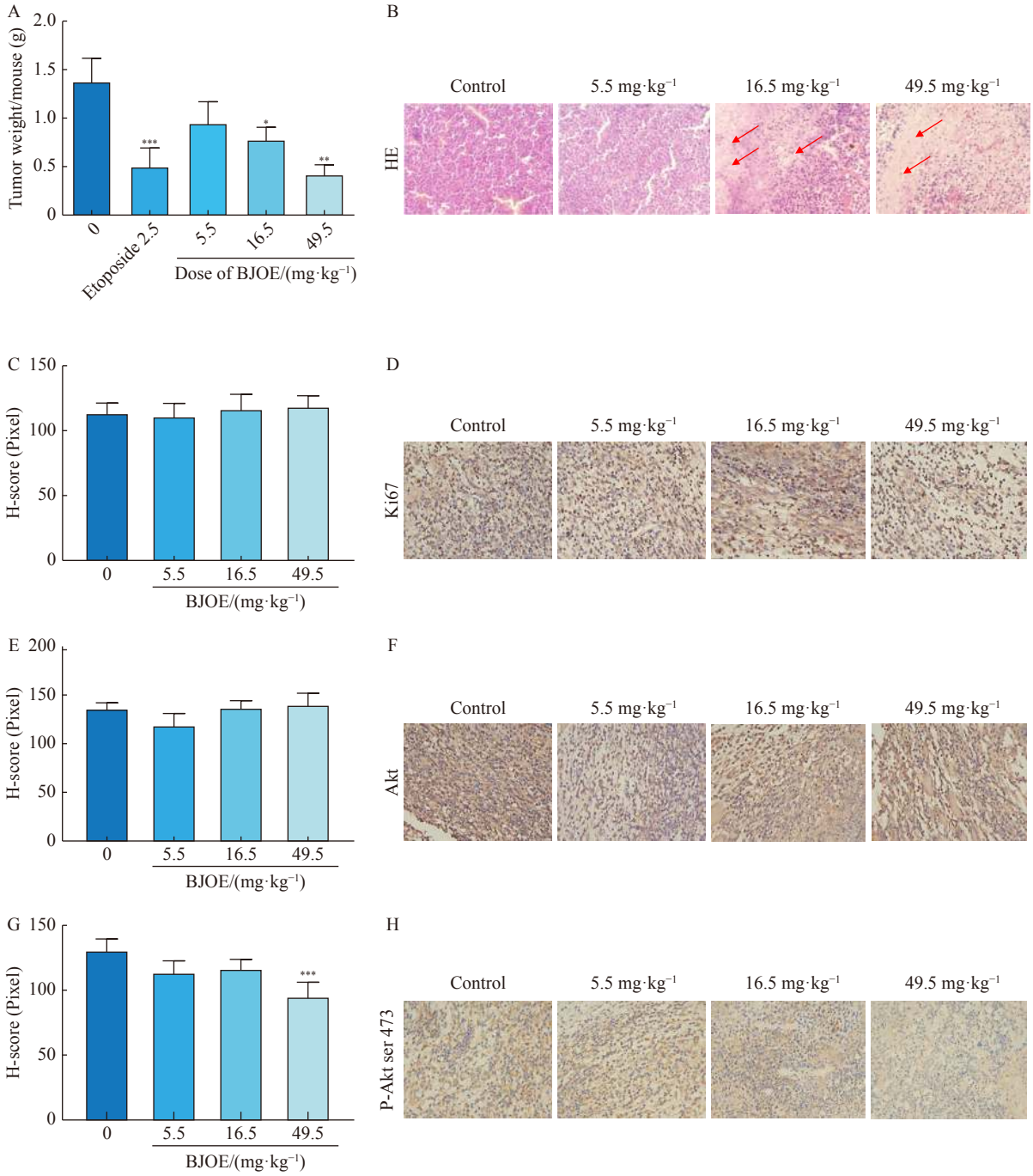



**Fig. 8 The *in vivo* anti-leukemia effect of BJOE in the p388 mouse leukemia transplant model. (A) Weights of tumors. (B) Representitive images of pathological sections with H&E staining. (C−D) The H-score and representitive images of Ki67-positive cells. (E−F) The H-score and representitive images of Akt-positive cells. (G−H)The H-score and representitive images of p-AKT-positive cells (Ser 473). Data are expressed as mean ± SEM ($n$ = 6–8 mice/group). $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* control (one-way ANOVA followed by Dunnett's *t*-test)**

ALL remains the most common type of leukemia in children or adolescents [50]. In recent years, the incidence of ALL has increased in both children and adults [51, 3, 4]. Although chemotherapy remains the primary indicator of ALL, its relapse rates remain high. As chemotherapy exerts negative effects on the bone marrow, the immune system, and the gastrointestinal system, botanical anti-cancer compounds now play an increasingly important role in leukemia treatment. BJOE is a medicinal herb extracted from *Brucea javanica* that has been used in China for the past twenty years, and effective against lung cancer, and brain metastases from prostate and gastrointestinal cancer. Recent studies have reported that BJOE also possesses therapeutic effects against acute and chronic myeloid leukemia cells [35, 43]. Therefore, we in-

vestigated the beneficial effects of BJOE in ALL patients and explored the mechanisms underlying BJOE activity.

We found that BJOE had cytotoxic effects in Jurkat cells in a dose-dependent manner. Morphological observation after AO/EB staining showed that Jurkat cells treated with BJOE displayed apoptotic characteristics, including the appearance of apoptotic bodies, cell shrinkage and nuclear condensation. BJOE treatment also led to a significant increase in the apoptotic cell population in a concentration-dependent manner after PI/Annexin V staining. Furthermore, we found that the apoptotic executor proteins caspase-9, caspase-8 and caspase-3 were activated after BJOE treatment. PARP, a substrate for caspase-3, was also cleaved. BJOE treatment increased the number of sub-$G_1$ phase cells in a concentration-dependent manner. All these data indicate that the cytotoxic effects of BJOE are mediated through the induction of apoptosis.

We further elucidated the mechanisms of BJOE-induced apoptosis. It has been reported that hyper-generation of ROS increases the permeability of mitochondria and disrupts mitochondrial function to initiate mitochondrial-related apoptosis [52]. After MMP collapse, mitochondria releases cytochrome c to activate caspase-9 and trigger apoptosis [53]. We found that BJOE treatment for 6 h increased intracellular ROS levels in Jurkat cells in a concentration-dependent manner. MMP also decreased in response to BJOE. BJOE treatment for 24 h also caused cytochrome c release from mitochondria to the cytosol. These data suggest that the apoptosis induced by BJOE is likely to be a mitochondrial response.

According to Western blot analysis, BJOE treatment down-regulated the phosphorylated form of Akt. The levels of FoxO1 and p53, which are substrates of activated Akt, increased in response to BJOE treatment. Bim, as a transcriptional target of FoxO1 was induced by BJOE. Bcl-2 is a downstream substrate of the Akt/p53 signaling pathway, and its levels decreased in response to BJOE. Bax and XIAP were also regulated by BJOE. Meanwhile, BJOE reduced the protein level of p-GSK3 (Ser9) to activate GSK3$\beta$. These results suggest that BJOE induced apoptosis is related to mitochondrial events and stimulated through the PI3K/Akt signaling pathway.

We used *in vitro* and *in vivo* studies to evaluate the clinical potential of BJOE to treat ALL. Primary leukemic cells from five newly diagnosed ALL patients were examined for their anti-leukemic effects *in vitro*. As shown, leukemic cells from the ALL patients were sensitive to BJOE treatment. BJOE increased the sub-$G_1$ percentage of cells and upregulated the cleavage of PARP proteins. These data suggest that BJOE induces apoptosis in primary leukemic cells from ALL patients *in vitro*. *In vivo*, we used the p388 mouse acute lymphocytic leukemia transplant model to examine the anti-leukemic effects of BJOE. We found that BJOE slowed down tumor growth leading to lower tumor weights compared to the control group. We also observed necrosis in the BJOE treatment groups upon pathological examination. The IHC staining results also showed that BJOE reduced the protein level of p-Akt (Ser473). These results confirm the anti-leukemic effects of BJOE, elucidate the underlying mechanism of inducing apoptosis through the PI3K/Akt pathway, and highlight its potential use in clinical setting.

## Conclusions

In summary, this study elucidates the molecular mechanisms through which BJOE exerts its cytotoxic effects on ALL cells. BJOE induces apoptosis in Jurkat cells through down-regulation of the PI3K/Akt signaling cascade. Our experimental data from BJOE-treated primary ALL cells *in vitro* and p388 mouse leukemia transplant models *in vivo* confirm the anti-leukemia effect of BJOE, and elucidate the role of the PI3K/Akt pathway in BJOE induced apoptosis. All the results will highlight the potential of BJOE as an anti-leukemia medication for the treatment of ALL.

## Ethics approval and consent to participate

This study was approved by the Institute Ethical Committee for Experimental Use of Animals in Shenyang Pharmaceutical University (Nos 20181022). The study was also approved by the Medical Ethical Committee of General Hospital of Northern Military Area (Permit Number: 20180825). All procedures were adhered to principles expressed in Declaration of Helsinki.

## References

[1] Gao C, Zhang RD, Liu SG, *et al*. Low CREBBP expression is associated with adverse long-term outcomes in paediatric acute lymphoblastic leukemia [J]. *Eur J Haematol*, 2017, **99**(2): 150-159.

[2] Jing SY, Wu ZD, Zhang TH, *et al*. *In vitro* antitumor effect of cucurbitacin E on human lung cancer cell line and its molecular mechanism [J]. *Chin J Nat Med*, 2020, **18**(7): 483-490.

[3] Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update [J]. *Blood Cancer J*, 2017, **7**(6): e577.

[4] Inaba H, Pui CH. Advances in the diagnosis and treatment of pediatric acute lymphoblastic leukemia [J]. *J Clin Med*, 2021, **10**(9): 1926.

[5] Wu FF, Cao W, Xu HP, *et al*. T-cell acute lymphoblastic leukemia/lymphoma cells apoptosis through the mitochondrial signaling pathway [J]. *Oncol Lett*, 2017, **13**(3): 1702-1710.

[6] Simioni C, Ultimo S, Martelli AM, *et al*. Synergistic effects of selective inhibitors targeting the PI3K/AKT/mTOR pathway or NUP214-ABL1 fusion protein in human Acute Lymphoblastic Leukemia [J]. *Oncotarget*, 2016, **7**(48): 79842-79852.

[7] Ding HM, Chen XJ, Chen HM, *et al*. Effect of *Sargassum fusiforme* polysaccharide on apoptosis and its possible mechanism in human erythroleukemia cells [J]. *Chin J Nat Med*, 2020, **18**(10): 749-759.

[8] Bongiovanni D, Saccomani V, Piovan E. Aberrant signalling pathways in T-cell acute lymphoblastic leukemia [J]. *Int J Mol Sci*, 2017, **18**(9): 1904.

[9] Guo HR, Cui HM, Peng X, *et al*. Modulation of the PI3K/Akt

pathway and Bcl-2 family proteins involved in Chicken's tubular apoptosis induced by Nickel Chloride [J]. *Int J Mol Sci*, 2015, **16**(9): 22938-23011.

[10] Rahmani M, Aust MM, Attkisson E, *et al*. Dual inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism [J]. *Cancer Res*, 2013, **73**(4): 1340-1351.

[11] Song HM, Song JL, Li DF, *et al*. Inhibition of FOXO1 by small interfering RNA enhances proliferation and inhibits apoptosis of papillary thyroid carcinoma cells *via* Akt/FOXO1/Bim pathway [J]. *Onco Targets Ther*, 2015, **8**: 3565-3573.

[12] Abraham AG, O'Neill E. PI3K/Akt-mediated regulation of p53 in cancer [J]. *Biochem Soc Trans*, 2014, **42**(4): 798-803.

[13] Tang B, Tang F, Wang ZR, *et al*. Upregulation of Akt/NF-kB-regulated inflammation and Akt/Bad-related apoptosis signaling pathway involved in hepatic carcinoma process: suppression by carnosic acid nanoparticle [J]. *Int J Nanomed*, 2016, **11**: 6401-6420.

[14] Beurel E, Jope RS. The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways [J]. *Prog Neurobiol*, 2006, **79**(4): 173-189.

[15] Rubio S, Quintana J, Lopez M, *et al*. Phenylbenzopyrones structure-activity studies identify betuletol derivatives as potential antitumoral agents [J]. *Eur J Pharmacol*, 2006, **548**(1-3): 9-20.

[16] Huang YF, Zhou JT, Qu C, *et al*. Anti-inflammatory effects of Brucea Javanical oil emulsion by suppressing NF-*κ*B activation on dextran sulfate sodium-induced ulcerative colitis in mice [J]. *J Ethnopharmaco*, 2017, **198**: 389-398.

[17] Kamperdick C, Sung TV, Thuy TT, *et al*. (20*R*)-*O*-(3)-*α*-L-arabinopyranosylpregn-5-*ene*-3*β*, 20-diol from *Brucea javanica* [J]. *Phytochemistry*, 1995, **38**: 699-701.

[18] Shi CY. The anti-cancer effect of *Brucea Javanica*: a summary of the clinical report [J]. *J Shenyang Coll Pharm*, 1978, **10**: 15-19.

[19] Li XY, Xie YH. *Brucea Javanica* oil can cure papilloma in acoustic duct, vocal cords, gum and nasal polyp: 5 case reports [J]. *Guangdong Med J*, 1984, **5**: 23.

[20] Yu YL, Lu Y, Tang X, *et al*. Formulation, preparation and evaluation of an intravenous emulsion containing *Brucea Javanica* oil and Coix seed oil for anti-tumor application [J]. *Biol Pharma Bull*, 2008, **31**(4): 673-680.

[21] Su XR, Yu QH, Zhao HD, *et al*. *Brucea Javanical* oil emulsion can inhibit DNA synthesis in 3H-TdR incorporated mouse Ehrlich's ascites carcinoma cells [J]. *J Shenyang Coll Pharm*, 1981, **14**: 8-12.

[22] Yang ZB, Su XR, Li PZ, *et al*. *Brucea Javanica* oil emulsion can affect the structure of 3H incorporated Ehrlich's ascites carcinoma cells [J]. *J Shenyang Coll Pharm*, 1986, **3**: 1-7.

[23] Su XR, Yao CS, Chen JM, *et al*. The pharmacokinetics study of $^{3}$H-oleic acid: the active components of *Brucea Javanica* oil [J]. *Chin Tradit Herb Drugs*, 1981, **12**: 21-24.

[24] Su SY, Wu SC. 100 Cases of *Brucea Javanical* oil emulsion treatment for patients with brain metastasis of lung cancer: clinical observation [J]. *J Pract Oncol*, 1989, **3**: 33-35.

[25] Ji ZQ, Huang XE, Wu XY, *et al*. Safety of *Brucea Javanica* and cantharidin combined with chemotherapy for treatment of NSCLC patients [J]. *Asian Pac J Cancer Prev*, 2014, **15**(20): 8603-8605.

[26] Nie YL, Liu KX, Mao XY, *et al*. Effect of injection of *Brucea Javanica* oil emulsion plus chemoradiotherapy for lung cancer: a review of clinical evidence [J]. *J Evid Based Med*, 2012, **5**(4): 216-225.

[27] Xu W, Jiang X, Xu Z, *et al*. The efficacy of *Brucea Javanica* oil emulsion injection as adjunctive therapy for advanced non-small-cell lung cancer: A meta-analysis [J]. *Evid Based Complement Altern Med*, 2016, **2016**: 5928562.

[28] Lu YY, Huang XE, Cao J, *et al*. Phase II study on Javanica oil emulsion injection (Yadanzi®) combined with chemotherapy in treating patients with advanced lung adenocarcinoma [J]. *Asian Pac J Cancer Prev*, 2013, **14**(8): 4791-4794.

[29] Liu J, Huang XE, Tian GY, *et al*. Phase II study on safety and efficacy of Yadanzi® (Javanica oil emulsion injection) combined with chemotherapy for patients with gastric cancer [J]. *Asian Pac J Cancer Prev*, 2013, **14**(3): 2009-2012.

[30] Chen Z, Zhou Z, Hu Z, *et al*. Effect of FOLFX4 combined with *Brucea Javanica* emulsion on VEGF in patients with gastric cancer [J]. *Oncol Lett*, 2018, **15**(1): 1079-1083.

[31] Wu JR, Liu SY, Zhu JL, *et al*. Efficacy of *Brucea Javanica* oil emulsion injection combined with the chemotherapy for treating gastric cancer: a systematic review and meta-analysis [J]. *Evid Based Complement Altern Med*, 2018, **2018**: 6350782.

[32] Yuan Y, Yang Y, Shi L, *et al*. Suppression of human hepatocellular cancer cell proliferation by *Brucea Javanica* oil-loaded liposomes *via* induction of apoptosis [J]. *Arch Med Sci*, 2015, **11**(4): 856-862.

[33] Lou GG, Yan HP, Xie LP. *Brucea javanica* oil induces apoptosis in T24 bladder cancer cells *via* upregulation of caspase-3, caspase-9, and inhibition of NF-kappa B and COX-2 expressions [J]. *Am J Chin Med*, 2010, **38**(3): 613-624.

[34] Ye HX, Liu XJ, Sun JC, *et al*. Enhanced therapeutic efficacy of LHRHa-targeted *Brucea javanica* oil liposomes for ovarian cancer [J]. *BMC Cancer*, 2016, **16**(1): 831.

[35] Pan P, Yang BX, Ge XL. *Brucea Javanica* seed oil enhances the radiosensitivity of esophageal cancer by inhibiting hypoxia-inducible factor 1*α*, *in vitro* and *in vivo* [J]. *Oncol Lett*, 2018, **15**(3): 3870-3875.

[36] Zhang H, Yang JY, Zhou F, *et al*. Seed oil of *Brucea javanica* induces apoptotic death of acute myeloid leukemia cells *via* both the death receptors and the mitochondrial related pathways [J]. *Evid Based Complement Altern Med*, 2011, **2011**: 965016.

[37] Dorsey JF, Jove R, Kraker AJ, *et al*. The pyrido[2, 3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells [J]. *Cancer Res*, 2000, **60**(12): 3127-3131.

[38] Yan Z, Zhang B, Huang Y, *et al*. Involvement of autophagy inhibition in *Brucea Javanica* oil emulsion-induced colon cancer cell death [J]. *Oncol Lett*, 2015, **9**(3): 1425-1431.

[39] Yu ZY, Wang R, Xu LY, *et al*. *N*-(b-Elemene-13-yl) tryptophan methyl ester induces apoptosis in human leukemia cells and synergizes with arsenic trioxide through a hydrogen peroxide dependent pathway [J]. *Cancer Lett*, 2008, **269**(1): 165-173.

[40] Han YH, Kim SZ, Kim SH, *et al*. Arsenic trioxide inhibits the growth of Calu-6 cells *via* inducing a $G_2$ arrest of the cell cycle and apoptosis accompanied with the depletion of GSH [J]. *Cancer Lett*, 2008, **270**(1): 40-55.

[41] Mackall CL, Fleisher TA, Brown MR, *et al*. Lymphocyte depletion during treatment with intensive chemotherapy for can-

cer [J]. *Blood*, 1994, **84**(7): 2221-2228.
[42] Yin SL, Wang R, Zhou F, *et al*. Bcl-xL is a dominant anti-apoptotic protein that inhibits homoharringtonine-induced apoptosis in leukemia cells [J]. *Mol Pharmacol*, 2011, **79**(6): 1072-1083.
[43] Filipski E, Amat S, Lemaigre G, *et al*. Relationship between circadian rhythm of vinorelbine toxicity and efficacy in P388-bearing mice [J]. *J Pharmacol Exp Ther*, 1999, **289**(1): 231-235.
[44] Zhang JY, Lin MT, Tung HY, *et al*. Bruceine D induces apoptosis in human chronic myeloid leukemia K562 cells *via* mitochondrial pathway [J]. *Am J Cancer Res*, 2016, **6**(4): 819-826.
[45] Thornberrry NA. The caspase family of cysteine proteases [J]. *Br Med Bul*, 1997, **53**(3): 478-490.
[46] Wang FY, Liu YJ, Wang LH, *et al*. Targeting procaspase-3 with WF-208, a novel PAC-1 derivative, causes selective cancer cell apoptosis [J]. *J Cell Mol Med*, 2015, **19**(8): 1916-1928.
[47] Dawick-Mckenna JM, Langelier MF, DeNizio JE, *et al*. PARP-1 activation requires local unfolding of an autoinhibitory domain [J]. *Mol Cell*, 2015, **60**: 755-768.
[48] Chipuk JE, Green DR. How do Bcl-2 proteins induce mitochondrial outer membrane permeabilization [J]. *Trends Cell Biol*, 2008, **18**(4): 157-164.
[49] Lee I, Bender Eand Kadenbach B. Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase [J]. *Mol Cell Biochem*, 2002, **234-235**(1-2): 63-70.
[50] Siege RL, Miller KD, Jemal A. Cancer statistics, 2017 [J]. *CA Cancer J Clin*, 2017, **67**(1): 7-30.
[51] Amitay EL, Keinan-Boker L. Breastfeeding and childhood leukemia incidence a meta-analysis and systematic review [J]. *JAMA Pediatr*, 2015, **169**(6): e151025.
[52] Pathak N, Khandelwal S. Role of oxidative stress and in cadmium induced thymic atrophy and splenomegaly in mice [J]. *Toxicol Lett*, 2007, **169**(2): 95-108.
[53] Dong Z, Saikumar P, Patel Y, *et al*. Serine protease inhibitors suppress cytochrome c-mediated caspase-9 activation and apoptosis during hypoxia-reoxygenation [J]. *Biochem J*, 2000, **347**(3): 669-677.

**Cite this article as:** ZHANG Hong, YIN Shi-Liang, WANG Li-Hui, JIA Li-Na, SU Guang-Yue, LIU Xiao-Qing, ZHOU Fan, BRESLIN Peter, MENG Ran, LI Qi-Yi, YANG Jing-Yu, WU Chun-Fu. Seed oil of *Brucea javanica* induces apoptosis through the PI3K/Akt signaling pathway in acute lymphocytic leukemia Jurkat cells [J]. *Chin J Nat Med*, 2021, **19**(8): 608-620.