

Effect of Petroleum Ether Extract from Rhizoma Amorphophalli (SLG) on Proliferation and Apoptosis of Non-small Cell Lung Cancer A549 Cells

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Abstract: Lung cancer has become one of the cancers that seriously threaten human life, with the highest morbidity and mortality in all countries of the world. At present, the treatment of lung cancer is mainly based on surgery, adjuvant chemotherapy and radiotherapy, but these treatments have adverse consequences for the prognosis and quality of life of patients with lung cancer. With the development of traditional Chinese medicine technology, traditional Chinese medicine and traditional Chinese medicine compound have achieved good clinical efficacy in the treatment of lung cancer. The traditional Chinese medicine Sheliugu has the effect of clearing heat and detoxifying, dispersing and detumescence, and the clinical curative effect is remarkable. In this paper, lung cancer A549 cells were used as the research object to explore the effect of petroleum ether extract of traditional Chinese medicine Heliugu on the proliferation and apoptosis of lung cancer A549 cells.

1. Introduction

According to the data released by the International Agency for Research on Cancer (IARC) of the World Health Organization in 2020, lung cancer is the second most common cancer and the first leading cause of death in the world, with 2.2 million cases and 1.8 million deaths^[1]. Non-small cell lung cancer (NSCLC) is the main type of lung cancer, ranking first in lung cancer, accounting for about 85 % of the total number of lung cancer^[2]. At present, surgery, radiotherapy and chemotherapy are still the main means of treating lung cancer, but the resulting adverse reactions seriously affect the healing of lung cancer patients. With the research and development of traditional Chinese medicine in recent years, traditional Chinese medicine and traditional Chinese medicine compound have a significant effect in the treatment of lung cancer. The traditional Chinese medicine Sheliugu is the dry tuber of Amorphophallus konjac or Amorphophallus konjac^[3]. It is warm and spicy, toxic,

and belongs to the lung, liver, and spleen meridians. It has the effects of resolving phlegm and removing blood stasis, softening and dispersing knots, detoxification and pain relief. The effective parts such as water extract, petroleum ether, ethyl acetate, n-butanol extract and so on can inhibit the growth of tumor^[4]. It is widely used in clinical practice, therapeutic effect is obvious. This experiment will study the effect of petroleum ether extract of Hexapoda on the proliferation and apoptosis of non-small cell lung cancer A549 cells from the cell point of view.

2. Information and methodology

Materials and reagents Human lung cancer A549 cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences; sheliugu decoction pieces were purchased from the pharmacy of our hospital; cisplatin (Tonghua Maoxiang Pharmaceutical Co., Ltd.); methyl thiazolyl tetrazolium (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma, USA. Apoptosis detection kit (Shanghai Jikai Biotechnology Co., Ltd.).

2.1 Experimental methods

Extraction of petroleum ether extract of *Amorphophallus amorphophallus* 100g dried *Amorphophallus amorphophallus* decoction pieces were weighed strictly and carefully, cut into coarse particles, soaked in 8 times the volume of 95 % ethanol overnight and reflux extracted twice. The extract was concentrated under negative pressure and extracted twice with 2 times the volume of petroleum ether, concentrated under negative pressure, evaporated and concentrated overnight in a petri dish, and dried under negative pressure vacuum to obtain the petroleum ether extract of *Amorphophallus amorphophallus*.

2.1.1 Culture of A549 cells

Human lung cancer A549 cells were resuscitated and cultured in a 37 °C saturated humidity, 5 % CO₂ incubator.

2.1.2 Experimental grouping processing

The concentrations of petroleum ether extracts of *Amorphophallus* were 100 µg / mL, 200 µg / mL, 300 µg / mL, 400 µg / mL, 500 µg / mL, and 600 µg / mL, respectively, as the experimental group (serpentine group). The concentrations of cisplatin in the control group were 1 µg / mL, 2 µg / mL, and 4 µg / mL, respectively, and the culture in the medium was used as the negative control group.

2.2 The inhibition rate of cell proliferation was detected by MTT assay.

The NSCLC A549 cells in the logarithmic growth phase were digested with trypsin and inoculated in a 96-well plate with a volume of 200 µL per well at a concentration of 5×10^5 cells / mL. The cells were cultured in a constant temperature cell incubator for 24 h. The supernatant was discarded and the petroleum ether extract of *Amorphophallus* was added at a concentration of (100,200,300,400,500,600 µg / mL) and cisplatin at a concentration of (1,2,4 µg / mL). The negative control group was added with the same amount of F12 culture medium, 200 µL per hole, and divided into 10 groups, with 5 holes in each group. The cells were cultured in the incubator for 24h, 48h and 72h respectively, and 20µL of 5mg / ml MTT solution was added to each well. The supernatant was discarded, 150µL DMSO was added, and the absorbance (OD) at 570 nm was measured by microplate reader. The cell inhibition rate (%) was calculated.

2.3 Detection of apoptosis rate by flow cytometry

Each group of human lung cancer A549 cells were digested with trypsin and planted in 6-well plates, 2ml per well. Two duplicate wells were designed for each group. After 24 hours of cultivation, petroleum ether extract (600 μ g / ml, 400 μ g / ml, 200 μ g / ml) and cisplatin (2 μ g / ml) were added respectively. The negative control group was not treated. The culture plate was taken out and washed twice with sterile PBS, and centrifuged at 800 r / min for 5 min. The centrifuged solution was carefully sucked out, and 1ml PBS solution was added for washing. The PBS supernatant was centrifuged, and the cells were fixed with 1ml 75 % ice alcohol. The cells were centrifuged at 4 $^{\circ}$ C and 800 r / min for 5 min, and the solution was taken to fully suspend the A549 cells in 3ml PBS solution. After filtration with a 400-mesh filter, the filtrate was collected, centrifuged at low speed for 5 min, and the solution was discarded. Add 0.1 % Ranse 1ml, add 1ml of 5ug / ml PI solution under the condition of 37 $^{\circ}$ C water bath box, 4 $^{\circ}$ C, avoid light staining for 0.5 hours. The apoptosis rate was detected by flow cytometry with excitation wavelength of 488 nm.

2.4 Statistical methods

SPSS22.00 statistical software was used for statistical analysis, and GraphPad Prism 9 software was used for data analysis and mapping. All measurement data were expressed in the form of (\pm s). F test and χ^2 test were used for comparison between multiple groups. LSD-t test was used for pairwise comparison between multiple groups. Correlation analysis showed that $P < 0.05$ was considered statistically significant.

3. Experimental results

3.1 Effects of petroleum ether extract of Amorphophallus on proliferation of human lung cancer A549 cells

The results of cell proliferation inhibition showed that compared with the control group, the petroleum ether extract of Amorphophallus had different degrees of inhibition on the proliferation of human non-small cell lung cancer A549 cells after 24 hours of administration. With the increase of the concentration of petroleum ether extract of Amorphophallus, the cell inhibition rate was higher, showing a better dose-effect curve (Fig. 1 and Fig. 2).

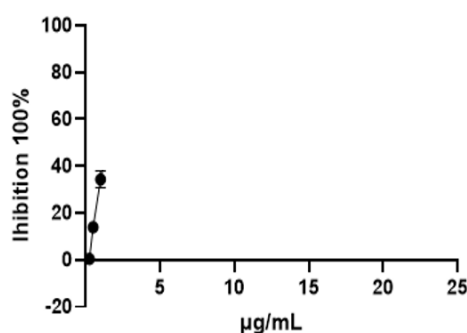
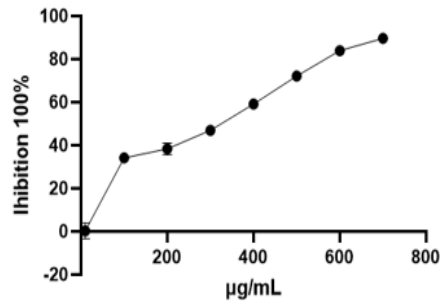


Figure 1: Control group



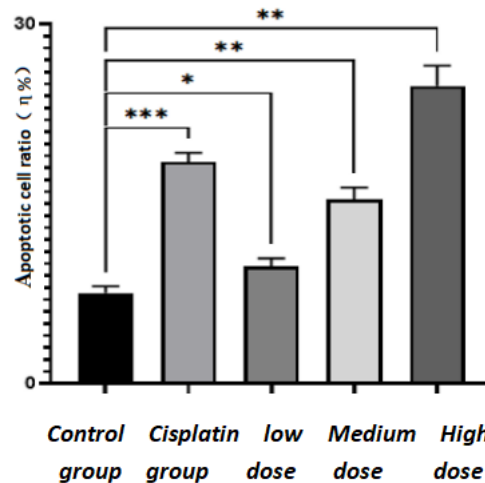
Note: Compared with the control group, * $P \leq 0.05$, $n = 10$.

Figure 2: Sheliugu petroleum ether extract group

Fig. 1 and Fig. 2 Effects of petroleum ether extract of Amorphophallus on proliferation of non-small cell lung cancer A549 cells

3.2 Effects of petroleum ether extract of *H.serrata* on apoptosis of human lung cancer A549 cells

The results of apoptosis experiment showed that the apoptosis rate of lung cancer A549 cells increased after 24 hours of petroleum ether extract of Amorphophallus (Fig.3), and the effect increased with the increase of drug concentration, showing a certain dose-effect relationship. Compared with the blank control group, there was a significant difference ($P < 0.05$), and with the increase of the concentration of petroleum ether extract, the apoptosis rate showed an increasing trend, suggesting that the petroleum ether extract of Sheliugu may have the effect of inducing the apoptosis of lung cancer A549 cells.



Note: Compared with the control group, * $P \leq 0.05$, $n = 5$.

Figure 3: Effect of petroleum ether extract of Amorphophallus on apoptosis of human lung cancer A549 cells

4. Discussions

Since ancient times, the records of tumors in traditional Chinese medicine literature have been involved, and there is a certain understanding of the name, etiology, pathogenesis, prevention and

treatment of tumors. As early as 3,500 years ago, the oracle bone inscriptions of the Shang Dynasty appeared, but at that time, there was only the word " tumor " without annotation. Han Dynasty Liu Xi 's ' Shiming ' said : ' tumor, flow, blood flow accumulation of tumor also. ' Modern doctors believe that the occurrence and development of cancer is a process of deficiency and toxicity. Deficiency of healthy qi is the root cause of tumor occurrence and development. When the body 's spleen qi is deficient, qi does not flow blood, blood stasis is blocked, body fluid does not melt, phlegm and dampness stagnation, phlegm and blood stasis can cause cancer. Phlegm and blood stasis are the pathological products formed in the process of disease and the main pathogenesis of tumor. Lung cancer has no such name in the literature of traditional Chinese medicine, but its clinical symptoms can be attributed to the categories of ' lung accumulation ', ' Xiben ', ' cough ' and ' lung atrophy ' in traditional Chinese medicine. The occurrence of lung cancer is caused by phlegm retention in the upper jiao, which hinders the lung from dispersing and descending. Therefore, cough, sputum, large amount, yellow or white tongue and other phlegm symptoms appear. The phlegm goes with the gas, so that the cancer toxin can spread to the whole body, but the characteristics of phlegm retention and viscosity make the cancer toxin easy to plant other organs. Obstinate phlegm should be soft. Sheliugu has the effect of resolving phlegm and removing blood stasis, softening and resolving hard mass, and has good curative effect on various malignant tumors ' phlegm dampness syndrome '.

From the perspective of traditional Chinese medicine, lung cancer belongs to the category of ' accumulation of lung and stagnation of stomach ' in traditional Chinese medicine. Deficiency of healthy qi is the basis of lung cancer, and phlegm and blood stasis are the key to the pathogenesis, and most of them are deficiency in origin and excess in superficiality^[5]. The main causes are internal deficiency of healthy qi and dysfunction of zang-fu organs. External factors including external evils, eating disorders, etc. can be incentives. Gradually lead to phlegm and blood stasis poison knot in the lung, over time and form a mass. In the pathogenesis of lung cancer, deficiency is the basis of lung cancer, phlegm and blood stasis are the key to the pathogenesis, and most of them are deficiency in origin and excess in superficiality. Many patients with lung cancer are mainly evil in the body in the early stage. The name of traditional Chinese medicine Sheliugu was first recorded in 'Zhejiang Pesticide Annals '. In the 'Chinese Herbal Medicine Dictionary ' and other documents, it is recorded by the alias of konjac, which belongs to the Araceae plant^[6]. The ancient book records that the snake Liugu, also known as the Konjac and the Konjac head, was first recorded in the Song Dynasty 's ' Kaibao Bencao ', Yun 's Konjac head (that is, the snake Liugu) was born in Wu and Shu, and the leaves were similar to the postscript and Pinellia, and the roots were as large as bowls and Yin. ' It tells the origin and shape of the drug. At the same time, it also records the main diseases, usage and decoction methods of the drug, such as the main carbuncle swelling and wind toxin, rubbing on the swelling. Mashed with ash juice boiled into a cake, five flavors to reconcile the food, the main thirst. 'After that, Songshu ' leizhengbencao ' recorded its taste, toxicity, attending diseases and specific usage, such as ' taste pungent, cold, toxic. Carbuncle swelling wind poison, Mo Fu Zhong Shang ' ^[7]. 'Zhejiang Pesticide Annals ' " Zhejiang Medicinal Plant Annals " believes that Sheliugu is the alias of *Arisaematis Rhizoma* and *Arisaematis heterophylla*. In Zhejiang Province, the medicinal part of *Arisaematis Rhizoma* is dry stem root, and its taste is pungent and warm. If *Arisaematis Rhizoma* is not decocted first, it will be decocted with other drugs, or improperly processed, and there will be toxic reaction after eating, which is similar to the characteristics of Sheliugu. Therefore, it can be considered that Sheliugu and *Arisaematis Rhizoma* are the alias of the same plant. The snake six grain has the effect of resolving phlegm and dispersing accumulation, removing blood stasis and detumescence. Modern pharmacological research has been widely used in the treatment of various malignant tumors^[8], and the clinical effect is remarkable. Yu Xiaoling^[9] et al. found that petroleum ether extract from *Agkistrodon acutus* could inhibit the proliferation,

promote apoptosis and induce differentiation of K562 cells through ERK signaling pathway. Lv Xiaokai^[10] is confirmed by experiments that Sheliugu inhibited the recurrence and metastasis of triple-negative breast cancer by intervening PI3K / Akt signaling pathway. Modern research has found that the traditional Chinese medicine Sheliugu has also played a very good role in the treatment process by combining with other traditional Chinese medicines. Cai Yong^[11] etc. selected Astragalus, Sheliugu, Hedyotis diffusa and other combined interventional treatment of breast cancer with liver metastasis in 40 cases, the final clinical improvement rate of up to 87.5 %. Professor Shu Qijin in the treatment of lung cancer patients stressed the clinical use of drugs, for patients with syndrome differentiation of phlegm and toxin, clinical often with Hedyotis diffusa-sheliugu as a drug pair, to clear heat and detoxify, detumescence Sanjie effect ^[12]. In the process of clinical treatment of lung cancer, the traditional Chinese medicine Sheliugu has the advantages of low price and easy access. It can not only improve the quality of life of lung cancer patients, but also extend the survival time of patients, but there is a lack of more experiments to confirm. In the future research, we should explore the mechanism of the treatment of non-small cell lung cancer from multiple angles, and provide the corresponding theoretical basis for clinical use. We need to make full use of the advantages of traditional Chinese medicine for the benefit of cancer patients.

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References

- [1] Xu Jing, Yu Xin, Ma Hongtao, et al. Trend analysis of lung cancer death in China from 2006 to 2020 based on age-period-cohort model [J]. *Tumor prevention and treatment research*, 2023, 50 (8): 788-793.
- [2] Broderick SR. Adjuvant and Neoadjuvant Immunotherapy in Non-small Cell Lung Cancer [J]. *Thorac Surg Clin*. 2020; 2: 215-220.
- [3] Pan Lei, Fang Meiling, Chen Peifeng. Experimental study on the apoptosis of human gastric cancer SGC-7901 cells induced by petroleum ether extract of traditional Chinese medicine Heliugu [J]. *Chinese Journal of Traditional Chinese Medicine*, 2012, 30 (6): 1259-1261.
- [4] Zhen Yan. Study on the anti-tumor efficacy of petroleum ether extract of *Amorphophallus hexandra* [J]. *Chinese Medicine and Clinical*, 2019, 19 (1): 151-152.
- [5] Cheng Quan, Fu Huazhou. Research progress on adjuvant treatment of non-small cell lung cancer with traditional Chinese medicine compound [J]. *Journal of Liaoning University of Traditional Chinese Medicine*, 2016, 18, (1):141-143.
- [6] Zou Wenyuan, Ge Xin, Fan Xiaoqiu, etc. Clinical application and mechanism of action of Sheliugu anti-tumor [J]. *World Traditional Chinese Medicine*, 2019, 14 (7): 1911-1914.
- [7] Xu Fei, Li Xuejun, Zhang Qiangqiang. The application of traditional Chinese medicine Sheliugu in the treatment of pancreatic cancer [J]. *Journal of Shaanxi University of Traditional Chinese Medicine*, 2022, 45 (1):132-136.
- [8] Chang Zhongfei, Chen Peifeng, Wu Qiaofeng, etc. Sheliugu petroleum ether extract column chromatography site to see the tumor active component screening again [J]. *Journal of Zhejiang University of Traditional Chinese Medicine*, 2011, 35 (5):753-755.
- [9] Yu Xiaoling, Zhao Yanna, Yin Liming, et al. The effect and mechanism of petroleum ether extract from *Agkistrodon acutus* on the biological characteristics of K562 cells [J]. *Chinese Journal of Experimental Hematology*, 2021, 29 (4):1028-1033.
- [10] Lv Xiaokai. Experimental study on the inhibition of recurrence and metastasis of triple-negative breast cancer by intervention of PI3K / Akt signaling pathway [D]. *Zhejiang University of Traditional Chinese Medicine*, 2013.
- [11] Cai Yong, Li Xiaoping. Jianpi Liqi Chinese medicine combined with interventional treatment of breast cancer liver metastasis in 40 cases [J]. *Shaanxi Traditional Chinese Medicine*, 2005, 26(6):491-493.
- [12] Xu Fei, Li Xuejun, Liu Yanfeng, etc. Application of Sheliugu in the treatment of lung cancer [J]. *Journal of Hubei University of Traditional Chinese Medicine*, 2021, 23(5):119-121.