

# Lily extracts inhibit the proliferation of gastric carcinoma SGC-7901 cells by affecting cell cycle progression and apoptosis via the upregulation of caspase-3 and Fas proteins, and the downregulation of FasL protein

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**Abstract.** The present study aimed to investigate the effect of alkaloids and carbinol extracts from lily on the proliferation of SGC-7901 cells, as well as the underlying mechanism. SGC-7901 cells were incubated with different concentrations of alkaloid or carbinol extracts for 24, 48 or 72 h. MTT assays were used to measure the inhibition rate of SGC-7901 cell proliferation. Inverted phase contrast and fluorescence microscopy was used to observe morphological changes of SGC-7901 cells. Flow cytometry was employed to detect cell cycle progression and apoptosis rates of SGC-7901 cells. Western blotting was performed to measure the expression of caspase-3, Fas and Fas ligand (FasL) proteins in SGC-7901 cells. The inhibition rate of SGC-7901 cell proliferation was significantly enhanced with increasing drug concentrations and time elapsed. Treatment with alkaloid or carbinol extracts deteriorated the morphology of SGC-7901 cells in a dose-dependent manner. Alkaloid and carbinol extracts arrested SGC-7901 cells in the G<sub>2</sub>/M phase, and induced apoptosis in a dose-dependent manner. Alkaloid and carbinol extracts enhanced caspase-3, and Fas expression, but reduced FasL expression in SGC-7901 cells. The present study demonstrated that alkaloids and carbinol extracts from lily inhibited the proliferation of gastric carcinoma SGC-7901 cells by arresting cells in the G<sub>2</sub>/M phase. The upregulation of

caspase-3 and Fas proteins, and the downregulation of FasL protein may be an important mechanism for the induction of SGC-7901 cell apoptosis.

## Introduction

Gastric carcinoma is one of the common types of malignant tumor that threaten human life and health (1). Gastric carcinoma has low eradication rates during surgery, with high recurrence and metastasis rates following surgery (2). It has a poor sensitivity to conventional radiotherapy, and chemotherapy serves an important role in the treatment of gastric carcinoma (3). In addition, there are not many effective drugs for the treatment of gastric carcinoma, and these drugs are usually associated with toxicity and unsatisfactory efficacy. By contrast, traditional Chinese medicine has demonstrated certain advantages in the treatment of tumors, including adverse reactions, low toxicity, little residue and unique pharmacological actions (4). It has been reported that traditional Chinese medicine enhances immunity (5), activates antioxidative signaling pathways associated with nuclear factor (NF)2 (6) and downregulates the NF-κB signaling pathway (7). The antitumor mechanisms include promotion of apoptosis (8), inactivation of telomerase, antioxidation, inhibition of angiogenesis and metastasis, and induction of differentiation of tumor cells to normal cells (9), increase of tumor necrosis factor receptor and Fas/Fas ligand (FasL) expression in death receptor/ligand regulation (10), reactive oxygen species-independent dysfunction of mitochondria and induction of reactive oxygen species production (11), activation of the caspase-3 signaling pathway, downregulation of Bcl-2 expression, and enhancement of apoptosis-associated protein phosphorylation (12). These mechanisms are associated in the treatment of tumors.

As a common plant, lily possesses high medicinal values. It has been demonstrated that specific components extracted from lily exhibit antitumor effects (4). However, the mechanism of action of the effective components extracted

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from lily remains unclear. To the best of our knowledge, the effect of lily on human gastric carcinoma SGC-7901 cells has not been previously studied. In the present study, the effect and underlying mechanisms of alkaloids, and carbinol extracts from lily on the proliferation of SGC-7901 cells were investigated.

## Materials and methods

**Cells.** SGC-7901 cells were purchased from the Institute of Digestive and Experimental Research, School of Medicine, Xi'an Jiaotong University (Xi'an, China). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100  $\mu\text{g/ml}$  penicillin and 100  $\mu\text{g/ml}$  streptomycin (all from Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C with 5% CO<sub>2</sub> in a humidified, sterile incubator. The medium was changed every 24 h. When 80% confluency was achieved, the cells were washed twice with 2 ml PBS, followed by addition of 0.25% trypsin (0.5 ml). When the cells were fully trypsinized, 2 ml fresh medium was added to resuspend the cells, which were then divided evenly into two culture flasks containing 6 ml medium each. Cells in the logarithmic phase growth were chosen for subsequent experiments.

**MTT assay.** To measure the proliferation of cells, an MTT assay was performed. Single cell suspension was prepared at a concentration of  $1 \times 10^5$  cells/ml, and then 100  $\mu\text{l}$ /well was added into a 96-well plate. For the blank group, no cells were added into the well. Following incubation for 24 h at 37°C, the medium was discarded. For the blank and control groups, only fresh medium was added. For the experimental groups, fresh medium containing 0.05, 0.075, 0.1, 0.125 and 0.15 g/l alkaloid or 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, and 1.6 g/l carbinol extracts were added. To prepare these extracts, lily bulb was washed and lyophilized to obtain a dry powder. Then, the dry powder (25 g) was dissolved in 300 ml 90% acid ethanol solution, and extracted with petroleum ether. The water layer was aspirated and extracted with chloroform. Then, the chloroform layer was dried, and 0.5 g dried powder was dissolved in 10 ml methanol to reach a concentration of 50 mg/ml. For the carbinol control groups, fresh medium containing 3.75 or 4% carbinol was added. Each group was repeated in six wells containing 200  $\mu\text{l}$ . After incubation for 24, 48 and 72 h, 20  $\mu\text{l}$  MTT (5 mg/ml) was added into each well, followed by another incubation for 4 h at 37°C. Then, the supernatants were discarded and 150  $\mu\text{l}$  dimethyl sulphoxide (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was added prior to agitating for 10-15 min at 37°C. The absorbance was measured using a microplate reader (Model 680; Bio-Rad Laboratories, Inc., Hercules, CA, USA) at a wavelength of 490 nm. The measurements of the control, experimental and carbinol control groups were adjusted according to the blank group. The cell proliferation inhibition rate was evaluated using the following formula: [(Absorbance of control group - absorbance of experimental group) / absorbance of control group] x 100%. The measurements were repeated for three times to calculate mean values. The cell proliferation inhibition rates under all concentrations and time points were calculated.

**Light and fluorescence microscopy.** Cells in the logarithmic growth ( $1 \times 10^5/\text{ml}$ ) were seeded into 6-well plates with 2 ml medium/well. For experimental groups, medium containing 0.9, 1.1 and 1.4 g/l carbinol extracts or 0.05, 0.1, and 0.15 g/l alkaloid was used to incubate the cells. For the control group, 2 ml normal medium was used. For the carbinol control group, 2 ml normal medium containing 4% carbinol was used. Following incubation for 24, 48 or 72 h, the cells were washed with PBS three times, and imaged under a light microscope (magnification, x10; CKX41; Olympus Corporation, Tokyo, Japan). For fluorescence microscopy, acridine orange/ethidium bromide staining liquid was dropped on the slide and kept at room temperature for 30 sec prior to observation under the microscope (magnification, x10).

**Flow cytometry.** Cells in the logarithmic growth were seeded into 25 ml culture flasks containing 3 ml medium. After incubation for 24 h, the medium was removed and fresh medium was added. After another 24 h of culture, the medium was discarded and fresh medium containing 0.9, 1.1, and 1.4 g/l carbinol extracts or 0.05, 0.1, and 0.15 g/l alkaloid was added to each experimental group. For the control group, only fresh medium was added. After incubation for 48 h, cells were trypsinized and the cell suspension was collected. After centrifugation at room temperature and 123 x g for 5 min, the supernatant was discarded and the cells were washed with PBS three times. Cell sediments were fixed with 70% pre-cold ethanol, and kept at -20°C for 24 h. Then, the cells were washed with PBS, and stained with propidium iodide (PI) for 30 min at room temperature in the dark. The DNA content was measured using flow cytometry. Cell cycles were analyzed using an excitation wavelength of 488 nm and emission wavelength of 630 nm. The experiments were performed in triplicate.

For Annexin V-FITC/PI staining (Beyotime Institute of Biotechnology, Shanghai, China), 50  $\mu\text{l}$  RNase (0.5 mg/ml) was added to samples and incubated at 37°C for 30 min. After centrifugation at room temperature and 192 x g rpm for 5 min, the supernatant was discarded and the cells were washed with PBS three times. Then, 10  $\mu\text{l}$  Annexin V-FITC was added and incubated at room temperature for 30 min, followed by centrifugation at room temperature and 192 x g for 5 min. Following the removal of the supernatant, 10  $\mu\text{l}$  PI (50  $\mu\text{g/ml}$ ) was added. Cell apoptosis rate was determined using flow cytometry using experiments performed in triplicate.

**Western blotting.** Cells were harvested and total protein extracts were prepared using the Total Protein Extraction kit (Wuhan Boster Biological Technology, Ltd., Wuhan, China) according to manufacturer's protocol. Total proteins were separated using 12% SDS-PAGE under 80-90 V and 250 mA for 30 min, and then transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc.). Then, the membranes were washed with PBS-Tween 20 twice (5 min/wash), and blocked with 1% bovine serum albumin at room temperature (Sigma-Aldrich, Merck KGaA) for 1 h. Subsequently, the membranes were incubated with caspase-3 (cat. no. BA2142), Fas (cat. no. BA0048), FasL (cat. no. BA0049) and GAPDH (cat. no. BA2913) primary antibodies (1:500/600; Wuhan Boster Biological Technology, Ltd.) at 4°C overnight. After washing

with PBS-Tween 20 twice (5 min/wash), the membranes were incubated with biotinylated goat anti-rabbit IgG secondary antibody (cat. no. BA1003; 1:40/50; Wuhan Boster Biological Technology, Ltd.) at 37°C for 30 min. After washing with PBS-Tween 20 three times (5 min/wash), the membranes were incubated with streptavidin-labeled horseradish peroxidase (1:40/50; Wuhan Boster Biological Technology, Ltd.) for 30 min, followed by washing with PBS-Tween 20 twice (5 min/wash). Then, the membranes were stained with DAB (1:20; Wuhan Boster Biological Technology, Ltd.).

**Statistical analysis.** The results were analyzed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). All data are expressed as the mean  $\pm$  standard deviation. Differences among groups were compared using one-way analysis of variance. For pairwise comparisons, the Fisher's least significant difference t-test was employed. Differences with  $P < 0.05$  were considered statistically significant.

## Results

*Inhibition of SGC-7901 cell proliferation is enhanced with increasing drug concentrations and duration of exposure.* To determine the proliferation rates of SGC-7901 cells, MTT assay was employed. The data demonstrated that the proliferation inhibition rate in the carbinol control group was not significantly different compared with that in the control group ( $P > 0.05$ ; Table I). In addition, different lily extract alkaloid concentrations (0.05 g/l vs. 0.1 g/l; 0.075 g/l vs. 0.05 g/l; 0.1 g/l vs. 0.075 g/l; 0.125 g/l vs. 0.1 g/l and 0.15 g/l vs. 0.125 g/l) or different incubation durations (48 h vs. 24 h and 72 h vs. 48 h) resulted in significantly different proliferation inhibition rates compared with the control group or among alkaloid experimental groups ( $P < 0.05$ ; Table II). Similarly, different carbinol extract concentrations (0.8 g/l vs. 0 g/l; 0.9 g/l vs. 0.8 g/l; 1.0 g/l vs. 0.9 g/l; 1.1 g/l vs. 1.0 g/l; 1.2 g/l vs. 1.1 g/l; 1.3 g/l vs. 1.2 g/l; 1.4 g/l vs. 1.3 g/l; 1.5 g/l vs. 1.4 g/l and 1.6 g/l vs. 1.5 g/l) or different incubation durations (48 h vs. 24 h and 72 h vs. 48 h) caused significantly different proliferation inhibition rates compared with the control group or among carbinol extract experimental groups ( $P < 0.05$ ; Table III). The results suggest that inhibition of SGC-7901 cell proliferation is enhanced as drug concentration or time elapsed increases.

*Treatment with alkaloid or carbinol extract deteriorates the morphology of SGC-7901 cells in a dose-dependent manner.* To investigate how alkaloid or carbinol extracts affect SGC-7901 cell morphology, the cells were observed under inverted phase contrast and fluorescence microscopes. The images demonstrate that SGC-7901 cells in the blank control group had polygonal shapes and uniform sizes, with faster proliferation compared with that in the experimental groups (Fig. 1A). In addition, the morphology of SGC-7901 cells in the carbinol control group was not distinct compared with that in the blank control group (Fig. 1B). After 48 h treatment with alkaloid or carbinol extracts, SGC-7901 cells demonstrated slower growth, deformed shapes and irregular cell edges, with the effect being increased with higher concentrations of extract (Fig. 1A and B). Under

Table I. Inhibition of SGC-7901 cell proliferation by different concentrations of carbinol as determined using an MTT assay.

Groups	Absorbance		
	24 h	48 h	72 h
0% carbinol	0.795 $\pm$ 0.002	1.120 $\pm$ 0.003	1.331 $\pm$ 0.011
3.75% carbinol	0.806 $\pm$ 0.014	1.117 $\pm$ 0.008	1.342 $\pm$ 0.009
4.00% carbinol	0.787 $\pm$ 0.011	1.118 $\pm$ 0.006	1.337 $\pm$ 0.003

Data are presented as the mean  $\pm$  standard deviation; n=12. Fisher's Least significant difference test=2.51, 0.16 and 1.45, respectively;  $P > 0.05$ .

the fluorescence microscope, SGC-7901 cells in the blank control group revealed uniformed sizes and even green fluorescence (Fig. 1C). The morphology of SGC-7901 cells in the carbinol control group was not distinct compared with that in the blank control group, also demonstrating even green fluorescence (Fig. 1D). After 48 h treatment with alkaloid or carbinol extracts, SGC-7901 cells demonstrated slower growth, deformed shapes, and red fluorescence, with the effect being increased with higher concentrations of extract (Fig. 1C and D). Furthermore, no apparent apoptosis was observed in the blank control group, but early apoptotic cells were observed in experimental groups, in which the nuclei were shrunk into crescent or granular shapes, stained green, and resided on one side of the cells. With increasing drug concentrations, the numbers of early and late apoptotic cells were increased, and the nuclei in the late apoptotic cells were shrunk, stained red, and resided on one side of the cells. Furthermore, the sizes of the necrotic cells were increased with vague edges, and the cells were decomposed with red-stained nuclei (Fig. 1E). These results indicate that treatment with alkaloid or carbinol extracts deteriorate the morphology of SGC-7901 cells in a dose-dependent manner.

*Treatment with alkaloid or carbinol extracts arrests SGC-7901 cells in G<sub>2</sub>/M phase.* Flow cytometry was performed in order to examine the effects of alkaloid or carbinol extracts on the cell cycle progression of SGC-7901 cells after 48 h treatment. Following treatment with alkaloid or carbinol extracts for 48 h, SGC-7901 cells were arrested in the G<sub>2</sub>/M phase. With increasing drug concentrations of either extract, cell cycle arrestment was promoted, with the percentage of cells in the G<sub>0</sub>/G<sub>1</sub> phase being significantly reduced, and that of cells in the G<sub>2</sub>/M phase being significantly increased, compared with control groups ( $P < 0.05$ ; Tables IV and V). These results suggest that treatment with alkaloid or carbinol extract arrests SGC-7901 cells in the G<sub>2</sub>/M phase.

*Treatment with alkaloid or carbinol extracts induces apoptosis of SGC-7901 cells in a dose-dependent manner.* To examine the effect of alkaloid or carbinol extract on SGC-7901 cell apoptosis, flow cytometry was performed in combination with Annexin V-FITC/PI staining. Following treatment

Table II. Inhibition of SGC-7901 cell proliferation by different concentrations of alkaloid as determined using an MTT assay.

Treatments (g/l)	Inhibition rate (%)			F	P
	24 h	48 h	72 h		
0	0	0	0		
0.05	21.50±0.85 <sup>a,d</sup>	28.27±1.87 <sup>a,b,d</sup>	37.13±3.89 <sup>a,c,d</sup>	28.60	0.00
0.075	31.87±0.83 <sup>a,d</sup>	40.63±2.19 <sup>a,c,d</sup>	49.30±1.72 <sup>a,c,d</sup>	81.28	0.00
0.1	40.90±0.87 <sup>a,d</sup>	50.13±1.57 <sup>a,c,d</sup>	58.10±1.52 <sup>a,c,d</sup>	122.24	0.00
0.125	51.33±1.46 <sup>a,d</sup>	65.60±0.58 <sup>a,c,d</sup>	80.07±1.59 <sup>a,c,d</sup>	372.07	0.00
0.15	65.30±1.23 <sup>a,d</sup>	74.06±1.21 <sup>a,c,d</sup>	91.93±1.05 <sup>a,c,d</sup>	409.35	0.00
F	1630.81	1033.15	803.49		
P	0.00	0.00	0.00		

<sup>a</sup>P<0.01 compared with control group; <sup>b</sup>P<0.05 and <sup>c</sup>P<0.01 compared with the previous time point group; <sup>d</sup>P<0.01 compared with the previous lower concentration group. Data are presented as the mean ± standard deviation; n=3. Fisher's Least significant difference test=1630.81, 1033.15 and 803.49, respectively; P<0.05.

Table III. Inhibition of SGC-7901 cell proliferation by different concentrations of carbinol extracts as determined using an MTT assay.

Treatments (g/l)	Inhibition rate (%)			F	P
	24 h	48 h	72 h		
0	0	0	0		
0.8	5.53±0.28 <sup>a,c</sup>	14.58±0.69 <sup>a-c</sup>	23.39±2.15 <sup>a-c</sup>	139.29	0.00
0.9	19.62±0.35 <sup>a,c</sup>	30.34±0.76 <sup>a-c</sup>	37.23±1.89 <sup>a-c</sup>	165.88	0.00
1.0	27.26±0.44 <sup>a,c</sup>	38.73±1.02 <sup>a-c</sup>	46.76±1.27 <sup>a-c</sup>	304.07	0.00
1.1	35.85±1.08 <sup>a,c</sup>	45.84±0.77 <sup>a-c</sup>	53.31±3.90 <sup>a-c</sup>	40.64	0.00
1.2	43.23±0.36 <sup>a,c</sup>	52.32±0.74 <sup>a-c</sup>	58.39±2.15 <sup>a-c</sup>	98.80	0.00
1.3	50.29±0.55 <sup>a,c</sup>	58.31±0.63 <sup>a-c</sup>	66.47±1.28 <sup>a-c</sup>	250.83	0.00
1.4	59.36±0.79 <sup>a,c</sup>	69.32±0.69 <sup>a-c</sup>	82.69±1.60 <sup>a-c</sup>	338.58	0.00
1.5	69.39±0.95 <sup>a,c</sup>	86.15±0.28 <sup>a-c</sup>	96.74±2.21 <sup>a-c</sup>	290.09	0.00
1.6	76.33±0.69 <sup>a,c</sup>	96.74±1.53 <sup>a-c</sup>	98.32±1.40 <sup>a</sup>	284.07	0.00
F	5062.53	4219.32	729.73		
P	0.00	0.00	0.00		

<sup>a</sup>P<0.01 compared with control group; <sup>b</sup>P<0.01 compared with the previous time point group; <sup>c</sup>P<0.01 compared with the previous lower concentration group. Data are presented as the mean ± standard deviation; n=3. Fisher's Least significant difference test=5062.53, 4219.32 and 729.73, respectively; P<0.05.

with different concentrations of alkaloid or carbinol extract for 48 h, SGC-7901 cell apoptotic rates were enhanced with increasing doses. The apoptotic rates of experimental groups were significantly higher compared with that of the control groups (P<0.05; Tables VI and VII). These results indicate that treatment with alkaloid or carbinol extracts induces apoptosis of SGC-7901 cells in a dose-dependent manner.

*Treatment with alkaloid or carbinol extracts enhances caspase-3 and Fas expression, but reduces FasL expression.* To determine how alkaloid or carbinol extract affects caspase-3, Fas and FasL protein expression, western blotting was used. The expression of caspase-3 and Fas proteins in SGC-7901

cells of blank control group was low, but that of FasL protein was high. After 48 h treatment with alkaloid or carbinol extracts, the expression levels of caspase-3 and Fas proteins in SGC-7901 cells were markedly enhanced with increasing drug concentrations, but that of FasL protein was decreased (Fig. 2). The results suggest that treatment with alkaloid or carbinol extracts enhances caspase-3 and Fas expression, but reduces FasL expression.

## Discussion

Apoptosis is the independent physiological self-destructive death of cells that involves the activation, expression and

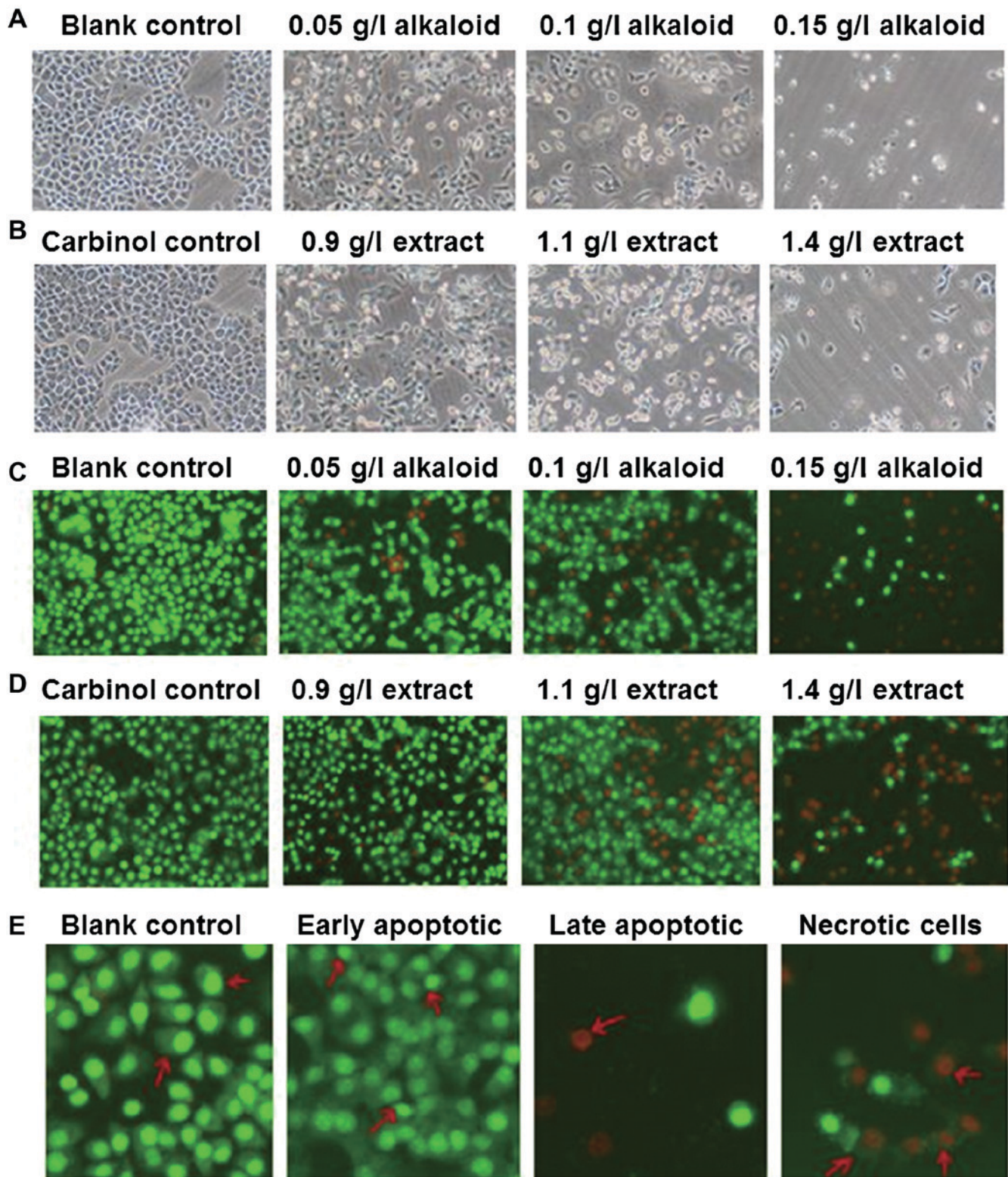


Figure 1. Morphology of SGC-7901 cells after treatment with different concentrations of alkaloid or carbinol extract for 48 h. Observation of SGC-7901 cells under an inverted phase contrast microscope after treatment with different concentrations of (A) alkaloid or (B) carbinol extract for 48 h (magnification, x100). Observation of SGC-7901 cells under a fluorescence microscope after treatment with different concentrations of (C) alkaloid or (D) carbinol extract for 48 h (magnification, x100). (E) Observation of early apoptotic, late apoptotic, and necrotic SGC-7901 cells under a fluorescence microscope after treatment with alkaloid for 48 h (magnification, x200). The treatment concentrations of alkaloid were 0.05, 0.1 or 0.15 g/l, while those for carbinol extract were 0.9, 1.1, or 1.4 g/l.

regulation of a series of genes. It is a type of death phenomenon that adapts the organism to the environment. Therefore, disordered apoptosis may directly or indirectly lead to

various diseases, including tumors (13). It is accepted that chemotherapy drugs kill tumor cells directly by cytotoxicity, but it also now understood that the primary mechanism of

Table IV. Effect of different concentrations of alkaloid on SGC-7901 cell cycle as determined using flow cytometry.

Treatments (g/l)	Cell cycle phase (%)		
	G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M
0	71.23±4.90	12.36±1.23	10.70±0.71
0.05	56.13±1.50 <sup>a,b</sup>	10.70±2.33	15.81±1.18 <sup>a,b</sup>
0.1	52.98±2.94 <sup>a</sup>	8.62±0.83 <sup>a</sup>	20.90±1.92 <sup>a,b</sup>
0.15	43.62±1.63 <sup>a,b</sup>	5.94±1.69 <sup>a</sup>	30.02±1.43 <sup>a,b</sup>
F	42.00	8.82	106.99
P	0.00	0.01	0.00

<sup>a</sup>P<0.01 compared with control group of the same phase; <sup>b</sup>P<0.01 compared with the previous lower concentration group of the same phase. The data were measured after treatment for 48 h. Data are presented as the mean ± standard deviation. Fishers Least significant difference test=42.00, 8.82 and 106.99, respectively; P<0.05.

Table V. Effect of different concentrations of carbinol extract on SGC-7901 cell cycle as determined using flow cytometry.

Treatments (g/l)	Cell cycle phase (%)		
	G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M
0	71.23±4.90	12.36±1.23	10.70±0.71
0.9	69.96±1.18	11.69±1.12	13.31±0.38 <sup>a,b</sup>
1.1	58.15±1.83 <sup>a,b</sup>	8.43±0.68 <sup>a,b</sup>	19.99±1.21 <sup>a,b</sup>
1.4	53.30±1.41 <sup>a,b</sup>	4.87±0.95 <sup>a,b</sup>	27.03±1.18 <sup>a,b</sup>
F	30.43	34.42	184.07
P	0.00	0.00	0.00

<sup>a</sup>P<0.01 compared with control group of the same phase; <sup>b</sup>P<0.01 compared with the previous lower concentration group of the same phase. The data were measured after treatment for 48 h. Data are presented as the mean ± standard deviation. Fisher's least significant difference test=30.43, 34.42 and 184.07, respectively; P<0.05.

action of these drugs involves the induction of apoptosis (14). It has been reported that certain active components in lily are used for the treatment of different cancer types, including lung cancer, lymphoma, esophageal cancer, leukemia, skin cancer and cervical cancer. Hou *et al* (15) have reported that lily polysaccharides in combination with genistein inhibit the proliferation of MCF-7 cells and arrest cells in the G<sub>2</sub> phase. In the present study, treatment with alkaloid or carbinol extracts inhibited the proliferation of SGC-7901 cells, with increased doses and longer treatment durations exhibiting higher inhibitory effects. In addition, the sizes of the cells were decreased with shrunken nuclei. The cells were arrested in the G<sub>2</sub>/M phase and apoptosis was induced. Of note, the MTT assay results revealed that alkaloid and carbinol extracts also exhibit direct killing effects on the cells.

Apoptosis is a gene expression cascade system involving multiple pathways to induce apoptosis (16). There are three

Table VI. Effect of different concentrations of alkaloid on SGC-7901 cell apoptotic rate as determined using flow cytometry.

Treatments (g/l)	Apoptotic rate (%)
0	1.57±0.64
0.05	6.54±0.48 <sup>a,b</sup>
0.1	11.14±2.00 <sup>a,b</sup>
0.15	20.35±1.50 <sup>a,b</sup>
F	111.18
P	0.00

<sup>a</sup>P<0.01 compared with control group; <sup>b</sup>P<0.01 compared with the previous lower concentration group. The data were measured after treatment for 48 h. Data are presented as the mean ± standard deviation.

Table VII. Effect of different concentrations of carbinol extract on SGC-7901 cell apoptotic rate as determined using flow cytometry.

Treatments (g/l)	Apoptotic rate (%)
0	1.57±0.64
0.9	5.36±0.62 <sup>a,b</sup>
1.1	11.78±1.13 <sup>a,b</sup>
1.4	20.32±1.21 <sup>a,b</sup>
F	227.22
P	0.00

<sup>a</sup>P<0.01 compared with control group; <sup>b</sup>P<0.01 compared with the previous lower concentration group. The data were measured after treatment for 48 h. Data are presented as the mean ± standard deviation.

signal transduction pathways that trigger apoptosis: The mitochondrial pathway, external death receptor pathway and endoplasmic reticulum pathway (17). Among the three pathways, Fas and FasL serve important roles in the external death receptor pathway (18). Fas and FasL belong to tumor-necrosis factor/nerve-growth factor receptor family. The cytoplasmic region of Fas has no catalytic activity prior to binding with its ligand FasL, after which a trimer is formed to induce the aggregation of the intracellular death domain. Following binding of Fas-associated protein with the death domain, caspase family cascade reactions are induced, leading to the degradation of DNA fragments and apoptosis (19,20). Caspase family members are involved in a protease system that leads to the disintegration of apoptotic cells, serving a central role in the large network of cell apoptotic mechanisms (21). It is considered that caspase-3 is the most essential terminal cut enzyme in caspase family cascade reactions and the deactivation or reduced expression of caspase-3 is correlated with the occurrence, and development of multiple tumor types (22).

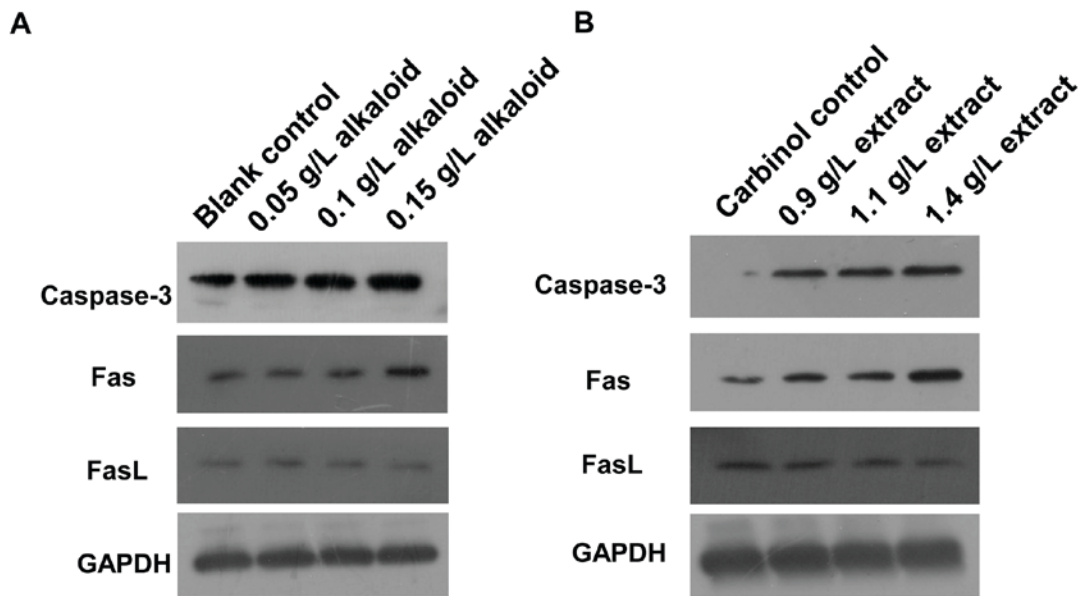


Figure 2. Effect of different concentrations of (A) alkaloid or (B) carbinol extract on the expression of caspase-3, Fas and FasL proteins in SGC-7901 cells after treatment for 48 h. Western blotting was employed to measure protein expression. The treatment concentrations of alkaloid were 0.05, 0.1 or 0.15 g/l, while those for carbinol extract were 0.9, 1.1, or 1.4 g/l. FasL, Fas ligand.

Normally, caspase-3 exists in an inactive zymogen form (23,24). Following external stimulation by oxidant or ultraviolet, caspase-3 is released from the mitochondria into cytoplasm, and activated (25). The activation of caspase-3 induces the irreversible stage of apoptosis (26). Following the activation of caspase-3, its cascade reactions are aggravated, and a series of protein substrates are degraded, leading to the deactivation of structural and functional proteins, and finally, apoptosis.

It is considered that the expression levels of caspase-3 or Fas proteins may indicate the malignancy and development stage of tumors (27). Reduced Fas expression inhibits the apoptosis of tumor cells, which is induced via the Fas signaling pathway, and inhibited apoptosis is considered to serve important roles in the occurrence and development of tumors (28,29). FasL is the ligand of Fas that is exclusively expressed on the surface of activated T cells. It has been revealed that high expression of FasL exists on the surface of numerous tumor cells, and leads to high expression of Fas on tumor-invasive TL, inducing TL apoptosis and immune inhibition (30). The results of the present study demonstrated that SGC-7901 cells in the control group exhibited low levels of caspase-3 and Fas expression, but high FasL expression levels. In addition, alkaloid or carbinol extract treatment enhanced the expression of caspase-3 and Fas, but reduced the expression of FasL. These results suggest that alkaloid or carbinol extract induces SGC-7901 cell apoptosis by upregulating caspase-3 and Fas expression, and downregulating FasL expression. Chinese herbal medicines have demonstrated certain advantages in the treatment of tumors. As a type of Chinese herbal medicine, lily has revealed specific effects in inhibiting tumor cell proliferation and promoting tumor cell apoptosis. Further studies on other active components of lily may also be beneficial for the treatment of patients with tumors in the future.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Authors' contributions

AW and MW performed the experiments, analyzed the data and wrote the manuscript. QP and LJ participated in the MTT assay and flow cytometry analysis. JZ and MC helped with western blot analysis and data collection. YZ conceived the idea, designed the study and helped to draft the manuscript. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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