

Antitumor activity of the *Ailanthus altissima* bark phytochemical ailanthone against breast cancer MCF-7 cells

RUXING WANG^{1,2}, YANJIE LU³, HONG LI⁴, LIXIN SUN⁴, NING YANG⁴,
MINGZHEN ZHAO⁴, MANLI ZHANG¹ and QINGWEN SHI¹

¹Department of Medicinal Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University, Shijiazhuang, Hebei 050017; ²Hebei Province Key Laboratory of Research and Development for Chinese Medicine, Institute of Chinese Materia Medica; ³Department of Pathology, Institute of Cancer Research, Chengde Medical College; ⁴Department of Central Laboratory, The Affiliated Hospital of Chengde Medical College, Chengde, Hebei 067000, P.R. China

Received March 18, 2016; Accepted November 16, 2017

DOI: 10.3892/ol.2018.8039

Abstract. Ailanthone is isolated from the bark of *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae). The mechanism that underlies the activity of ailanthone on MCF-7 cells was investigated by MTT assay. Breast cancer MCF-7 cells were treated with 0.5, 1.0, 2.0, 4.0 and 8.0 $\mu\text{g/ml}$ ailanthone for 24, 48 and 72 h. The inhibition of proliferation induced by treatment with ailanthone was assessed by MTT assay. Apoptosis and cell cycle distribution in MCF-7 cells with the same doses of ailanthone for 48 h were determined by flow cytometry. Expression of apoptosis-associated genes and proteins were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis, respectively. The results revealed that ailanthone inhibited MCF-7 cell proliferation. Flow cytometry assay demonstrated that ailanthone induced apoptosis and G₀/G₁ cell cycle arrest in MCF-7 cells. Western blotting and RT-PCR assays demonstrated that upregulation of pro-apoptotic caspase-3 and Bcl-associated X, and downregulation of anti-apoptotic apoptosis regulator B-cell lymphoma-2 in MCF-7 cells may be associated with the induction of apoptosis and inhibition of proliferation. To the best of our knowledge, the present study is the first to investigate the antitumor activity of ailanthone from *A. altissima* on MCF-7 cells and to attempt to elucidate the underlying mechanism. The present study revealed the presence of ailanthone-mediated antitumor effects, indicating that ailanthone may be a novel phytomedicine with potential use in breast cancer therapy.

Introduction

Breast cancer is one of the most common cancers and the leading cause of cancer-associated mortality among women, causing significant morbidity worldwide (1). Nearly 5.8/100,000 new breast cancer cases are diagnosed in developed countries and >10.5 new breast cancer cases per 100,000 individuals occur worldwide each year (1). In the early stages of the disease, breast cancer, as in case of a number of other cancers, tends to spread asymptotically throughout the body and at the point of diagnosis there is an increased likelihood of identification of metastatic lesions (2). Conventional therapies, including surgery, radiotherapy and chemotherapy, have served roles in treatment of breast cancer, but there remain numerous problems in tumor therapy. For example, cyclophosphamide, methotrexate and 5-fluorouracil chemotherapy for breast cancer have various side effects (3,4).

Despite progress in anticancer therapeutics, there are few efficient drugs with low toxicity available to treat cancer. Plants have been previously used in cancer therapy (5). Traditional medical phytotherapy has been used for cancer treatment for several millennia in Asia, including in China, Japan and Thailand, and is currently being used for cancer prevention and therapy worldwide (6-8). Throughout the centuries, certain plant extracts have been tested for antitumor potential (9). Plants are a source of a number of effective anticancer agents in current use, including taxanes, irinotecan, topotecan, vincristine and vinblastine (10-12). Plant products such as these can aid in discovery and development of novel anticancer drugs. In addition, plant products demonstrate fewer side effects compared with chemical drugs. There has been an increasing interest in identifying and isolating natural compounds from medicinal plants with an aim to develop novel anticancer drugs (13).

Ailanthus altissima is an example of a plant that has been used in tumor therapy (14). *A. altissima*, which grows in Northeast and Central China, can also be present in Europe and the USA and has been used as a traditional Chinese medicine for a number of years (15). The bark of *A. altissima* has been used for the treatment of ascariasis, diarrhea, spermatorrhea, bleeding and gastrointestinal diseases in China (16).

Correspondence to: Dr Qingwen Shi, Department of Medicinal Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University, 361 Zhong Shan East Road, Shijiazhuang, Hebei 050017, P.R. China
E-mail: 1716023668@qq.com

Key words: quassinoid, breast cancer, inhibition of proliferation, apoptosis, cell cycle

The antitumor effect that has enabled the use of this plant in the treatment of colonic, cervical, and rectal cancer has been previously described (17). In the present study, ailanthone was successfully isolated from *A. altissima*. Ailanthone has a wide spectrum of biological activities, including anti-allergic, anti-malarial, anti-HIV, anti-inflammatory, anti-ulcer and anti-microbial activities (18,19). Ailanthone has been demonstrated to exhibit *in vitro* growth-inhibitory effects against several cancer cell lines, including HepG2, Hep3B, R-HepG2, HeLa and A549 cells (18,20). Rosati (20) demonstrated that ailanthone could induce mitochondrial membrane depolarization and caspase-3 activation in Jurkat cancer cells. However, the antitumor activity in breast cancer treatment and the underlying molecular mechanism remain to be elucidated. In the present study, MCF-7 cells were used to evaluate the antitumor activity of ailanthone and the underlying molecular mechanism.

Materials and methods

Plant material and extraction. The bark of *A. altissima* (catalog no., NMC-2012-2) was purchased from Anguo Zhong Sheng Medicinal Material Co., Ltd. (Anguoshi, China). The bark of *A. altissima* was powdered, a 10-fold volume of methanol was added to the powdered sample and allowed to soak for 12 h. Hot reflux was performed two times serially, each time using a rotary evaporator with 95% ethanol at 80°C for 2 h, filtered, and the filtrate was merged. The combined filtrates were concentrated *in vacuo*, and then treated with saturated salt solution at concentration of 20%. It was extracted with petroleum ether and dichloromethane for 2 h, and dried *in vacuo* at 40°C respectively. The extract with was separated with 50 times the amount of 200-300 mesh silica gel column (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). The mobile phase was a solution of dichloromethane and methanol in ratios of 50:1, 30:1, 15:1, 10:1, 5:1, and 500 ml was collected using each ratio. A total of 211 fractions were collected, and similar fractions were combined on the basis of TLC to obtain 35 major fractions. Following further purification by crystallization, ailanthone was purified as previously described (21).

Cell preparation. Breast cancer MCF-7 cells were purchased from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China), and grown in Dulbecco's Modified Eagle's medium (DMEM, Gibco; Thermo Fisher Scientific, Inc.). DMEM was supplemented with 100 µg/ml streptomycin, penicillin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 10% (v/v) FBS (Gibco; Thermo Fisher Scientific, Inc.). Cells were cultured at 37°C and 5% CO₂ in a humidified environment.

Cell viability assay. Cell viability was assessed by MTT colorimetric assay (22). The cells were treated with various concentrations (0.5, 1.0, 2.0, 4.0 and 8.0 µg/ml) of ailanthone for 24, 48 and 72 h. At the end of the treatment, 50 µl MTT (5 mg/ml) was added to each well (1x10⁵ cells/ml) and the samples were incubated for an additional 1 h. The culture medium was removed and dissolved by adding 100 µl dimethyl sulfoxide. The absorbance was measured at a wavelength of 490 nm.

Cell apoptosis. The level of cell apoptosis was determined by flow cytometry with annexin V (BD Biosciences, Franklin Lakes, NJ, USA) labeling and propidium iodide (PI) exclusion staining. MCF-7 cells were treated with 0.5, 1.0, 2.0, 4.0 and 8.0 µg/ml ailanthone for 48 h, collected, washed with PBS and suspended in binding buffer (BD Biosciences). Subsequently, the cells (1x10⁵ cells/ml) were stained with 10 µl annexin V-fluorescein FITC and 5 µl PI, incubated in dark at room temperature for 15 min according to the manufacturer's protocol and subjected to flow cytometry using a flow cytometer (ModFit 6.0, BD Biosciences, USA). Cells undergoing apoptosis were stained with annexin V and treated as described above.

Cell cycle distribution. Cells were seeded at a density of 1x10⁶/ml in 6-well microplates and treated with 0.5, 1.0, 2.0, 4.0 and 8.0 µg/ml ailanthone for 48 h. A total of 1x10⁵ cells were collected for each treatment, washed with PBS, trypsinized with 0.25% trypsin and harvested by centrifugation for 5 min at 70 x g at room temperature. Cells were re-suspended with 0.5 ml PBS, fixed overnight with cold 70% ethanol at -20°C and stained with PI solution containing 10 µg/ml RNase A and 50 µg/ml PI for 60 min at room temperature. Cells were analyzed by flow cytometry, as previously described (23).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. MCF-7 cells were treated by ailanthone (0.5, 1.0, 2.0, 4.0 and 8.0 µg/ml), and following extraction of total cellular RNA from the MCF-7 cells using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 2 µg of total RNA was reverse transcribed to generate cDNA using the PrimeScript RT reagent kit (Takara Bio, Inc.). The following thermocycling conditions were used for RT-PCR: Initial denaturation at 94°C for 30 sec, followed by 30 cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 45 sec, and the final extension at 72°C for 8 min. The PCR products were resolved in 2% agarose gels and visualized by staining with ethidium bromide (24). To semiquantify the PCR products, the bands representing the amplified products were analyzed by Quantity One analysis software (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The relative level of the target mRNA expression was defined as the ratio of the absorbance of the target band to that of the β-actin band. The following primer sequences were used for PCR: (Takara RNA PCR Kit AMV, Ver.3.0) Caspase-3 (442 bp), forward 5'-ATCTCGGTCTGGTACAGATGTCGAT-3' and reverse 5'-TGAATTTCCGCAAGAATAATACCA-3'; Bcl-associated X (Bax; 258 bp), forward 5'-CAC CAGCTCTGAGCAGATCA-3', reverse 5'-ATGTCAGCTGCC ACTCGGA-3'; B-cell lymphoma 2 (Bcl-2; 383 bp), forward 5'-TACGAGTGGGATGCGGGAGATGT-3', reverse 5'-CCA CCGAACTCAAAGAAGGC-3'; GAPDH (135 bp), forward 5'-CAATGACCCCTTCATTGACC-3', reverse 5'-TGGAAG ATGGTGTATGGGATT-3'.

Western blot analysis. MCF-7 cells were treated by ailanthone (0.5, 1.0, 2.0, 4.0 and 8.0 µg/ml), and lysed in lysis buffer [50 mmol/l Tris-HCl (pH 8.0), 150 mmol/l NaCl, 0.5% NP40, 0.5% sodium deoxycholate, 0.1% SDS with protease inhibitor]. Determination of total protein content was performed using a BCA assay (Beijing Solarbio Science and Technology, Co., Ltd., Beijing China). The proteins were separated by 12%

SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% milk in Tris-buffered saline-0.05% Tween-20 (TBST) for 3 h at room temperature. Following washing in TBST, the membranes were incubated for 2 h at room temperature with 1:1,000 dilutions of rabbit monoclonal antibodies for Bcl-2, (cat no. 2872; Cell Signaling Technology, Inc., Danvers, MA, USA), Bax (cat no. 2772; Cell Signaling Technology, Inc.) and Caspase-3, (cat no. 9662; Cell Signaling Technology, Inc.), and a mouse monoclonal antibody for β -actin (ab8226; Abcam, Cambridge, UK). Following washing in TBST, the blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G secondary antibody (rabbit monoclonal antibody; mouse monoclonal antibody, 1:3,000; Abcam) for 1 h at room temperature. The signal was detected using an ECL Western Blotting Substrate kit (Applygen Technologies, Inc., Beijing, China), and exposed to X-ray film at room temperature. Protein expression levels were quantitated using Quantity-one software (v 4.62; Bio-Rad Laboratories, Inc.).

Statistical analysis. SPSS software (version 16; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis of the data. Data are presented as the mean \pm standard deviation. One-way analysis of variance followed by Dunnett's test was used for statistical analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Inhibitory effect of ailanthon on proliferation of tumor cells. The effect of ailanthon on viability of MCF-7 cells was measured by MTT assay. Compared with the untreated control, the viability of MCF-7 cells treated with 0.5, 1.0, 2.0, 4.0 or 8.0 $\mu\text{g/ml}$ of ailanthon was 92.62, 88.46, 86.36, 73.74 and 64.05%, respectively, following 24 h of treatment; 77.27, 75.65, 69.89, 61.40 and 52.40%, respectively, following 48 h of treatment; and 64.36, 62.48, 57.64, 50.24 and 43.24%, respectively, following 72 h of treatment (Fig. 1). The data indicated that ailanthon inhibits MCF-7 cell proliferation.

Effects of ailanthon on apoptosis of MCF-7 cells. Following 48 h of treatment with ailanthon, 22.28, 27.99, 35.88, 49.77 and 75.51% of cells were apoptotic at doses of 0.5, 1.0, 2.0, 4.0 and 8.0 $\mu\text{g/ml}$, respectively (Fig. 2). In total, 2.13% of control cells were apoptotic at 48 h, which is significantly different to all treatment groups ($P < 0.01$).

Effects of ailanthon on cell cycle of MCF-7 cells. Following treatment with 0.5, 1.0, 2.0, 4.0 and 8.0 $\mu\text{g/ml}$ ailanthon for 48 h, all treatment groups exhibited statistically significant differences compared with the control. Following treatment with ailanthon, the proportion of cells in the G_0/G_1 phase increased and percentage of cells in S and G_2/M phases decreased significantly compared with the control group (Fig. 3).

Alteration of expression levels of Bcl-2 and Bax mRNA. Following 48 h of treatment with different doses of ailanthon, RT-qPCR demonstrated that in the MCF-7 cells, expression of the Bax and caspase-3 genes increased, whereas expression

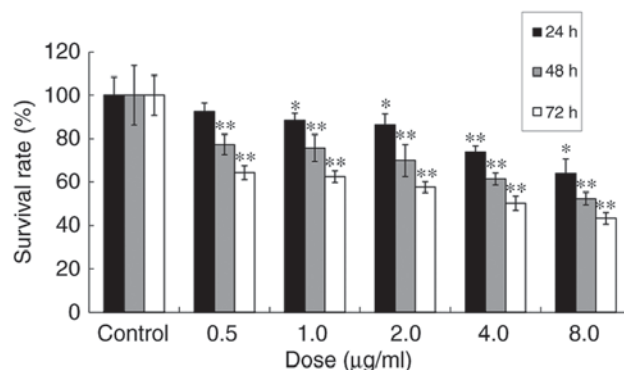


Figure 1. Effect of ailanthon on MCF-7 cell survival. Cells were treated with 0.5, 1, 2, 4 and 8 $\mu\text{g/ml}$ ailanthon for 24, 48, and 72 h. Cell survival was determined by MTT. Data are presented as the mean \pm standard deviation ($n=3$). * $P < 0.05$ and ** $P < 0.01$ vs. the respective control group.

levels of the Bcl-2 gene decreased. Doses of 1.0, 2.0, 4.0 and 8.0 $\mu\text{g/ml}$ of ailanthon resulted in significantly altered expression, compared with the control group (Fig. 4).

Effects of ailanthon on protein expression levels by western blotting. Following 48 h of treatment with 0.5, 1.0, 2.0, 4.0 or 8.0 $\mu\text{g/ml}$ ailanthon, it was observed that ailanthon promoted the expression of Bax and caspase-3 proteins, whereas the expression of Bcl-2 protein was inhibited. The inhibitory effect on Bcl-2 protein expression increased with the increase of the doses ($P < 0.05$). The Bax/Bcl-2 ratios were 0.27, 0.53, 0.56, 0.80, 0.93 and 1.25 at dosages of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 $\mu\text{g/ml}$, respectively, and thus increased following treatment with ailanthon in a dose-dependent manner (Fig. 5).

Discussion

Breast cancer is the primary cause of mortality among women in the world, according to statistics from 2009 (2). Currently, certain cytotoxic drugs are used for the treatment of breast cancer, including daunorubicin, doxorubicin, cisplatin and bleomycin (25). However, these agents are costly and have been demonstrated to induce several side effects, including emesis, anemia, myelosuppression and cellular resistance (3). Therefore, it is necessary to identify alternative drugs or therapies to minimize side effects (26). As a result, natural medicines that cause fewer side effects have been attracting substantial attention (27). Previous research has demonstrated that natural antitumor drugs may serve important roles in the future. A phytochemical study demonstrated the presence of quassinoids in *A. altissima* and these compounds have been previously demonstrated to exhibit potent antitumor properties (28). To the best of our knowledge, the present study is the first to report that ailanthon isolated from *A. altissima* inhibits proliferation of MCF-7 cells.

The majority of drugs achieve antitumor effects by inducing apoptosis in tumor cells (29). Cell cycle analysis is used to determine the distribution of cells in different phases of the cell cycle and enables investigation of tumor proliferation as opposed to apoptosis. There are three cell cycle regulation points in G_1 , S and G_2 phases, which can modulate cell cycle progression. Induction of tumor cell cycle arrest in G_0/G_1

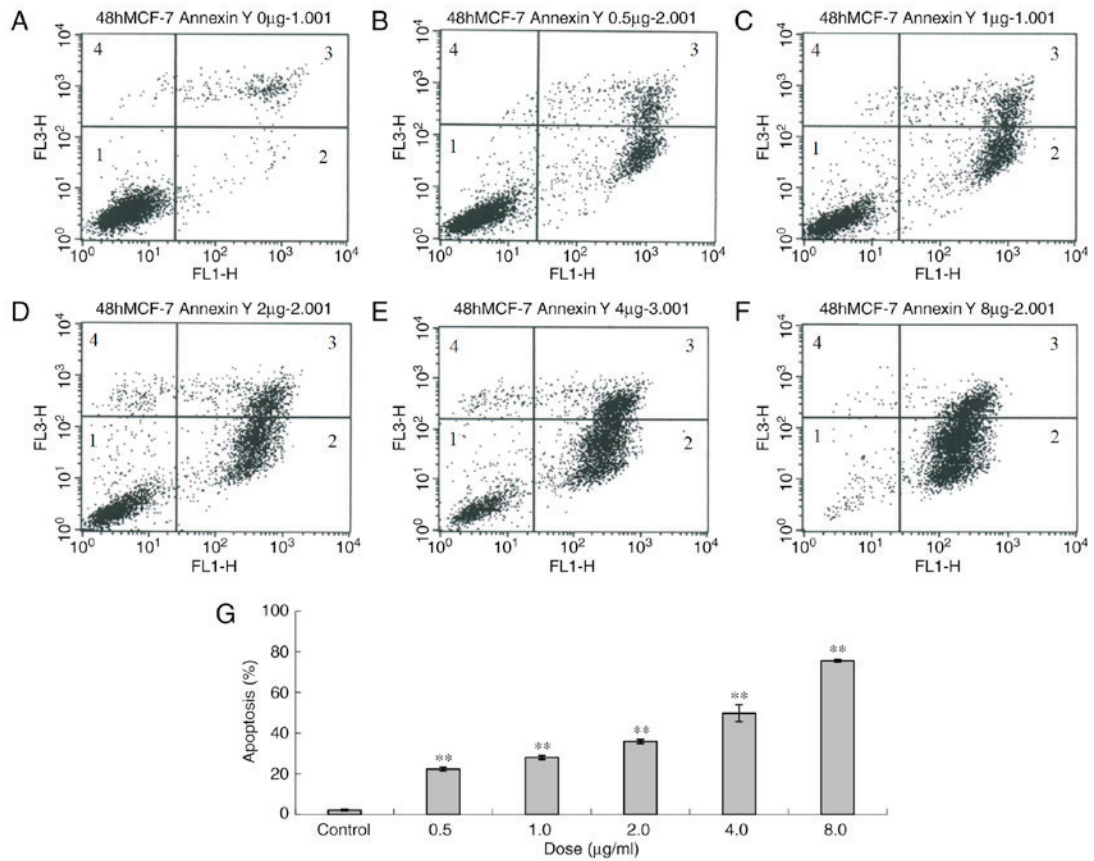


Figure 2. Apoptosis of MCF-7 cells following treatment with different doses of ailanthone (0.5, 1, 2, 4 and 8 μg/ml) for 48 h, measured by flow cytometry. (A) Control (0 μg/ml) and (B) 0.5 μg/ml, (C) 1.0 μg/ml, (D) 2.0 μg/ml, (E) 4.0 μg/ml and (F) 8.0 μg/ml ailanthone. (G) Histogram of apoptosis of MCF-7 cells. Data are presented as the mean ± standard deviation (n=3). **P<0.01 vs. the control and treatment groups. 1, normal cells; 2, early apoptosis; 3, late apoptosis; 4, dead cells.

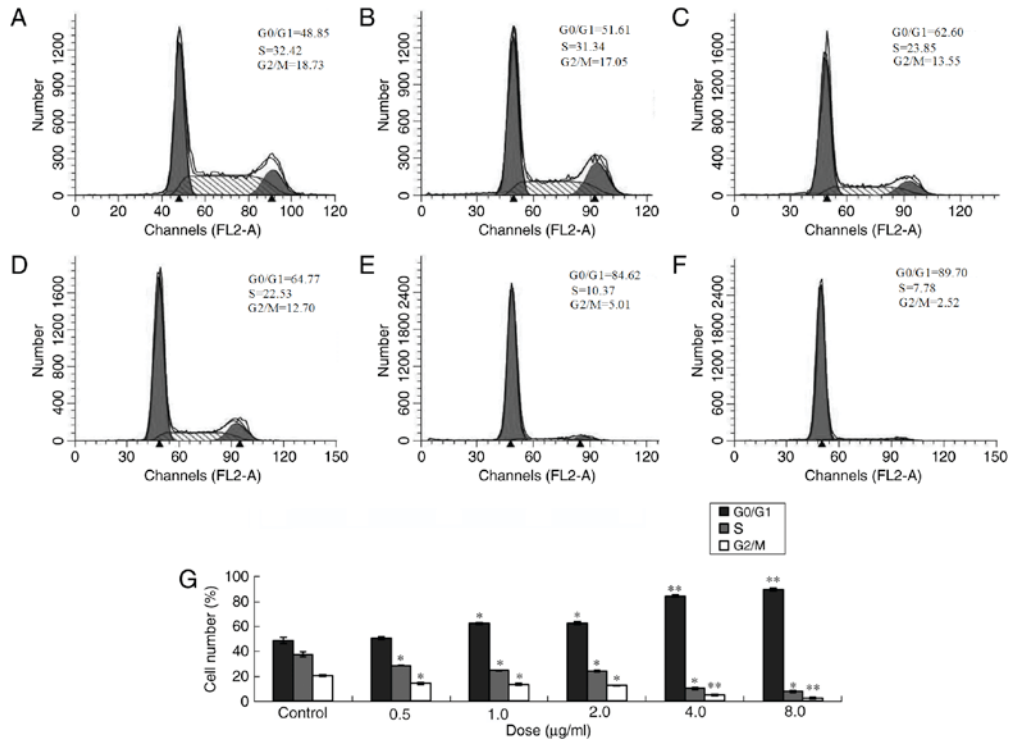


Figure 3. Cell cycle of MCF-7 cells following treatment with different doses of ailanthone. MCF-7 cells were treated with different doses of ailanthone for 48 h and the cell cycle distribution was measured by flow cytometry. (A) Control (0 μg/ml) and (B) 0.5 μg/ml, (C) 1.0 μg/ml, (D) 2.0 μg/ml, (E) 4.0 μg/ml and (F) 8.0 μg/ml ailanthone. (G) Cell cycle distribution of MCF-7 cells. Data are presented as the mean ± standard deviation (n=3). *P<0.05 and **P<0.01 vs. the respective control group.

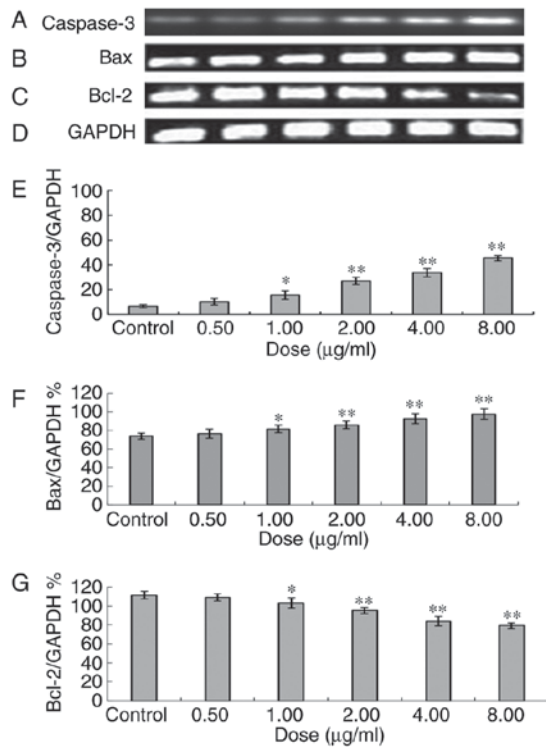


Figure 4. Caspase 3, Bax and Bcl-2 mRNA in MCF-7 cells following treatment with different doses of ailanthonone. Following 48 h of treatment with ailanthonone, levels of (A) caspase-3, (B) Bax, (C) Bcl-2 and (D) reference GAPDH mRNA in MCF-7 cells were measured by densitometric analysis of reverse transcription-polymerase chain reaction. Quantitative analysis of expression of (E) Caspase-3, (F) Bax and (G) Bcl-2. Data are presented as the mean \pm standard deviation (n=3). *P<0.05 and **P<0.01 vs. the control group. Bcl-2, B-cell lymphoma-2; Bax, Bcl-associated X.

phase is a target for the development of antitumor therapy (30). Certain molecules, including tumor protein p53, serve a role in cell cycle inhibition and induction of apoptosis; cells may be arrested in the G₁ phase and apoptosis may be induced by p53 (31). In the present study, the results of flow cytometry revealed that, following treatment with ailanthonone, apoptosis in MCF-7 cells increased. Analysis of the cell cycle by flow cytometry demonstrated an increase in the number of MCF-7 cells in G₀/G₁ phase following treatment with ailanthonone and a decrease in the number of cells in S phase, indicating that the cells were arrested in the G₀/G₁ phase by so that the cells could not enter S phase or perform DNA synthesis, thus inhibiting proliferation. This checkpoint may be involved in the effects of ailanthonone on the cell cycle of MCF-7 cells (32), but this potential mechanism remains to be further investigated. Caspases are mediators of apoptosis, of which caspase-3 are frequently activated death protease that catalyzes specific cleavage of numerous cellular proteins (33). Tumor inhibition and apoptosis, as well as the expression of caspase-3, Bcl-2 and Bax were determined by flow cytometry. The results demonstrated that ailanthonone can induce apoptosis in tumor cells. Apoptosis is an active cell suicide process that is regulated by p53 (34). The effect of Bcl-2 depends on the ratio of its expression with Bax; this ratio determines whether cells undergo apoptosis or survival upon signal stimulation (35). Excessive Bax expression in cells promotes apoptosis, whereas excessive Bcl-2 expression promotes survival (36).

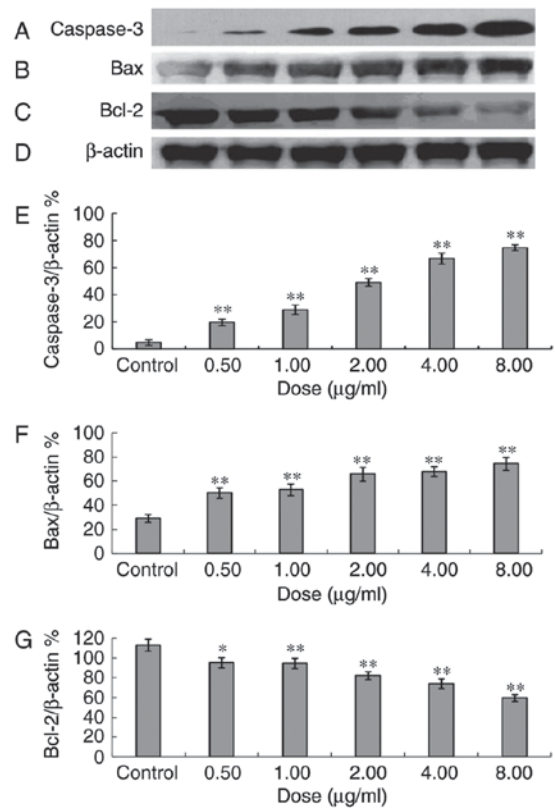


Figure 5. Caspase-3, Bax and Bcl-2 proteins in MCF-7 cells following intervention with different doses of ailanthonone. Following 48 h of treatment with different doses of ailanthonone, protein expression levels of (A) caspase 3, (B) Bax, (C) Bcl-2 and (D) β -actin in the MCF-7 cells were measured by western blotting. (E) Quantitative analysis of protein expression levels of caspase-3, (F) Bax and (G) Bcl-2. Data are presented as the mean \pm standard deviation (n=3). *P<0.05 and **P<0.01 vs. the control group. Bcl-2, B-cell lymphoma-2; Bax, Bcl-associated X.

Western blot analysis was used to detect levels of Bax and Bcl-2 protein expression; it was demonstrated that following treatment of MCF-7 cells with 0.5, 1.0, 2.0, 4.0 or 8.0 $\mu\text{g/ml}$ ailanthonone for 48 h, Bax expression increased, whereas that of Bcl-2 decreased markedly. The results indicated that the mechanism underlying ailanthonone-induced MCF-7 cell apoptosis may be associated with the adjusting of the Bax and Bcl-2 family proteins. Experimental results demonstrated that ailanthonone exhibited an inhibitory effect on cellular proliferation and induced apoptosis. The promotion of Bax and the inhibition of Bcl-2 proteins may further enhance the antitumor effect.

Genetic abnormalities in the phosphatidylinositol 3-kinase (PI3K)/RAC serine/threonine-protein kinase (AKT) signaling pathway are frequently observed in human tumors; previous studies indicate that this pathway is involved in the development of multiple cancer types (37). The role of the PI3K/AKT pathway and its potential as a therapeutic target for tumor treatment has been investigated in preclinical studies into a number of tumor types, including lung, breast and renal cancer, neuroblastoma and glioblastoma. The results of these studies indicate that the PI3K/AKT signaling pathway and those downstream of it are potential targets for therapeutic intervention (38-41). The PI3K/AKT pathway serves a role in apoptosis, cell cycle progression and tumorigenesis; therefore, we hypothesize that ailanthonone-induced apoptosis may also involve the PI3K/AKT

pathway, demonstrating that the ailanthone treatment of Huh7 cells resulted in a decrease in the expression of PI3K and AKT phosphorylation at threonine-408 and serine-473.

Treatment of MCF-7 cells with ailanthone resulted in cell apoptosis. In the present study, the antitumor effect of ailanthone indicated that this compound may be beneficial for the treatment of breast cancer. Further investigation is required to identify the mechanism underlying the antitumor activity.

In conclusion, the present study demonstrated that ailanthone, isolated from *A. altissima*, exhibited an inhibitory effect on MCF-7 cells and promoted cell apoptosis by upregulating Bax protein and mRNA. Ailanthone inhibited the protein and mRNA expression of Bcl-2, indicating that it has potential antitumor activity. Ailanthone may be a novel phytomedicine for tumor therapy.

Acknowledgements

The authors would like to thank Professor Jun-Qing Liang (Shijiazhuang Yiling Pharmaceutical Co., Ltd., Hebei, China) for providing necessary facilities to perform experiments in the present study. The present study was supported by the National Natural Science Foundation of China (grant no., 81302664 and 81703001) and the Hebei Medical University Development Project (2016-kyfz111). We are grateful for the key discipline construction project of the Universities in Hebei (ZD 2017003); Chengde medical college high level talent research startup fund (201705) and Key Discipline Construction Projects of Higher Schools in Hebei. The authors also wish to extend thanks for the financial support of Syngenta Ltd. (2017-Hebei Medical University-Syngenta-04).

Competing interests

The authors declare that they have no competing interests.

References

- Khoobchandani M, Ojeswi BK, Sharma B and Srivastava MM: Chenopodium album prevents progression of cell growth and enhances cell toxicity in human breast cancer cell lines. *Oxid Med Cell Longev* 2: 160-165, 2009.
- Tokgun O, Akca H, Mammadov R, Aykurt C and Deniz G: Convolvulus galaticus, crocus antalyensis, and lilium candidum extracts show their antitumor activity through induction of p53-mediated apoptosis on human breast cancer cell line MCF-7 Cells. *J Med Food* 11: 1000-1005, 2012.
- Binkley JM, Harris SR, Levangie PK, Pearl M, Guglielmino J, Kraus V and Rowden D: Patient perspectives on breast cancer treatment side effects and the prospective surveillance model for physical rehabilitation for women with breast cancer. *Cancer* 8: 2207-2216, 2012.
- Sitzia J and Huggins L: Side effects of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) chemotherapy for breast cancer. *Cancer Pract* 6: 13-21, 1998.
- da Rocha AB, Lopes RM and Schwartzmann G: Natural products in anticancer therapy. *Curr Opin Pharmacol* 1: 364-369, 2001.
- Efferth T, Li PC, Konkimalla VS and Kaina B: From traditional Chinese medicine to rational cancer therapy. *Trends Mol Med* 13: 353-361, 2007.
- Olaku O and White JD: Herbal therapy use by cancer patients: A literature review on case reports. *Eur J Cancer* 47: 508-514, 2011.
- Dennis T, Fanous M and Mousa S: Natural products for chemopreventive and adjunctive therapy in oncologic disease. *Nutr Cancer* 61: 587-597, 2009.
- Ho JW, Leung YK and Chan CP: Herbal medicine in the treatment of cancer. *Curr Med Chem Anticancer Agents* 2: 209-214, 2002.
- Pezzuto JM: Plant-derived anticancer agents. *Biochem Pharmacol* 53: 121-133, 1997.
- Kinghorn AD, Farnsworth NR, Doel Soejarto D, Cordell GA, Pezzuto JM, Udeani GO, Wani MC, Wall ME, Navarro HA, Kramer RA, *et al*: Novel strategies for the discovery of plant-derived anticancer agents. *Pure Appl Chem* 71: 611-618, 1999.
- Lee KH: Anticancer drug design based on plant-derived natural products. *J Biomed Sci* 6: 236-250, 1999.
- Bishayee A: Editorial: Current advances in cancer prevention and treatment by natural products. *Curr Pharm Biotechnol* 13: 115-116, 2012.
- Efferth T, Li PC, Konkimalla VS and Kaina B: From traditional Chinese medicine to rational cancer therapy. *Trends Mol Med* 13: 353-361, 2007.
- De Feo V, De Martino L, Quaranta E and Pizza C: Isolation of phytotoxic compounds from tree-of-heaven (*Ailanthus altissima* swingle). *J Agric Food Chem* 51: 1177-1180, 2003.
- Rahman S, Fukamiya N, Ohno N, Tokuda H, Nishino H, Tagahara K, Lee KH and Okano M: Inhibitory effects of quassinoid derivatives on Epstein-Barr virus early antigen activation. *Chem Pharm Bull (Tokyo)* 45: 675-677, 1997.
- Wang Y, Wang WJ, Su C, Zhang DM, Xu LP, He RR, Wang L, Zhang J, Zhang XQ and Ye WC: Cytotoxic quassinoids from *Ailanthus altissima*. *Bioorg Med Chem Lett* 23: 654-657, 2013.
- Okunade AL, Bikoff RE, Casper SJ, Oksman A, Goldberg DE and Lewis WH: Antiplasmodial activity of extracts and quassinoids isolated from seedlings of *Ailanthus altissima* (Simaroubaceae). *Phytother Res* 17: 675-677, 2003.
- Kundu P and Laskar S: A brief resume on the genus *Ailanthus*: chemical and pharmacological aspects. *Phytochem Rev* 9: 379-412, 2010.
- Rosati A, Quarantam E, Ammirante M, Turco MC, Leone A and De Feo V: Quassinoids can induce mitochondrial membrane depolarisation and caspase 3 activation in human cells. *Cell Death Differ* 11 (Suppl 2): S216-S218, 2004.
- Casinovi CG, Ceccherelli P, Grandolini G and Bellavita V: On the structure of ailanthone. *Tetrahedron Lett* 5: 3991-3997, 1964.
- Bishayee A, Háznagy Radnai E, Mbimba T, Sipos P, Morazzoni P, Darvesh AS, Bhatia D and Hohmann J: Anthocyanin-rich black current extract suppresses the growth of human hepatocellular carcinoma cells. *Nat Prod Commun* 5: 1613-1618, 2010.
- Kim MJ, Kim YJ, Park HJ, Chung JH, Leem KH and Kim HK: Apoptotic effect of red wine polyphenols on human colon cancer SNU-C4 cells. *Food Chem Toxicol* 44: 898-902, 2006.
- Lu YJ, Xu Q, Chen L, Zuo Y, Liu S, Hu Y, Li X, Li Y and Zhao X: Expression of semaphorin 6D and its receptor plexin-A1 in gastric cancer and their association with tumor angiogenesis. *Oncology* 12: 3967-3974, 2016.
- Moysich KB, Beehler GP, Zirpoli G, Choi JY and Baker JA: Use of common medications and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 7: 1564-1595, 2008.
- Kim DW, Hong GH, Lee HH, Choi SH, Chun BG, Won CK, Hwang IK and Won MH: Effect of colloidal silver against the cytotoxicity of hydrogen peroxide and naphthazarin on primary cultured cortical astrocytes. *Int J Neurosci* 117: 387-400, 2007.
- Duan JA, Su SL and Qian DW: Approaches and advances in the resources chemistry of Chinese medicinal material. *Chin J Nat Med* 7: 333-340, 2009.
- Zhuo Z, Hu J, Yang X, Chen M, Lei X, Deng L, Yao N, Peng Q, Chen Z, Ye W and Zhang D: Ailanthone inhibits Huh7 cancer cell growth via cell cycle arrest and apoptosis in vitro and in vivo. *Sci Rep* 5: 16185, 2015.
- James BR and Griffith TS: Activation of systemic antitumor immunity via TRAIL-induced apoptosis. *Oncol Immunology* 17: 1178-1180, 2012.
- Chen M, Xu XY, X D, *et al*: Inhibiting Bcl-2 gene expression enhance radiosensitivity of non-small cell lung cancer NCI-H460 cells. *China Oncology* 20: 641-5, 2010.
- Bai X, Che F, Li J, Ma Y, Zhou Y, Zhai J and Meng L: Effects of adenovirus-mediated p16 and p53 genes transfer on apoptosis and cell cycle of lung carcinoma cells. *Zhonghua Bing Li Xue Za Zhi* 29: 354-358, 2000 (In Chinese).
- Zhang Z, Leonard SS, Huang C, Vallyathan V, Castranova V and Shi X: Role of reactive oxygen species and MAPKs in vanadate-induced G(2)/M phase arrest. *Free Radic Biol Med* 34: 1333-1042, 2003.
- Porter AG and Jänicke RU: Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 6: 99-104, 1999.

34. Alvarez S, Drane P, Meiller A, Bras M, Deguin-Chambon V, Bouvard V and May E: A comprehensive study of p53 transcriptional activity in thymus and spleen of gamma irradiated mouse: High sensitivity of genes involved in the two main apoptotic pathways. *Int J Radiat Biol* 82: 761-770, 2006.
35. Raisova M, Hossini AM, Eberle J, Riebeling C, Wieder T, Sturm I, Daniel PT, Orfanos CE and Geilen CC: The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. *J Invest Dermatol* 117: 333-340, 2001.
36. Samarghandian S, Nezhad MA and Mohammadi G: Role of caspases, Bax and Bcl-2 in chrysin-induced apoptosis in the A549 human lung adenocarcinoma epithelial cells. *Anticancer Agents Med Chem* 14: 901-909, 2014.
37. Wong KK, Engelman JA and Cantley LC: Targeting the PI3K signaling pathway in cancer. *Curr Opin Genet Dev* 20: 87-90, 2010.
38. Rao E, Jiang C, Ji M, Huang X, Iqbal J, Lenz G, Wright G, Staudt LM, Zhao Y, McKeithan TW, *et al*: The miRNA-17-92 cluster mediates chemoresistance and enhances tumor growth in mantle cell lymphoma via PI3K/AKT pathway activation. *Leukemia* 26: 1064-1072, 2012.
39. Markman B, Dienstmann R and Tabernero J: Targeting the PI3K/Akt/mTOR pathway-beyond rapalogs. *Oncotarget* 1: 530-543, 2010.
40. Neri LM, Cani A, Martelli AM, Simioni C, Junghanss C, Tabellini G, Ricci F, Tazzari PL, Pagliaro P, McCubrey JA and Capitani S: Targeting the PI3K/Akt/mTOR signaling pathway in B-precursor acute lymphoblastic leukemia and its therapeutic potential. *Leukemia* 28: 739-748, 2014.
41. Slomovitz BM and Coleman RL: The PI3K/AKT/mTOR pathway as a therapeutic target in endometrial cancer. *Clin Cancer Res* 18: 5856-5864, 2012.