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ORIGINAL ARTICLE

Full Extract of *Euphorbia esula* Reversed Chemoresistance, Inhibited Cell Migration/Invasion, and Induced Apoptosis of Multidrug-resistant SGC7901/VCR Cells

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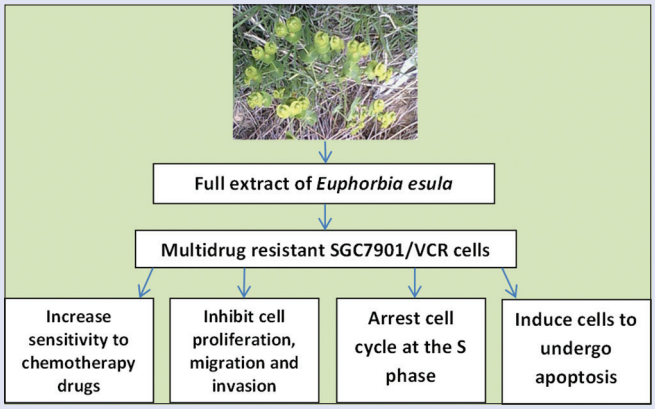
ABSTRACT

Background: The plant *Euphorbia esula* has been used by ancient Chinese people to treat cancer and other ailments and is used by present doctors or folks as an assistant treatment for some kind of cancers, but the mode of action is unclear. **Objective:** To investigate the influence of *E. esula* full extract on chemoresistance, cell migration/invasion, and apoptosis of multidrug-resistant human stomach cancer cells. **Materials and Methods:** After treating multidrug-resistant human stomach cancer SGC7901/VCR cells with *E. esula* extract at varying concentrations, the inhibition of cell proliferation was detected using thiazolyl blue. Sensitivity to the chemotherapeutic drugs, adriamycin and paclitaxel, was evaluated using half maximal inhibitory concentration. Cell cycle progression was analyzed by flow cytometry. The inhibitions of cell migration and invasion were examined by Transwell method. The induction of apoptosis and the apoptotic rate were studied by electron microscopy and flow cytometry, respectively. Activation of caspase 3 enzyme was inspected by ultraviolet spectrophotometry. **Results:** *E. esula* extract could increase the sensitivity of SGC7901/VCR cells to the chemotherapeutic drugs, adriamycin and paclitaxel. The proliferation, migration, and invasion of SGC7901/VCR cells were significantly inhibited by *E. esula* extract ($P < 0.01$ compared with negative control), which showed time- and dose-dependent manners ($P < 0.05$ and $P < 0.01$, respectively). Cell cycle was arrest at the S phase. *E. esula* extract also induced apoptosis in SGC7901/VCR cells, and the apoptotic rate was increased significantly with drug concentration and with treatment time ($P < 0.01$ compared with negative control, $P < 0.05$ between concentrations and time points). *E. esula* extract upregulated enzymatic activity of caspase 3. **Conclusion:** *E. esula* extract could reverse SGC7901/VCR cell's resistance to chemotherapeutic drugs and could inhibit cell proliferation, migration, and invasion, interfere with cell cycle progression, and induce apoptosis in SGC7901/VCR cells.

Key words: Apoptosis, caspase 3, cell migration and invasion, *Euphorbia esula*, multidrug resistance reversal, stomach cancer

SUMMARY

- This study aims to explore the effects of the full extract of the Chinese medicinal herb *Euphorbia esula* on SGC7901/VCR cells to reversing multidrug resistance. The experimental results showed that *Euphorbia esula* extract significantly increased the sensitivity of SGC7901/VCR cells to the chemotherapeutic drugs Adriamycin and Paclitaxel. *Euphorbia esula* extract significantly inhibited cell proliferation, migration and invasion, arrested cell cycle at the S phase, and induced cell apoptosis in SGC7901/VCR cells.



Abbreviations used: *E. esula*: *Euphorbia Esula*; FCS: fetal calf serum; IC50: half maximal inhibitory concentration; OD: optical density.

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INTRODUCTION

Stomach cancer is one of the most common cancers in the world, and the incidence is especially high in some East Asian countries.[1-3] Chemotherapy is regularly used in combination with surgical operation for the treatment of early stomach cancers; for advanced stomach cancers, chemotherapy is usually the first choice or the only choice.[4,5] However, many stomach cancers develop resistance to multiple chemotherapeutic drugs, making the treatment less effective or ineffective.[6,7] Therefore, looking for means to counteract or reverse stomach cancer multidrug resistance is a pressing subject for stomach cancer treatment, and finding natural products that have this function is one of the mainstreams of the current investigations.[8-12]

Euphorbia esula Linn. is an herbaceous plant originally found in Asian and European countries. The aboveground part of the plant is used as an herbal medicine in traditional Chinese medicine. In recent years, it was reported that the extract of *E. esula* could inhibit cell proliferation and induce apoptosis in a nonresistant human stomach cancer cell line and

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several other cancerous cell lines.[13,14] In the present paper, we studied the effects of *E. esula* full extract on multidrug-resistant human stomach cancer cell line SGC7901/VCR in reversion of chemotherapeutic drug resistance; in inhibition of cell proliferation, migration, and invasion; in interference of cell cycle progression; and in induction of cell apoptosis; aiming to find out a therapeutic natural product that can be used to counteract or reverse stomach cancer multidrug resistance.

MATERIALS AND METHODS

Herb extract and cell line

The aboveground parts of *E. esula* plant in the wild were gathered from the mountainous area of North Shaanxi in early May. The fresh herb (stems, leaves, and flowers) was washed first with tap water and then with ultrapure water. The cleaned herb was cut into pieces and was extracted with squeezing machine in ultrapure water. The extracted solution was filtered with a slow-type analytical filter paper at 4°C overnight. The filtered liquid was dried with air blower at 50°C. The dry extract was dissolved with ultrapure water. The resulting solution was made aseptic by filtration with a 220-nm ultrafiltration membrane (Minisart, Germany). The aseptic extract was stored at −20°C for future use.

SGC7901/VCR cells (a multidrug-resistant human stomach cancer cell strain that was derived from human stomach cancer SGC7901 cell line, and the multidrug resistance was induced by the chemotherapeutic drug vincristine) were obtained from Xijing Hospital of the Fourth Military Medical University (Xian, China). The cells were cultivated using RPMI 1640 (Thermo Fisher Scientific, Beijing, China) supplemented with 10% fetal calf serum (FCS; Zhejiang Tianhang Biotechnology Co., Huzhou, Zhejiang, China), 1000 U/L penicillin and 100 mg/L streptomycin at 37°C, 5% CO_2, and saturated humidity. For all cultivations, 1 mg/L (final concentration) of vincristine (Zhejiang Hisun Pharmaceutical Co., Taizhou, Zhejiang, China) was added to maintain cellular drug resistance.

Assay of cell proliferation/viability using thiazolyl blue

SGC7901/VCR cells cultivated to 80% confluence were harvested with 2.5 g/L trypsin. The cells at density of 4×10^7 cells/L were seeded in 96-well plates, 100 μL per well, and incubated at 37°C and 5% CO_2 routinely. After 24 h, the old medium was replaced with fresh medium (RPMI 1640 supplemented with 10% FCS) containing *E. esula* extract at concentrations of 10, 20, 40, 80, 160, 320, and 640 mg/L; each concentration had six repeats. The negative control was set in which the medium contained no *E. esula* extract. After 24 h and 48 h incubation, cell viability experiment was performed: 5 mg/mL thiazolyl blue (Sigma-Aldrich, St Louis, MO, USA) was added to all wells of the cell culture plates, 20 μL per well. After 4 h at 37°C, the thiazolyl blue containing-medium was cast off and 150 μL dimethyl sulfoxide (Toray Fine Chemicals Company, Cangzhou, Hebei, China) was added to all the wells. The plate was shaken slowly for 10 min. Then, the optical density (OD) was measured at λ = 490 nm with a microplate reader (Model 680; Bio-Rad, USA). Proliferation inhibition rates were calculated with the formula: inhibition rate = (mean OD value of control well − mean OD value of drug-containing well)/mean OD value of control well.

Evaluation of drug sensitivity using half maximal inhibitory concentration

E. esula extract was diluted with culture medium RPMI 1640 supplemented with 10% FCS into three concentrations: 10, 20, and 40 mg/L. The chemotherapeutic drug adriamycin was diluted into six concentrations with the same culture medium: 32, 16, 8, 4, 2, and 1 μmol/L, and the chemotherapeutic drug paclitaxel was diluted into six concentrations with the same culture medium: 64, 32, 16, 8, 4, 2 μmol/L.

SGC7901/VCR cells at logarithmic growth phase were made into 4×10^7 cells/L suspension. The cells were grouped into four groups: negative control, *E. esula* low, medium, and high concentrations and were inoculated in a 96-well plate at 100 μL/well and cultured routinely. After 24 h of cultivation, the original culture medium was discarded, and RPMI 1640 medium supplemented with 10% FCS and *E. esula* extracts at 10, 20, and 40 mg/L were added to each of the four groups, respectively (negative control, *E. esula* extract low, medium, and high concentrations), 100 μL per well. Then, gradient dilutions of adriamycin and gradient dilutions of paclitaxel dilution were added to each group, respectively, 100 μL per well. Each group has three repeats. The plates were incubated in 37°C, 5% CO_2, and saturated humidity for 48 h. The cell viability experiment was performed in the same way as detailed above, and the half maximal inhibitory concentration (IC50) was calculated according to the Bliss method.

Analysis of cell cycle progression with flow cytometry

SGC7901/VCR cells were seeded in six-well plates. After 24 h incubation, the old medium was replaced with the medium containing 40 and 80 mg/L of *E. esula* extract and the cells were incubated for 48 h. Cells without *E. esula* treatment were used as control. Cells were then harvested with trypsin and washed twice with ice-cold phosphate-buffered saline (PBS). The cells were fixed in ice-cold 70% ethanol at 4°C for 12 h and were washed with ice-cold PBS. The cells were resuspended in 1 mL propidium iodide solution (1 mg/mL sodium citrate, 50 μg/mL propidium iodide, 10 μg/mL RNase A, 0.5% Triton X-100) and placed in dark at 4°C for 30 min. The population of cells in each cell cycle phase was measured using a flow cytometer (CyFlow® Cube, Am Flugplatz 13, 02828 Görlitz, Germany).

Measurement of cell migration and invasion with transwell

Cell migration assay

The lower chamber of Transwell (BD Biosciences, San Jose, CA, USA) was filled with RPMI 1640 containing 10% FCS, and 100 μL of SGC7901/VCR cells at a density of 1×10^5 cells/mL in RPMI 1640 (serum-free)-containing *E. esula* extract at 0 mg/L (used as negative control), 10, 20, and 40 mg/L was added to the upper chamber. After 24 h cultivation at 37°C, the cells remained on the upper Transwell chamber were cleaned away and the cells migrated to the lower side of the membrane were fixed with 95% ethyl alcohol for 10 min and stained with 5 g/L crystal violet. Six microscopic fields were randomly chosen, and the cell number was counted.

Cell invasion assay

The upper Transwell chamber was coated with Matrigel gel (BD Bioscience, San Jose, CA, USA), and the lower chamber was filled with RPMI 1640 containing 10% FCS. After the gel became solidified, 200 μL of SGC7901/VCR cells at a density of 1×10^5 cells/mL in RPMI 1640 (serum-free)-containing *E. esula* extract at 0 mg/L (negative control), 10, 20, and 40 mg/L was added to the upper chamber. After 48 h cultivation at 37°C, cells on the upper chamber and the gel were cleaned away and cells on the lower side of the membrane were fixed with 95% ethyl alcohol for 10 min and stained with 5 g/L crystal violet. Six microscopic fields were randomly chosen, and the cell number was counted.

Electron microscopic observation of apoptotic morphology

Logarithmic phase SGC7901/VCR cells were seeded in a six-well plate, each well 3×10^4 cells in 2 mL, and cultivated at 37°C and 5% CO_2.

After 24 h cultivation, the old medium was replaced with 10% FCS RPMI-1640 medium-containing 80 mg/L of *E. esula* extract; 10% FCS RPMI-1640 without *E. esula* extract was used as control. The cells were cultured at 37°C and 5% CO_2. After 48 h, the cells were digested with 2.5% trypsin, washed in PBS, pelleted at 1200 rpm for 6 min, and fixed with 1.5 g/L glutaraldehyde for 90 min. Then, the cells were washed with 0.18 mol/L sucrose for three times and overnight, postfixed with 1 g/L osmium tetroxide for 1 h, and then washed. The cells were then dehydrated in series of gradient concentration ethyl alcohol, soaked in a mixed solution of ethyl alcohol and embedding medium at 37°C for 3 h, and finally embedded in epoxy resin. A Leica ultra-microtome was used to cut the blocks; a mixture of uranium acetate and lead citrate was used to stain the cells. The slices were examined using a transmission electron microscope (Model JEM-1011, JEOL Ltd., Japan).

Flow cytometry examination of apoptotic rate

The SGC7901/VCR cells were cultured in a six-well plate. When the cells grew to 80% confluency, *E. esula* extract diluted with 10% FCS RPMI-1640 at 40, 80, and 160 mg/L was added to each well to replace the old medium, and the cells were incubated for 24, 48, and 72 h. The cells were collected with 2.5% trypsin and centrifuged at 1200 rpm for 6 min. The cells were washed with 3 mL of PBS and pelleted again. Precooled 70% ethyl alcohol was used to fix the cells at 4°C. After 1 h, the ethyl alcohol was removed by centrifugation, and 3 mL of PBS was added to resuspend the cells. The cells were centrifuged at 3000 rpm for 3 min, and the supernate was discarded. Then, 1 mL of 100 mg/L propidium iodide was added and the cells were suspended with a vortex mixer and stained in the dark at 4°C for 30 min. The cells were examined by a flow cytometer (CyFlow® Cube, SysmexPartec GmbH, Germany) at λ = 488 nm for excitation wavelength and λ = 630 nm for emission wavelength. The average of three measurements was used to calculate apoptotic rate.

Ultraviolet spectrophotometry inspection of caspase 3 enzyme activity

SGC7901/VCR cells were cultivated using RPMI 1640 medium supplemented with 10% FCS at 37°C, 5% CO_2, and saturated humidity. When the cells grew to logarithmic phase, old culture medium was replaced by fresh medium and 80 mg/L of *E. esula* extract (final concentration) was added. The cells were cultivated continually for 24, 48, and 72 h. At each of the three time points, caspase 3 enzyme activity was inspected in accordance with the operation instructions of the detection kits (Caspase 3 Activity Assay Kit, Biyuntian Biological Technology Co. Ltd., Zhejiang, China). The cells were harvested with 2.5 g/L trypsin and washed with ice-cooled PBS for two times. A cell suspension of 2×10^6 cells/L was made and after precooled lysis buffer was added, was placed in ice bath for 30 min. During this period, the cell suspension was gently shaken intermittently for three times, 10 s each time. At the end of the treatment, the cell lysate solution was centrifuged at 12000 rpm and 4°C for 20 min. The supernate was moved to new centrifugal tubes. The OD values at λ = 405 nm were measured using a microplate reader (Model 680; Bio-Rad, USA).

Statistical analysis

The statistical software package SPSS 19.0 (IBM Corporation, Armonk, New York, United States) was used for statistical analysis. The data were expressed as mean ± standard deviation. The *t*-test was used to compare two groups, and analysis of variance was used to compare multiple groups. $P < 0.05$ was considered statistically significant.

RESULTS

Inhibition of cell proliferation by *Euphorbia esula* extract

The thiazolyl blue assay indicated that *E. esula* extract significantly inhibited the proliferation of the multidrug-resistant human stomach cancer SGC7901/VCR cells, which showed an apparent time- and dose-dependent manner. All SGC7901/VCR cells treated with *E. esula* extract showed a significant decrease of viability compared with control ($P < 0.01$). When the *E. esula* concentration is higher than 40 mg/L, with the increase of concentration, there was more significant inhibition of cell viability ($P < 0.01$); in addition, the inhibition was more obvious when treatment was prolonged from 24 to 48 h ($P < 0.05$) [Table 1].

Reversal of SGC7901/VCR cell's drug resistance by *Euphorbia esula* extract

Reversal of SGC7901/VCR cell's resistance to the chemotherapeutical drugs adriamycin and paclitaxel by *E. esula* extract was evaluated using IC50. For adriamycin, the IC50 values of the three *E. esula* extract concentrations were significantly different from the negative control ($P < 0.01$), and there were significant differences ($P < 0.05$) between different concentrations of *E. esula* extract as well [Figure 1]. For paclitaxel, the IC50 values of the three *E. esula* extract concentrations were also significantly different from the negative control ($P < 0.01$), and there were significant differences ($P < 0.05$) between different concentrations of *E. esula* extract too. These results demonstrated that *E. esula* extract could reverse drug resistance of SGC7901/VCR cells to adriamycin and paclitaxel, and the effect was dependent on the concentration of *E. esula* extract [Figure 1].

Interference of cell cycle progression by *Euphorbia esula* extract

Analysis of cell cycle arrest in SGC7901/VCR cells was carried out using flow cytometry. The results showed that the population of cells at the S phase increased from 18.16% in the control group to 23.19% and 25.72% in the *E. esula* extract-treated groups, while the population of cells at the G2/M phase was reduced from 34.84% in the control group to 29.87% and 28.01% in the *E. esula* extract-treated groups [Figure 2]. The differences were statistically significant ($P < 0.01$ between treated groups and the control, $P < 0.05$ between the two treatment concentrations). The results showed that *E. esula* extract could interfere with cell cycle progression of SGC7901/VCR cells and more cells accumulated at the S phase.

Inhibition of cell migration and cell invasion by *Euphorbia esula* extract

The results of Transwell experiments showed that the migration of SGC7901/VCR cells was inhibited significantly by *E. esula* extract

Table 1: Inhibition of SGC7901/VCR cells by *Euphorbia esula* extract (thiazolyl blue assay results)

Euphorbia Esula (mg/L)	OD_{490} (mean±SD)		Inhibition rate (%)	
	24 h	48 h	24 h	48 h
0 (control)	0.821±0.058	0.803±0.060	-	-
10	0.765±0.062	0.755±0.064	6.82	5.98
20	0.736±0.056[a]	0.724±0.069[a]	10.4[a]	9.84[a]
40	0.692±0.049[a]	0.650±0.053[a,b]	15.7[a]	19.1[a,b]
80	0.611±0.051[a,b]	0.492±0.059[a,b,c]	25.6[a,b]	38.7[a,b,c]
160	0.526±0.055[a,b]	0.418±0.056[a,b,c]	35.9[a,b]	47.9[a,b,c]
320	0.463±0.057[a,b]	0.359±0.061[a,b,c]	43.6[a,b]	55.3[a,b,c]
640	0.384±0.059[a,b]	0.301±0.057[a,b,c]	53.2[a,b]	62.5[a,b,c]

[a]$P<0.01$, *vs.* control; [b]$P<0.01$, *vs.* lower concentration; [c]$P<0.05$ vs. 24 h $n=6$

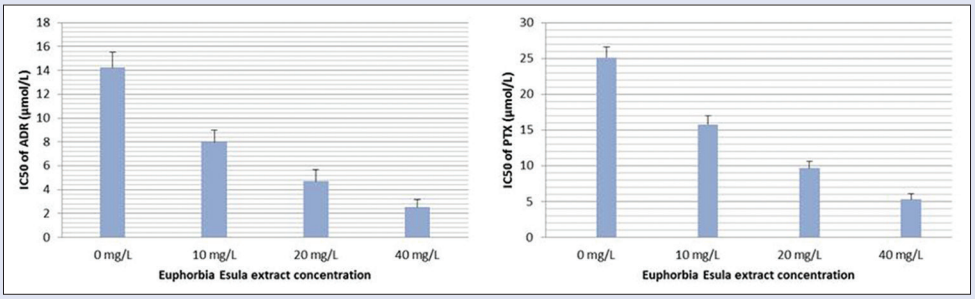
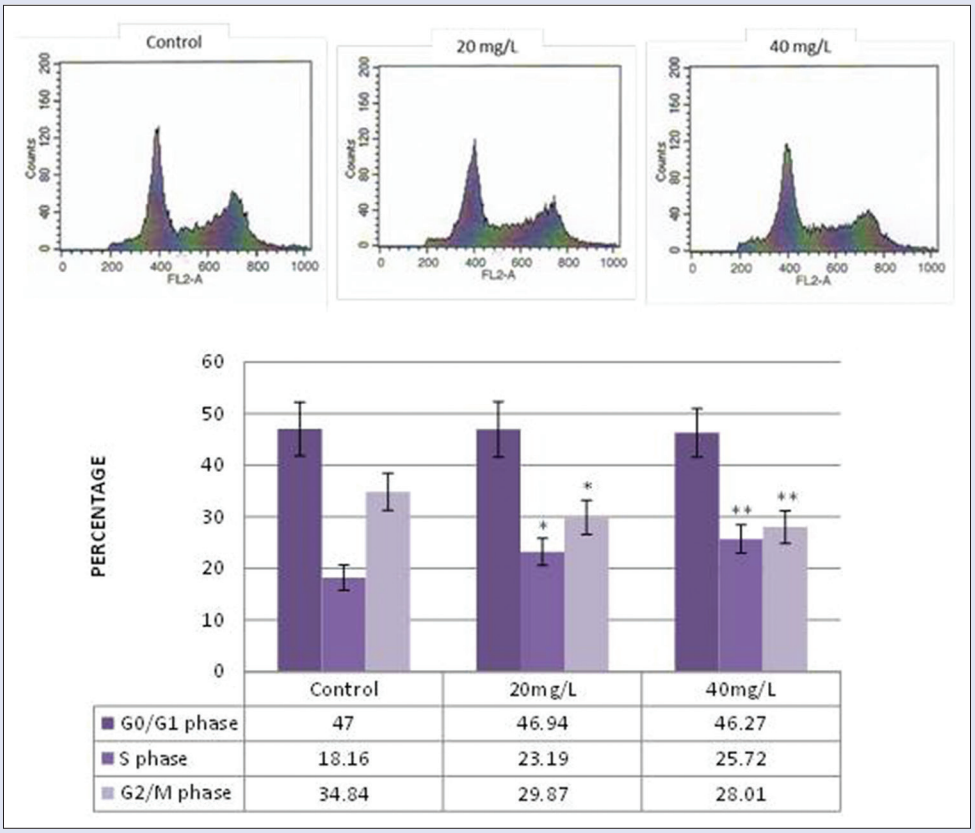


Figure 1: The effects of *Euphorbia esula* extract on SGC7901/VCR cells in reversal of resistance to chemotherapeutical drugs as measured with half maximal inhibitory concentration. $P < 0.01$ between *Euphorbia esula* extract three concentrations and the control (0 mg/L *Euphorbia esula* extract), $P < 0.05$ between different concentrations; $n = 3$. ADR: Adriamycin; PTX: Paclitaxel



	Control	20mg/L	40mg/L
G0/G1 phase	47	46.94	46.27
S phase	18.16	23.19	25.72
G2/M phase	34.84	29.87	28.01

Figure 2: Interference of cell cycle progression of SGC7901/VCR cells by *Euphorbia esula* extract as measured with flow cytometry. *$P < 0.01$, versus control; **$P < 0.05$, versus lower concentration

compared to the negative control ($P < 0.01$); the inhibition of migration was more significant with increased *E. esula* concentrations ($P < 0.05$). The invasion of SGC7901/VCR cells showed the similar manner as well. When the cells were treated by *E. esula* extract, cell invasion was inhibited significantly compared to the negative control ($P < 0.01$); the inhibitory effect was more significant with increased *E. esula* concentrations ($P < 0.05$) [Figure 3].

Induction of apoptosis by *Euphorbia esula* extract

The morphological changes of SGC7901/VCR cells following treatment with *E. esula* extract were observed with transmission electron microscopy, and the apoptotic rates were examined with flow cytometry. After treating the SGC7901/VCR cells with 80 mg/L *E. esula* extract for 48 h, it could be observed under the electron microscope that cells showed some characteristic morphological changes of apoptosis. Some of these changes included foaming or bubbling of cytoplasm [Figure 4] and chromatin condensation or marginalization. The cells in the control group did not show these morphological changes [Figure 4].

SGC7901/VCR cell apoptotic rate induced by *Euphorbia esula* extract

Flow cytometry analysis showed that the *E. esula* extract-treated SGC7901/VCR cells had a significantly higher apoptotic rate than the negative control group ($P < 0.01$). The apoptotic rate increased with *E. esula* extract concentration and with treatment time, and the differences were statistically significance ($P < 0.05$). These results demonstrated that the

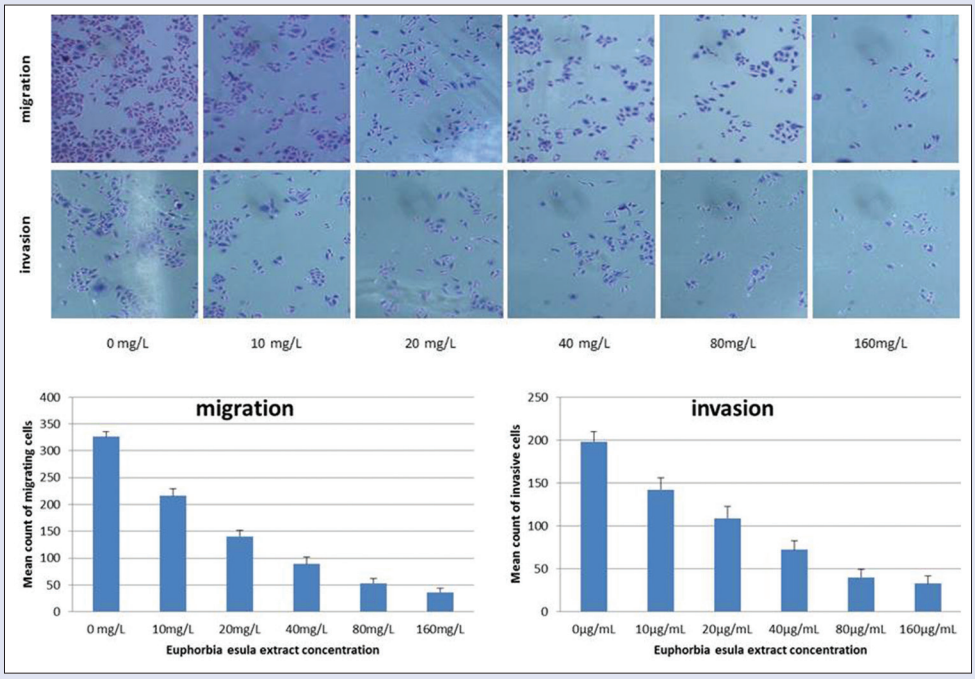


Figure 3: The inhibition of SGC7901/VCR cell migration and invasion by *Euphorbia esula* extract as measured with Transwell method. $P < 0.01$ between *Euphorbia esula* treatment and control (0 mg/L); $P < 0.05$ between *Euphorbia esula* concentrations ($n = 3$)

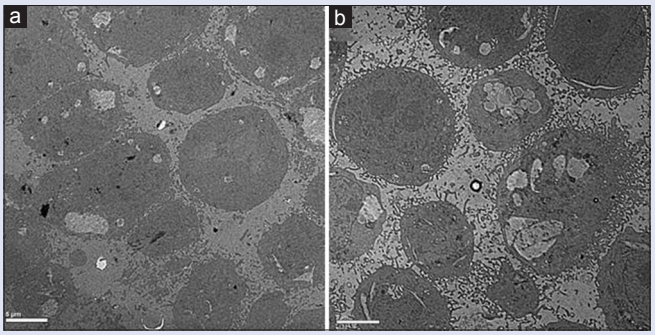


Figure 4: Apoptotic morphology of SGC7901/VCR cells after *Euphorbia esula* extract treatment observed with transmission electron microscope. (a) Untreated cells as control; (b) cells treated with 80 mg/L *Euphorbia esula* extract for 48 h. Bar = 5 μm. Note the bubbling or foaming phenomenon of the treated cells: numerous blebs are seen and the cell surface is not smooth; in contrast, the surface of the control cells is largely smooth

extract of *E. esula* could effectively induce SGC7901/VCR cells to undergo apoptosis [Figure 5].

Upregulation of caspase 3 enzyme activity by *Euphorbia esula* extract

To determine if the apoptotic enzyme caspase 3 was activated by *E. esula* extract, ultraviolet spectrophotometry inspection of caspase 3 enzymatic activity was performed. The result showed that the caspase 3 enzyme activity was upregulated significantly after 80 mg/L of *E. esula* extract treatment ($P < 0.01$ compared with negative control) for 24, 48, and 72 h. In addition, the activation of the enzyme activity became more obvious with time progress: there were significant differences between the three time points ($P < 0.05$) [Figure 6].

DISCUSSION

Cancer multidrug resistance is defined as the resistance of cancer cells against a variety of anticancer agents that are structurally and mechanistically unrelated. The resistance can be acquired in the course of cancer chemotherapy, or it may occur intrinsically/naturally.[15] The mechanisms involved in multidrug resistance of cancer cells are complex and diverse and include at least increase in drug efflux resulted from overexpression of membrane transporter proteins, decrease in drug uptake, defect in apoptotic machinery, alteration of drug metabolism, and activation of detoxifying enzymes.[16-18] Some cancers such as stomach cancer are more easily to develop multidrug resistance. Cancer multidrug resistance has become a serious troublesome problem for cancer drug therapy, which eventually leads to cancer relapse and death. Numerous measures have been taken to overcome cancer cell multidrug resistance, but the problem remains unsolved.[19-21]

Recently, there has been an increased interest in the exploration of herbal medicines or other traditional medicines to find out bioactive components that can combat cancer multidrug resistance.[22,23] Many experts believe that development of natural products is one of the most promising approaches in the discovery of counter-multidrug-resistant drugs.[24,25]

E. esula L. is a perennial herb in the family of *Euphorbiaceae* [Figure 7]. The entire plant is 20–40 cm high with a thin (2–3 mm) and round stem. The base of the stem is purple and hard. The stem contains a kind of white sap or latex and is easily broken. The stem branches from the base or near the top for several times. Leaves grow alternately. Leaf blade is linear, about 3 cm long, <3 mm wide; the apex is slightly acute or obtuse. The plant has a pleiochasial cyme (cyathium) with 4–9 umbrella stems. The inflorescence grows apically or in the upper axillary. The basal leafy bracts are half-moon shaped or kidney shaped. The ovoid or triangular shaped capsule is smooth with a special but light odor.[26,27] In traditional Chinese medicine, the aboveground part of *E. esula* is processed as a herbal medicine, which is slightly cold,

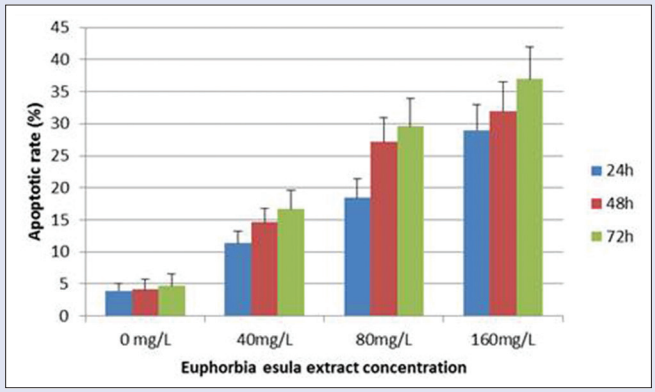


Figure 5: The apoptotic rate of SGC7901/VCR cells treated by *Euphorbia esula* extract, analyzed with flow cytometry. $P < 0.01$ compared between each testing group and the control group (0 mg/L); $P < 0.05$, between different treatment concentrations; $P < 0.05$ between 24, 48, and 72 h treatment time ($n = 3$)

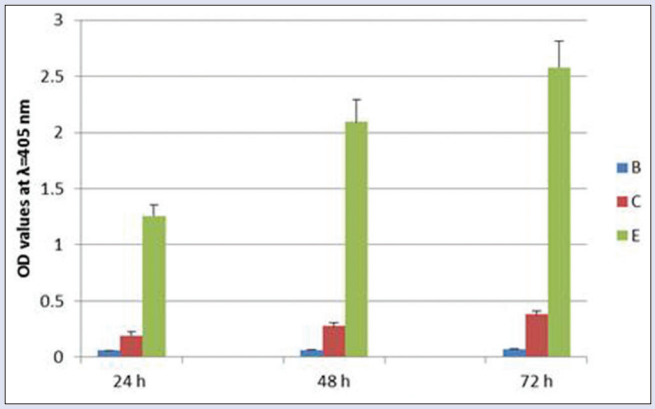


Figure 6: Activation of caspase 3 enzyme by *Euphorbia esula* extract. $P < 0.01$ between *Euphorbia esula* extract and negative control, $P < 0.05$ between different treatment time. $n = 3$. B: blank; C: negative control; E: *Euphorbia esula* extract at 80 mg/L

Figure 7: The plant of *Euphorbia esula* Linn. as seen in or gathered from the mountainous area of China's Northern Shaanxi Province in May

tastes bitter, and is slightly toxic; the herbal medicine of *E. esula* goes to the meridians and collaterals of the stomach, liver, spleen, and lung; It is able to remove the stasis, eliminate the stagnation, and has the function of detumescence.[28,29] *E. esula* and several other species of the *Euphorbiaceae* family have been shown to inhibit cell proliferation and induce apoptosis by our previous studies and by a number of other research groups.[30-33]

Two classes of extracts isolated from the *E. esula* herb have been shown to have anticancer functions: one is diterpenoids and the other is flavonoids. In our recent paper, we have shown that the ethanol/hexane extract of *E. esula*, which contains diterpenoids, could induce apoptosis and inhibit cell proliferation of SGC7901/ADR cells.[34] Researches have also shown that the flavonoids extract from *E. esula* could inhibit the transporter protein P-gp in multidrug-resistant cancer cell membrane to decrease drug efflux and increase cellular drug sensitivity. P-gp or Pgp refers to permeability glycoprotein or P-glycoprotein 1, also known as ATP-binding cassette subfamily B member 1 or multidrug resistance protein 1 or cluster of differentiation 243. It is an important cell membrane protein that can pump many alien substances out of cells. P-gp is an ATP-dependent efflux pump with wide substrate specificity and is probably evolved as a defense mechanism against harmful substances.

In the present paper, we studied the effects of the full extract of *E. esula* on the multidrug-resistant human stomach cancer cell strain SGC7901/VCR, which is derived from the parental human stomach cancer cell line SGC7901 and the multidrug-resistant was induced by vincristine, a common chemotherapeutic drug. The full extract of *E. esula* should contain diterpenoids, flavonoids, and other active ingredient in addition, and so, its function must be munificent. We studied the inhibition of cell proliferation, inhibition of cell migration and cell invasion, interference of cell cycle progression, and induction of cell apoptosis by the *E. esula* extract. We also studied the activation of caspase 3 enzyme by the extract. The results clearly demonstrated that the full extract of *E. esula* could reverse SGC7901/VCR cell's resistance to chemotherapeutic drugs adriamycin and paclitaxel; could potently inhibit SGC7901/VCR cell proliferation, migration, and invasion; and could induce cell apoptosis. This study prepared the way for further research on the reversal treatment of multidrug-resistant human stomach cancers and other human cancers or the adjuvant therapy of these cancers using *E. esula* preparations.

CONCLUSION

The full extract of *Euphorbia esula* could significantly increase chemosensitivity, inhibit cell proliferation, migration and invasion, arrest cell cycle progression, and induce cell apoptosis in SGC7901/VCR cells.

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Conflicts of interest

There are no conflicts of interest.

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