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# Safflower polysaccharide induces cervical cancer cell apoptosis *via* inhibition of the PI3K/Akt pathway☆

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ABSTRACT

Safflower polysaccharide (SPS) is a major active component of *Carthamus tinctorius* L. Previous reports have demonstrated that SPS shows cytotoxic activity against various types of cancer cells; however, its anti-cervical cancer effect remains unclear. Therefore, we explored the effect of SPS treatment of a cervical cancer cell line (HeLa). SPS significantly inhibited cell proliferation and increased the rate of cell apoptosis as determined by TdT-mediated dUTP nick-end labeling assay, Hoechst 33342 staining, and double staining with annexin V-fluorescein isothiocyante/propidium iodide. Furthermore, western blot and real-time polymerase chain reaction revealed that SPS down-regulated mammalian target of rapamycin and protein kinase B and up-regulated Bcl-2-associated death promoter. These results suggest that SPS-induced apoptosis may occur through down-regulation of the phosphatidylinositol-3-kinase/AKT pathway. Collectively, these findings implicate SPS as a candidate for the development of anti-cervical cancer drugs.



## 1. Introduction

Cervical cancer is the most common malignant tumor of the female reproductive system (Siegel et al., 2015), accounting for 73–93% of all malignant tumors of the female reproductive system, with a higher incidence in younger women (Patel et al., 2012). There are more than 130,000 new cases of cervical cancer diagnosed in China annually. Therefore, the current focus of research on cervical cancer is to identify reliable, efficient methods and drugs for prevention or treatment. However, commonly used anti-cancer drugs have substantial toxicity, inducing side effects that cause significant damage to the patient's body and reduce quality of life (Abudayyak et al., 2016; Jamesdaniel et al., 2016). Traditional Chinese medicine has been used in the treatment of cervical cancer with good results. For example, *Hedyotis diffusa* inhibits the proliferation of cervical cancer HeLa cells by reducing the expression of Ki-67 *in vivo* and *in vitro* (Zhang et al., 2015). Therefore, the development of drugs derived from natural Chinese herbal medicines has become an important strategy in cervical cancer treatment.

Safflower (*Carthamus tinctorius* L.) is a well-known and widely used traditional Chinese medicine that has been verified to have anti-inflammatory, antioxidant, and cytotoxic functions (Chen et al., 2012; Esmail Al-Snafi, 2015). Safflower polysaccharide (SPS) is one of the active ingredients extracted from safflower and has been reported to enhance immunomodulatory activity and block the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway in the non-small cell lung cancer cell lines (NSCLC) A549 and YTMLC-90 to suppress tumor growth (Li et al., 2016). SPS also inhibits the metastasis of MCF-7 breast cancer cells (Luo et al., 2015). However, different types of tumors show various degrees of sensitivity to the same drug, and the cytotoxic effect of SPS on cervical cancer cells remains unknown. Therefore, in this study, we aimed to determine whether SPS has an inhibitory effect on the growth of the cervical cancer HeLa cells *in vitro* and to elucidate the underlying mechanism. Specifically, as SPS has been shown to exert inhibitory effects by inducing apoptosis *via* the PI3K/AKT pathway, particularly through the regulation of mammalian target of rapamycin (mTOR) and Bcl-2-associated death promoter (BAD) signaling (Fu et al., 2016; Wu et al., 2016), we hypothesized that SPS might also inhibit the growth of cervical cancer cells *via* the PI3K/AKT pathway.

*Abbreviations:* SPS, Safflower polysaccharide; IR, Inhibition rate; TUNEL, TdT-mediated dUTP Nick-end Labeling.

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## 2. Materials and methods

### 2.1. SPS extraction from safflower

SPS was extracted from safflower (*C. tinctorius* L) purchased from Shiyitang (Harbin, China). After dehydration, the safflower was smashed and ultrasonicated for 2 h, and the supernatant was collected and stored with a 5-times volume of 95% ethanol for 24 h. The mixture was centrifuged for 10 min at 2000 × *g*, and the same volume of 5% trichloroacetic acid was added, followed by stirring for 15 min and storage for 12 h at 4 °C. After filtration, the extracted polysaccharide was discolored with 15% $H_2O_2$ for 3 h at 60 °C. The polysaccharide was then washed with anhydrous ethanol, acetone, and anhydrous ether. Finally, the polysaccharide was dialyzed with distilled water for 48 h; the water was replaced with new distilled water every 4 h. The purity of SPS was determined by a UV-4802 double-beam ultraviolet–visible light spectrophotometer [Unico (Shanghai) Instrument Co., Ltd., Shanghai, China].

### 2.2. Confirmation of SPS

SPS (10 mg) was dissolved in a 100-ml volumetric flask, and 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, or 0.8 ml of the extract was added to 1 ml of distilled water. Subsequently, 0.5 ml of 5% phenol and oil of vitriol was added to the samples, which were heated for 15 min, and then the optical density (OD) values were measured to determine the calibration curve. The relative standard deviation values of precision and reproducibility were measured using the OD values of 2 ml of the extract measured 5 and 6 times, respectively. The stability test was conducted by measuring the OD values at 10, 20, 30, 60, 90, 120, 150, and 180 min according to the steps described above. The average recycle rate was determined by measuring the OD values 6 times and comparing the values to those of glucose as a reference.

### 2.3. Cell culture

Human cervical cancer cells (HeLa cells) were purchased from Ruiqi Biological Technology Co. (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium/high glucose containing 4.0- nM L-glutamine (Hyclone Laboratories, Inc., Logan, UT, USA) with 10% fetal bovine serum (Gibco Life Technologies, Grand Island, NY, USA) in a 5% $CO_2$ atmosphere at 37 °C.

### 2.4. Proliferation assay

The ability of SPS to inhibit cell proliferation was determined with the 3-(4,5-dimethylthaizol-yl-)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, cells were grown in a 96-well plate ($5 \times 10^3$/100 μl per well). The cells were treated with different concentrations of SPS (0.16, 0.32, and 0.64 mg/ml) or 5 μg/ml cis-platinum as a positive control for 24, 48, or 72 h each, and then 10 μl of MTT (5 mg/ml) reagent was added to the wells and incubated for 4 h. The precipitate was placed in 150 μl of dimethyl sulfoxide (Sigma, St. Louis, MO, USA) and agitated for 10 min to dissolve. Cell growth was determined based on the OD determined on an automatic microplate reader at 490 nm (ELx800,

**Table 1**
PCR primer sequences for mRNA amplification.

| Gene | Primers |
|---|---|
| *GAPDH* | Forward: 5′-CATGAGAAGTATGACAACAGCCT-3′<br>Reverse: 5′-AGTCCTTCCACGATACCAAAGT-3′ |
| Human *mTOR* | Forward: 5′-TCCGAGAGATGAGTCAAGAGGAGTC-3′<br>Reverse: 5′-GCTGGAAACCAATTCAAAAATGTG-3′ |
| Human *BAD* | Forward: 5′-CGGAGGATGAGTGACGAGTTTGT-3′<br>Reverse: 5′-ATCCCACCAGGACTGGAAGACTC-3′ |
| Human *AKT* | Forward: 5′-ACCTTCCATGTGGAGACTCCTGAG-3′<br>Reverse: 5′-GTCCATCTCCTCCTCCTCCTGC-3′ |
| Homan BCL-2 | Forward: 5′-CATGTGTGTGGAGAGCGTCAAC-3′<br>Reverse: 5′-CTTCAGAGACAGCCAGGAGAAATC-3′ |

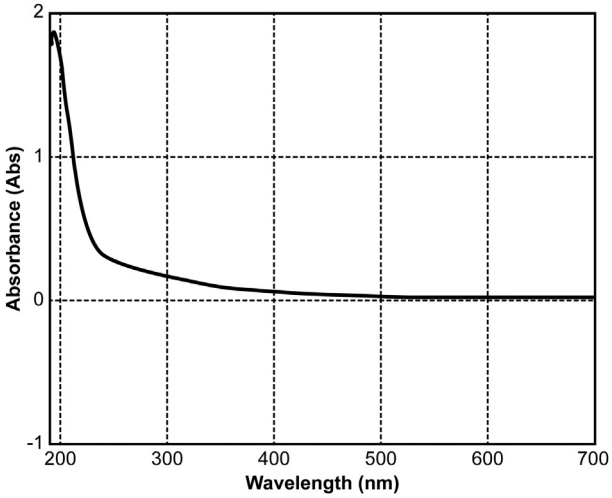


**Fig. 1.** Ultraviolet spectrum used to determine the purity of SPS, indicating its suitability for experiments.

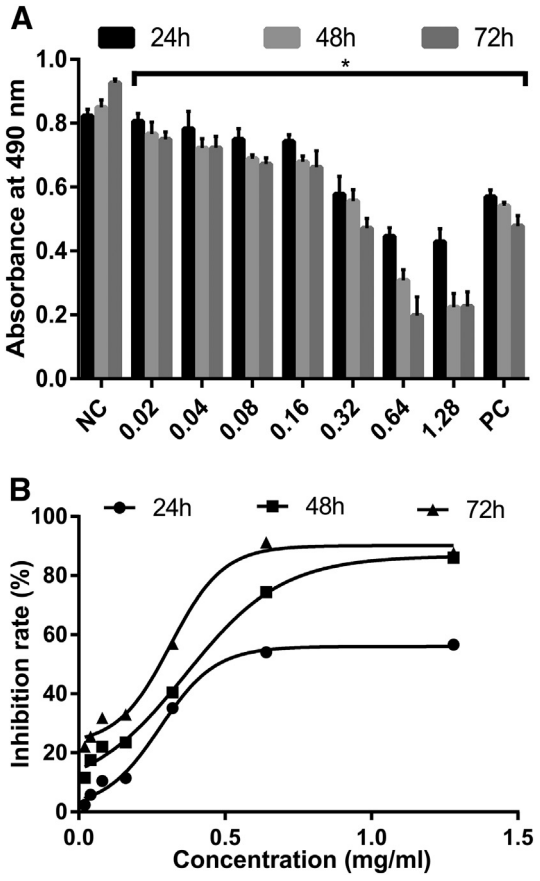


**Fig. 2.** SPS inhibition of HeLa cell proliferation in a time- and concentration-dependent manner. Cells were treated with different concentrations of SPS as indicated (n = 3). (A) Absorbance was measured at 24 h, 48 h, and 72 h for analysis of the MTT assay. (B) The half maximal inhibitory concentration ($IC_{50}$) values for SPS were determined at 24 h, 48 h, and 72 h. Cells treated with cis-platinum were used as a positive control.