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ORIGINAL ARTICLE

Total Alkaloids of *Sophora alopecuroides* Inhibit Growth and Induce Apoptosis in Human Cervical Tumor HeLa Cells *In vitro*

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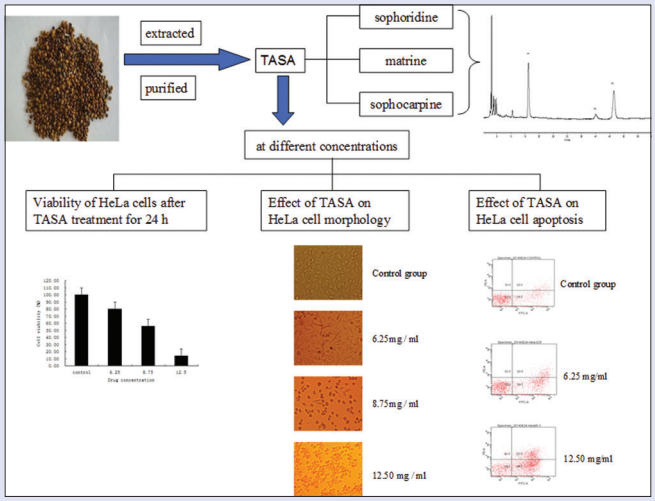
ABSTRACT

Background: Uygur females of Xinjiang have the higher incidence of cervical tumor in the country. Alkaloids are the major active ingredients in *Sophora alopecuroides*, and its antitumor effect was recognized by the medical profession. Xinjiang is the main site of *S. alopecuroides* production in China so these plants are abundant in the region. Studies on the antitumor properties of total alkaloids of *S. alopecuroides* (TASA) can take full use of the traditional folk medicine in antitumor unique utility. **Objectives:** To explore the effects of TASA on proliferation and apoptosis of human cervical tumor HeLa cells *in vitro*. **Materials and Methods:** TASA was extracted, purified, and each monomer component was analyzed by high-performance liquid chromatography. The effect of TASA at different concentrations on the survival of HeLa cells was determined after 24 h using the Cell Counting Kit-8. In addition, cells were photographed using an inverted microscope to document morphological changes. The effect of TASA on apoptotic rate of HeLa cells was assessed by flow cytometry. **Results:** Monomers of TASA were found to be sophoridine, matrine, and sophocarpine. On treatment with 8.75 mg/ml of TASA, more than 50% of HeLa cells died, and cell death rate increased further with longer incubation. The apoptotic rates of HeLa cells in the experimental groups were 16.0% and 33.3% at concentrations of 6.25 mg/ml and 12.50 mg/ml, respectively. **Conclusion:** TASA can induce apoptosis in cervical tumor HeLa cells, and it has obvious inhibitory effects on cell growth.

Key words: Apoptosis, cell growth, HeLa cells, inhibition, total alkaloids of *Sophora alopecuroides*

SUMMARY

- Total alkaloids of *Sophora alopecuroides* (TASA) exhibits anti-human cervical tumor properties
- Monomer component of TASA was analyzed by high-performance liquid chromatography, and its main effect component are sophoridine, matrine, and sophocarpine
- TASA inhibits growth and induces apoptosis in HeLa cells.



Abbreviations used: TASA: Total alkaloids of *S. alopecuroides*, CCK-8: Cell Counting Kit-8, FBS: Fetal bovine serum, PBS: Phosphate buffered saline, DMEM: Dulbecco's modified Eagle medium

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INTRODUCTION

Sophora alopecuroides is a perennial herb among leguminous plants of the genus *Cassia*.[1] Also called *Sophora* root, *Sophora*, or "Papua" (by Uighurs), it is now mentioned in the "Chinese Pharmacopoeia," "Xinjiang herbal," and "Uygur medicine."[2] Alkaloids are the major active ingredients in *S. alopecuroides*, most of which belong to quinolizidine alkaloids and include sophoridine, matrine, oxymatrine, sophocarpine, oxysophocarpine, and sophoramine, totaling more than 20 species.[3-6] There is growing interest in the antitumor properties of alkaloid monomers (8.75 mg/ml) found in *S. alopecuroides*. Research in recent years has shown that different alkaloids in *S. alopecuroides* inhibit proliferation of human gastric tumor MGC-803 cells, colon tumor SW620 cells, human leukemia cell line K562, hepatoma cells HepG2, Lewis lung tumor, bladder tumor, prostate tumor, and other tumor cells.[7-9] Leukemia, liver cancer, and gastric cancer are managed relatively well in clinical practice, but the characteristics and the mechanisms of cervical tumor gynecologic malignancies are poorly known.

The morbidity and mortality rates of the cervical tumor are second only to those of mammary tumor worldwide. Uygur females of Xinjiang have the higher incidence of cervical tumor in the country. The disease was discovered late and have a high mortality rate, which is the leading cause of death due to illness in the nation of women.[10] Drugs currently used in cancer treatment are not always effective, have severe side effects, and are expensive. Xinjiang is the main site of *S. alopecuroides* production

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in China, so these plants are abundant in the region. Studies on the antitumor properties of total alkaloids of *S. alopecuroides* (TASA) can provide the necessary foundation for future research on its potential use for clinical treatment of cervical tumor.

In this study, we investigate the inhibitory effect of TASA on cell growth of human cervical tumor HeLa cells *in vitro*.

MATERIALS AND METHODS

Materials

Herbs

S. alopecuroides was provided by the Xinjiang Institute of Materia Medica. It was identified as being genuine by Dr. He J, the Research Associate of Xinjiang Institute of Materia Medica.

Reagents

HP D500 macroporous resin was purchased from Cangzhou Bao En adsorbent Ltd., and 0.25% trypsin was provided by Beijing Concord Cell Resource Center. Fetal bovine serum (FBS) was purchased from Braun Biotech Ltd., Lanzhou. Dulbecco's modified Eagle medium (DMEM) was purchased from Thermo Fisher. Cell Counting Kit-8 (CCK-8) was provided by DOJINDO laboratories. Annexin-V-FLUOS Staining Kit was purchased from the biotechnology company Xin Bo Sheng.

Cells

Human cervical tumor HeLa cells were purchased from the Beijing concord Cell Resource Center.

Methods

Extraction and purification of total alkaloids of Sophora alopecuroides

S. alopecuroides was crushed into a meal at a solid-liquid ratio of 1:8 (m/V), and then extracted thrice in 70% ethanol at 1 h interval. The filtrate was combined and filtered, and then under vacuum conditions of 65°C, concentrated to 1 ml per concentrate containing crude drug 0.4 g. Isolated and purified using HP D500 macroporous resin at an adsorption rate of 1–2 bed volume (BV)/h and adsorption time >6 h, then eluted with distilled water 4 BV. After elution with 5 BV of 40% ethanol, elution rate was changed to 1–3 BV/h. The eluent in 40% ethanol eluent was collected, concentrated, dried to get TASA powder, and finally placed in a desiccator.

Preparation of the total alkaloids of Sophora alopecuroides drug solution

TASA powder was dissolved in phosphate buffered saline (PBS) at a concentration of 25 mg/ml. After complete dissolution, it was centrifuged at 12,000 rpm for 5 min. The supernatant was filtered using 0.22 μm sterile filter to obtain the final drug solution.

Chromatographic conditions of high-performance liquid chromatography

High-performance liquid chromatography was performed on a phenomenex C18 (4.6 mm × 250 mm, 5 μm) column in which a binary mobile phase, consisting of 0.01 mol/L ammonium carbonate (solvent A) and acetonitrile (solvent B) (80:20), the detection wavelength was 220 nm, the column temperature was 30°C, the flow rate was 1.0 ml/min, and the injection volume was 10 μl.

Determination of cell viability

HeLa cells in logarithmic growth phase and up to 95% confluency were plated in 96-well plates (100 μl/well), and then placed in an incubator with 5% CO_2 and 37°C. After adherent growth of the cells for 24 h, the drug solution was added at various concentrations to the cells. The experiment consisted of three groups: Blank group, control group, and experimental group. The 6 wells in the blank group were supplemented with DMEM containing 10% FBS culture medium at 100 μl/well. The 6 wells in the experimental group were added with 100 μl of 6.25, 8.75, and 12.50 mg/ml of the drug. The control group added 100 μl cells at a concentration of 5000 cells. After 24 h, add a mixed solution 100 μl to each well, the mixed solution was cell culture medium mixed with 10% CCK-8. After incubating for 3 h, absorbance was measured using a microplate reader at 450 nm, and at 650 nm as reference wavelength. Then, cell viability was calculated as follows:

$$\text{Cell viability} = 1 - ([OD_{experimental} - OD_{blank}]/[OD_{control} - OD_{blank}]) \times 100\%$$

HeLa cell morphology

The drug was added at different concentrations (6.25, 8.75, and 12.50 mg/ml) to HeLa cells at the logarithmic growth phase. Cells were then incubated under 5% CO_2 and 37°C conditions for 24 h. The cells were placed under the microscope, and their morphology was photographed.

Determination of cell apoptosis

HeLa cells at the logarithmic growth phase were digested into single cell suspension using pancreatin. Cells were collected by centrifugation (1100 rpm, 4 min), and the density was adjusted by adding media (FBS: DMEM = 10:90). They were then added to a 12 hole cell plate (10,000 cells per hole and 1 ml per hole) and placed in a cell culture incubator for 24 h at 37°C. Set up three control groups and two experimental groups were added 6.25 mg/ml and 12.50 mg/ml solution of different concentrations of TASA drugs 100 μl, after placed in the incubator of cell culture in 24 h (37°C), each cell is collected by 5 mL centrifuge tube. The cell culture medium was aspirated, and the cells were washed once with PBS. The wash was collected, and 0.5 ml trypsin was added to digest the cells. About 1 ml culture medium was added and mixed by pipetting back and forth 20 times. Then, the suspension was collected by centrifugation (1100 rpm, 4 min) followed by aspiration of

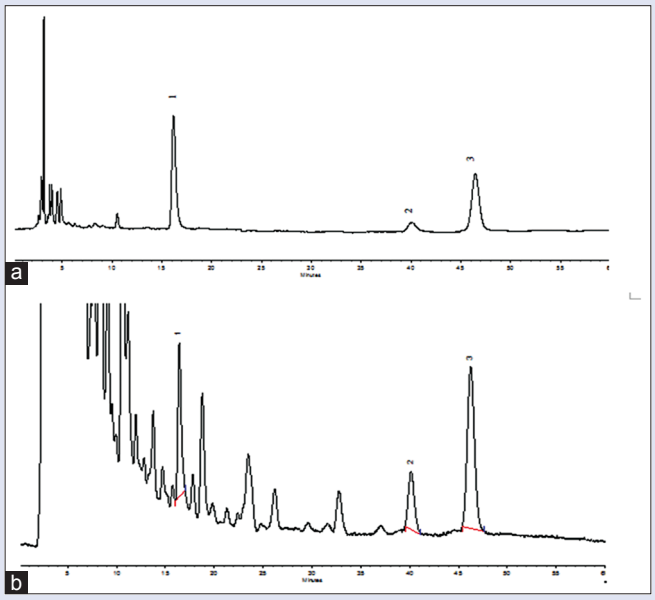

Figure 1: (a) Chromatograms of mixed reference; and (b) chromatograms of total alkaloids of *Sophora alopecuroides*. 1: Sophoridine; 2: Matrine; 3: Sophocarpine

the supernatant. The cell pellets were washed thrice with 1 ml PBS. Then, 500 µl of binding buffer mix was added. Annexin V-FITC (5 µl) mix was added, which was followed by staining with 5 µl of propidium iodide at room temperature in the dark. Finally, the cells were passed through a 300-mesh screen and immediately analyzed by flow cytometry.

Statistical analysis

Data are expressed as mean ± standard deviation and analyzed by one-way analysis of variance using SPSS version 16.0 (IBM Software). $P < 0.05$ was considered statistically significant.

RESULTS

Monomer components of total alkaloids of *Sophora alopecuroides*

The TASA drug solution contains sophoridine, matrine, and sophocarpine [Figure 1].

Viability of HeLa cells after total alkaloids of *Sophora alopecuroides* treatment for 24 h

Compared to the untreated control group, cell viability decreased significantly with increase in drug concentration [Figure 2]. At a drug concentration of 8.75 mg/ml, more than 50% of HeLa cells died. These results showed that TASA has significant inhibitory effect on HeLa cells.

Effect of total alkaloids of *Sophora alopecuroides* on HeLa cell morphology

TASA was added at different concentrations (6.25, 8.75, and 12.50 mg/ml) to cultured HeLa cells, and cell morphology was observed after 24 h under an inverted microscope [Figure 3]. Compared with HeLa cells untreated control groups, those treated with TASA exhibited decrease in cell volume, change in cell shape from the original spindle gradient shape to a round shape, and increases in cell gap. With increase in TASA concentration, morphological changes became more obvious.

Effect of total alkaloids of *Sophora alopecuroides* on HeLa cell apoptosis

Apoptotic rates in the experimental group were 16.0% and 33.3%, which were significantly higher compared to the control group. With increases in the concentration of administration, the proportion of early apoptotic, and late apoptotic cells increased significantly [Figure 4].

DISCUSSION

In this study, we found that TASA can significantly inhibit the proliferation of HeLa cells. Apoptosis is one of the major mechanisms targeted by anticancer therapies.[11] Apoptosis signal transduction pathways consist of an extrinsic pathway (death receptor-mediated) and an intrinsic pathway (activated by mitochondria-related proteins).[12] In this study, HeLa cells exhibited increased activity with decreased concentrations of TASA. Based on flow cytometry analysis after TASA treatment, the apoptotic rate was found to be significantly higher in the experimental group compared to the control group. With increase in drug concentration, early apoptotic and late apoptotic rates both increased significantly. In summary, this study confirmed that TASA can promote apoptosis of tumor cells and shows potential for use as anticervical tumor therapy.

TASA can inhibit a variety of tumor cells very well and displays more specific anticancer effects on some of them. Thus, TASA may be a broad-spectrum anticancer drug and merits further research. Different extraction methods of TASA may lead to differences in alkaloid compositions. Therefore, future studies should examine this. Compared TASA with monomer of TASA acting on tumor cells, then explore whether the efficacy of TASA can better than certain monomer, achieve the same efficacy with monomer in smaller doses but fewer side effects as much as possible.

CONCLUSION

In summary, the TASA extracted and purified using HP D500 macroporous resin, and its effect component includes sophoridine, matrine, and sophocarpine. The results of this study have confirmed TASA can induce apoptosis in cervical tumor HeLa cells, and it has obvious inhibitory effects on cell growth. In addition, further studies are required to compare the efficacy and the side effects between TASA and monomers.

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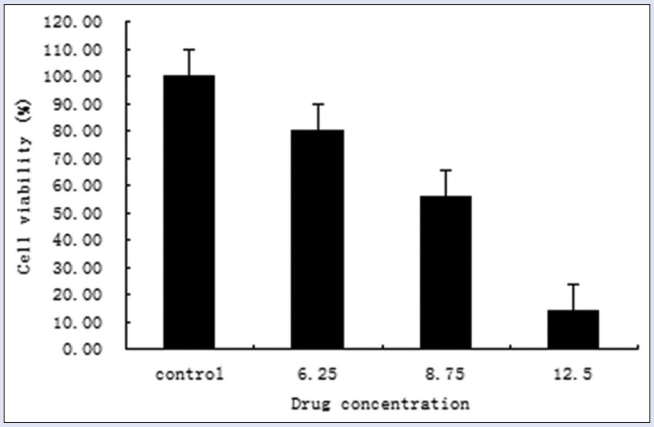

Figure 2: Effects of total alkaloids of *Sophora alopecuroides* at different concentrations on HeLa cell viability: Cell viability was determined under different concentrations of the drug (6.25, 8.75, and 12.50 mg/ml) after 24 h by Cell Counting Kit-8 viability assay

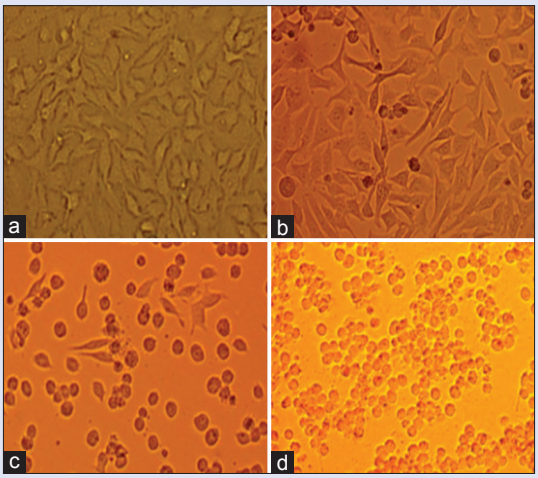

Figure 3: Different concentrations of the drug solution resulted in morphological changes in HeLa cells. (a) Control group: Normal HeLa cells; experimental groups: Cells treated with (b) 6.25 mg/ml drug solution, (c) 8.75 mg/ml drug solution, and (d) 12.50 mg/ml drug solution

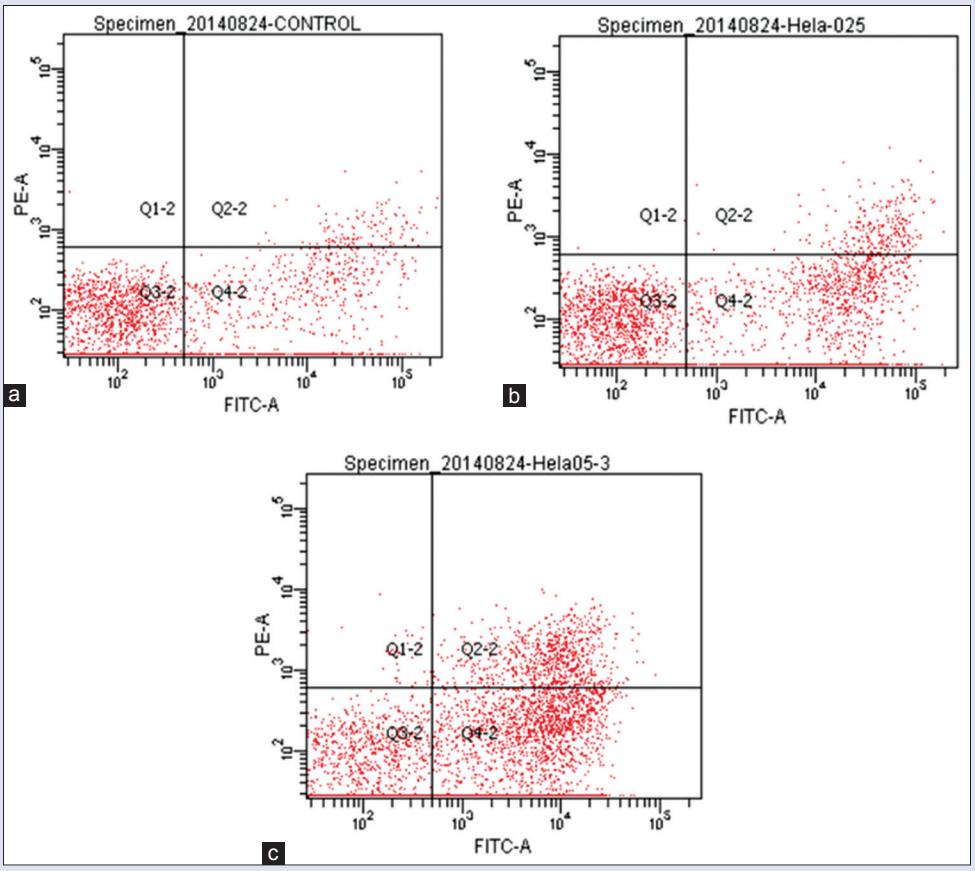

Figure 4: Total alkaloids of *Sophora alopecuroides* drug solution was added at different concentrations (6.25 and 12.50 mg/ml), and the apoptotic rate was determined after 24 h by flow cytometry. (a) Control group, (b) total alkaloids of *Sophora alopecuroides* concentration of 6.25 mg/ml, and (c) total alkaloids of *Sophora alopecuroides* concentration of 12.50 mg/ml

Conflicts of interest

There are no conflicts of interest.

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