Mechanism of Coixen Ester Inducing Apoptosis of Human Cervical Cancer Hela Cells

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Cervical cancer is a serious threat to women's health. In recent years, the incidence rate has increased year by year, and the incidence of cervical cancer tends to be younger. Because of its characteristics of easy recurrence and easy metastasis, the drug therapy of cervical cancer has attracted increasing attention. Coixen ester has anti-cancer and immunomodulatory effects. After years of clinical research, it has been shown to be effective against a variety of cancers and has a growth inhibitory effect on tumor cells. This article aims to study the mechanism of Coixen ester-induced apoptosis of human cervical cancer HeLa cells. This article puts forward what are the causes of cervical cancer, and analyzes the positive effects of traditional Chinese medicine in the prevention and treatment of cervical cancer. During the experiment, the MTT method was used to study the effect of the complex on the proliferation of HeLa cells. The experimental results in this article show that a certain concentration of Coixen ester solution can inhibit cell proliferation and can also reduce cell viability to about 0.35.

Keywords: Cervical Cancer, Coixan Ester, HeLa Cells, Cell Apoptosis, Cell Proliferation

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Nowadays, cancer has become the "first killer" that endangers the health of people in China and other countries. According to reports, in China alone, 2.2 million people die every year due to cancer. Since the 1990s, the number of cancer patients around the world has been on the rise, while the growth rate in developing countries is particularly fast, especially China is facing a war with cancer. Experts say that according to the current human living environment and social pressure, the number of cancer patients in China will continue to rise every year, and there will be tens of thousands of new patients every day after 2030, which will affect the overall quality of life of the society and reduce the human happiness index. Cervical cancer seriously endangers the health and lives of women in the world today, and the number of cervical cancer patients in China continues to increase. Approximately 500,000 new cases and 270,000 deaths occur each year. In recent years, due to the increase in human papillomavirus (HPV) infection, the incidence rate has increased at a rate of 2% to 3% per year, and the average age of onset has dropped from about 10 to 10 years old. Cervical cancer patients under 35 the number is gradually increasing. In the face of this severe situation, finding effective anticancer drugs has become one of the most pressing problems in the medical field today.

Based on the current severe situation and human health and safety considerations, it is of great practical significance to explore new and effective methods for the prevention and treatment of cervical cancer. In 2008, German scientists and two French scientists discovered that HPV virus is the main cause of cervical cancer. The first-generation preventive vaccines Gardasil and Cervarix vaccines have been approved for sale, which can prevent 70% of cervical cancer, but these two vaccines only target HPV16 and HPV18 high-risk HPV vaccines and are

not expensive. Due to the high incidence of adverse injection reactions, the second-generation vaccine is accelerating its participation in clinical trials, but the safety and immunogenicity of the vaccine need to be further improved. At present, it is safe to use HPV and there is no effective cure. Current cervical cancer treatment methods usually use traditional surgery, radiation therapy and chemotherapy, but these therapies may cause side effects and serious complications. Moreover, in recent years, patients with cervical cancer are getting younger and younger, and naturally they have a relatively high demand for quality of life after treatment. In addition, efforts must be made to ensure the normal operation of patients' reproductive endocrine and reproductive functions, which provide an effective basis and final indicators for evaluating the effectiveness and quality of cervical cancer treatment. Finding effective treatments and minimizing irreversible damage to patients during the treatment process has become the primary goal of many experts and scholars. Chinese herbal medicine is more and more important because of its unique advantages, such as being taken from nature, rich in resources, and less toxic and side effects than chemical synthetic drugs. Chinese medicine starts from the overall mechanism to prevent tumor recurrence and avoid the pain of chemotherapy, thereby improving the quality of life, especially for patients with advanced stages, regaining the desire to survive and prolonging the survival period. Therefore, screening and extracting high-efficiency anti-cancer components of traditional Chinese medicine, and strengthening exploratory and basic research on the anti-tumor effect of natural ingredients and their mechanisms will lay a good foundation for finding safer, effective and inexpensive anti-cancer drugs.

Lu Z is to study the regulatory effect of walnut ketone on the apoptosis of cervical cancer Hela cells and its molecular mechanism. Hela cells of cervical cancer were cultured and treated with different doses of juglone and c-Jun N-terminal kinase (JNK) inhibitor SP600125. Then detect the proliferation activity of the cells and the expression of JNK/c-Jun pathway molecules and apoptosis molecules in the cells for research. The disadvantage is that there are still some factors in the process of this research that have an impact on the experimental results [1]. Bai C significantly induces the apoptosis of cervical cancer Caski cells through the mitochondrial pathway. This study aims to further evaluate the effect of RCE-4 on human cervical cancer HeLa cells. Through the use of nuclear factor-kB (NF) to study the anti-cervical cancer effect of RCE-4, activation and key factors related to inflammation in HeLa cells. But through this study, it can only show that RCE-4 has a beneficial anti-cervical cancer effect on HeLa cells, and it does not explain how this effect is achieved [2]. HMQTF2 (F2) is a derivative of Taxpin, which has excellent anti-cancer activity in human cervical cancer. Dai, Yu's research aimed to evaluate the effect of F2 on the migration of HeLa cells in vitro. F2 inhibits the migration of HeLa cells by negatively regulating the Wnt signaling pathway and reversing EMT. F2 not only mediates the expression of Frizzled8, pLRP6 and LRP6, but also down-regulates the phosphorylation of GSK3β, and at the same time reduces the nuclear protein expression of MMP2, MMP3, MMP7, MMP9 and cMyc. But through these, it can only be considered that F2 may be a potential therapeutic agent against cervical cancer, which is still uncertain [3].

The innovation of this paper is (1) After culturing HeLa cells in vitro, and then treating them with coixen ester, the MTT method is used to study the effect of coixen ester on the proliferation of HeLa cells, and the optimal concentration ratio is screened, and then the isoline is used graphical analysis method to judge the anticancer effect of Coixen ester. (2) Taking death receptor-mediated extracellular apoptosis pathway and mitochondrial-mediated intracellular apoptosis pathway as research clues, the effect and mechanism of Coixen ester-induced HeLa cell apoptosis were preliminarily discussed.

RESEARCH METHODS OF THE MECHANISM OF COIXEN ESTER INDUCING APOPTOSIS OF HUMAN CERVICAL CANCER HELA CELLS

The Incidence of Cervical Cancer

With the acceleration of the pace of life of modern people and the changes in the surrounding environment, the etiology of cervical cancer has not

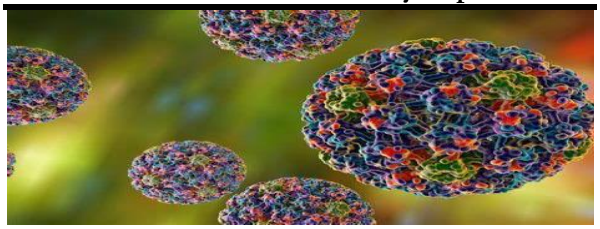
yet been fully clarified, and the uncertainty of many factors has become a stumbling block to overcome cervical cancer [4, 5]. After a preliminary analysis of the living habits and living environment of patients with cervical cancer, it was concluded that the factors leading to the onset of cervical cancer may include:

High-risk human papillomavirus (HR-HPV)

HPV is a double-stranded closed-loop small DNA virus without envelope [6]. The morphology of HPV is shown in Figure 1. Spread through close contact,

It can infect skin and epithelial tissues, cause verrucous hyperplasia, and lead to cancer [7].

Figure 1.
Morphology of HR-HPV (This picture is borrowed from Baidu Encyclopedia)



At present, about 100 types of HPV have been identified, roughly divided into two types: low-risk type and high-risk type [8, 9]. Humans are currently the only identified host of HPV, but it does not develop into cervical cancer once infected with HPV. For people infected with HPV virus, there is a 60% chance of self-healing. HPV can be eliminated through autoimmunity, about 6 months to 1 year; one third of infected people may have high-grade epithelial lesions due to defects in the defense mechanism. Only one in ten people may develop cervical intraepithelial neoplasia (CNI) or cervical cancer [10, 11]. However, persistent infection with high-risk HPV will lead to the occurrence of cancer, that is, the pathological process of cervical cancer is a long-term slow process. As long as attention is paid to prevention and screening, the occurrence of the disease can be effectively avoided [12].

Behavioral factors

Bad habits in daily life can also lead to the occurrence of diseases, such as multiple sexual partners, premature sex, multiple pregnancy, multiple pregnancy, smoking, improper use of contraceptives, etc., which are important risk factors for cervical cancer [13]. In addition, irregular diets or nutritional imbalances cause the body's immunity to decline and induce the occurrence of cervical cancer.

Genetic factors

Studies have shown that the occurrence of cervical cancer is not only related to the external environment and lifestyle, but also closely related to the individual's genetic background, with a certain degree of family aggregation [14].

Environmental factors

With the rapid development of modern industry and economy, changes in the ecological environment have affected people's daily lives, and have become a common concern of society, especially with the increasing risk of carcinogenic factors [15, 16]. For example, food additives, pesticides, polluted air and water, nitroso compounds and polycyclic carbohydrates and other chemical and radioactive substances can cause cancer in the body.

Treatment of Cervical Cancer

The current treatment methods for malignant tumors have their own characteristics, scope of application, and definite effectiveness in terms of a single treatment method; for different individual patients, choose the treatment that the patient can bear, and apply them in combination to achieve better results. Good treatment effect [17]. Surgical treatment is still the most effective method for the treatment of malignant tumors, namely Wertheim radical hysterectomy and pelvic lymphadenectomy, but because some patients find that the disease is late, the focus is too large, or there are metastases outside the pelvis, at this time it is no longer suitable for surgery. Chemotherapy is the most widely used tumor treatment method. Chemotherapy plays an important role in improving the survival of patients, but it kills cancer cells and affects normal cells at the same time [18, 19]. Radiotherapy plays an important role in the treatment of cervical cancer important role. Approximately 80% of cervical cancer patients require radiotherapy, and the treatment effect is satisfactory. Radiotherapy can delay recurrence and

relieve patients' pain, but it is not sensitive to the treatment of cervical adenocarcinoma, has irreversible destructive power to the ovaries and vagina, and is accompanied by complications after radiotherapy.

Biological therapy is most suitable for patients with multiple lesions and cervical cancer that has been extensively metastasized. It is mainly a therapy that uses and stimulates the patient's host individual's own immune system to inhibit cancer cells and ultimately kill them [20, 21]. Immunotherapy has become a new treatment model in the treatment of cervical cancer. This method uses molecularly targeted drugs for treatment, which can prevent the progression of tumors. It has a clear target and is uniquely targeted for specific cancer cells, but it has almost no treatment for normal cells. Influence [22, 23] for patients with advanced tumors, combining surgery with this therapy can improve the patient's quality of life and prolong survival.

Traditional Chinese medicine treatment pays attention to the theory of syndrome differentiation, adjusts the body's comprehensive functions from the overall level, and relieves clinical symptoms. Most of the tumor tissues are surgically removed first, and radiotherapy and chemotherapy are used as auxiliary methods to make the remaining small lesions disappear and improve immunity throughout the treatment [24, 25]. Therefore, looking for a safe, effective, and low-toxicity anti-tumor drug from natural herbal medicines has become a research hotspot for gynecological experts and scholars at home and abroad.

Prevention and Treatment of Cervical Cancer with Traditional Chinese Medicine

The mechanism of TCM treatment of lung cancer is reflected in:

Improve the immune function of the body

Enhancing immunity is a unique feature of traditional Chinese medicine. It can strengthen the body's immune function by strengthening the body to enhance the body's lethality against tumor cells [26, 27]. Studies have confirmed that the Chinese patent medicine Kanglaite injection enhances the body's immune function by promoting the proliferation of T cells and regulating the levels of factors such as IL-2 and NF-kB. In addition to directly acting on immune cells, traditional Chinese medicines that focus on strengthening the body can also enhance the body's immunity by enhancing the role and function of tumor killer cells and play an indirect anti-tumor effect [28].

Inhibit tumor cell proliferation or promote its apoptosis and differentiation

Cell cycle imbalance is one of the important causes of cell carcinogenesis. Traditional Chinese medicine exerts an anti-tumor effect by interfering with the normal cycle of tumor cells [29, 30]. The regulation of apoptosis is mainly achieved by blocking the cell cycle and regulating apoptotic genes.

Inhibit tumor angiogenesis

The formation of new blood vessels is necessary for tumor cell growth and proliferation, especially for highly malignant lung cancer cells. New blood vessels provide energy support for their proliferation, metastasis and invasion, and increase their malignancy. Shenmaiye can reduce the migration ability of tumor vascular endothelial cells by down-regulating the level of tumor cell MMP-2 and up-regulating the level of TIMP-1. Biejia Jianwan contains a large number of traditional Chinese medicines for promoting blood circulation, removing blood stasis and dissipating masses. Studies have shown that it can reduce the expression of VEGF in tumor masses of tumor-bearing mice, and has a corresponding dose-effect relationship. Therefore, traditional Chinese medicine can inhibit tumor blood vessels by inhibiting the activity of protein degrading enzymes and inhibiting vascular growth factors. In addition, the drugs that inhibit tumor angiogenesis are mostly traditional Chinese medicines for promoting blood circulation and removing blood stasis, which fully embodies the essence of activating blood circulation and removing blood stasis.

Intervene in cell signaling pathways

Many signal pathways related to cancer stem cells

are related to the self-renewal ability of a variety of tumor cells. Cancer stem cells have the characteristics of clonal proliferation, migration and drug resistance. Therefore, the discovery of specific markers related to lung cancer, including related proteins and signal pathways, and inhibiting these related proteins and markers can indirectly inhibit the growth and proliferation of tumor stem cells, thereby inhibiting the development of cancer.

Inhibit the metastasis and invasion ability of malignant tumor cells

Cancer cell infiltration and metastasis refers to the phenomenon that cancer cells in the main location are metastasized to other organs or tissues through lymphatic metastasis, blood metastasis, implant metastasis or direct metastasis, and it is still growing. It is the most important malignant tumor organism academic characteristics.

MECHANISM OF COIXEN ESTER INDUCING APOPTOSIS OF HUMAN CERVICAL CANCER HELA CELLS

Experimental Materials

Experimental subjects

Human Cervical Cancer HeLa Cells (purchased from Cancer Hospital Cell Bank)

Test equipment

BHC-1600IIA/B3 biological safety cabinet; B600A medical low-speed centrifuge; CT-2J-A autoclave; HH.W21.420AS water bath box; HERO-85 ultra-low temperature refrigerator; Sartorius electronic scale; Parafilm sealing Membrane; IMT2 phase contrast microscope; ST-360 automatic multifunctional microplate reader; MCO-175 carbon dioxide incubator; TS-1000 decolorizing shaker shaker.

Test reagent

Premium fetal bovine serum; Dmem/High Glucose medium; DMEM medium; special grade fetal bovine serum; trypsin digestion solution (with 0.02% EDTA); dimethyl sulfoxide (DMSO); penicillin-streptomycin solution; PBS ; Trypan Blue; MTT; Coixen ester.

Test Method

Reagent preparation

Prepare trypan blue solution

Weigh 0.2 g of trypan blue powder, dissolve it in 5 ml of ultrapure water, add water while stirring, let it stand for a few minutes before use, and store the remaining part at 4°C. Before use, it was diluted with PBS buffer into a trypan blue solution with a concentration of 0.4%, and filtered with filter paper before use.

Preparation of various concentrations of drugs

The lyophilized coixen ester powder was dissolved in DMSO, prepared as a 30mg/ml stock solution in a sterile environment, filtered and sterilized with a filter with a pore size of 0.22μm, and stored at -20°C for later use. In all Coixen ester medication groups, they were diluted with sterile medium to ensure that the concentration of DMSO was less than 1.0%. The mother liquors with final concentrations of Coixen esters of 25μg/ml, 50μg/ml, 75μg/ml, 100μg/ml, and 150μg/ml were prepared for later use.

Test Grouping

In order to explore the effect of coixen ester, this test is divided into no coixen ester group, 25μg/ml coixen ester group, 50μg/ml coixen ester group, 75μg/ml coixen ester group, 100μg/ml coixen ester group, 150μg/ml Coixen ester group. There are 5 groups in the experimental group, and the negative control group is sterile complete medium.

MTT Method to Detect Cervical Cancer HeLa Cells Proliferation

The MTT method was used to detect the inhibitory effect of Coixen ester on the growth of HeLa cells. MTT analysis is based on MTT in living cells. As an experimental method to detect cell viability or drug toxicity, tetramethylazolyzole is a dye that can accept hydrogen atoms. Mitochondrial enzymes have a strong reducing ability and can reduce MTT to formazan. This is a blue-violet crystal, hardly soluble in water, and soluble in organic solvents. Since this reaction process only occurs in living cells, the number of crystals produced is proportional to the number of living cells, and the difference between living and dead cells

can be estimated. After dissolving the formazan deposited in the cells, the absorbance value (A570) at the wavelength of 570 nm is detected by the microplate reader, and the absorbance value is used to characterize the number of living cells. Using this principle to measure the change in absorbance value (OD value) of cervical cancer HeLa cells treated with coixen ester, and calculate the survival rate of HeLa cells, thereby predicting the sensitivity of HeLa cells to coixen ester and judging anti-tumor drugs Killing effect on HeLa cells.

MTT was used to detect the effect of coixen ester on the proliferation of cervical cancer HeLa cells. The specific operations are as follows:

(1) Inoculate HeLa cells in logarithmic phase into 96-well culture plates, select 2 wells on each plate, add 200μl medium, do not inoculate the cells in blank wells, disinfect the surrounding wells, and fill the liquid with PBS;

(2) After culturing in an incubator for several hours, wait for HeLa cells to attach to the wall, aspirate the old medium, and add 100μl of Coixen ester. There are 6 parallel holes for each concentration. After 24 hours of incubation, 10μl of each MTT solution was added to the wells.

(3) After incubating in a carbon dioxide incubator at 37°C for 4 hours, add 100μl of formazan solution and shake at room temperature for 10 to 20 minutes at low speed until all the purple crystals are dissolved.

(4) When using a microplate reader, perform two-wavelength detection (490nm, 630nm) on the OD value of each well to calculate the drug growth inhibition rate in HeLa cells.

(5) Draw a regression curve based on the inhibitory rate of each drug concentration point on HeLa cell proliferation, and calculates the half inhibitory concentration from the regression curve.

Cervical Cancer HeLa Cells Viability Detection

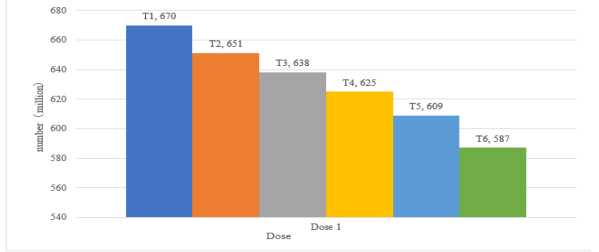
Cell viability is an important indicator to measure cell status. In this test, trypan blue staining is used to judge cervical cancer HeLa cell viability. The biggest difference between dead and living cells is the integrity of the cell membrane. Living cells are colorless and transparent, in sharp contrast with blue dead cells. The number of dead cells and living cells is counted according to different colors to obtain cell viability. Using trypan blue staining to detect the changes in cell viability of HeLa cells under the action of coixen ester, the specific operations are as follows:

(1) After calculating the cell viability, inoculate the logarithmic HeLa cells into a 24-well culture plate, wait for the wall to attach, aspirate from the old medium, and add 0.5ml of coixen ester solution, 6 in each group. Create a negative control group and add the same amount of medium.

(2) After stimulating the HeLa cells with drugs for 24h and 48h, aspirate the old solution, digest and centrifuge, add 1ml medium to resuspend the cells, and use the suspension as the mother solution for later needs;

(3) Remove the clean platelet counting plate and cover, and then place the cover to completely cover the measurement area.

Figure 2.
The number of cells in each time period at a concentration of 25μg/ml



(4) Gently press the cells until the cells are evenly mixed. Take out the cell suspension and the blue diamond stain at a ratio of 1:1. After mixing, take 20μl of the mixed solution, pour it into the measuring range, and then let it stand for 2 minutes.

(5) Measure the number of dead cells and live cells under an optical microscope, and calculate cell viability.

MECHANISM OF COIXEN ESTER INDUCING APOPTOSIS OF HUMAN CERVICAL CANCER HELA CELLS

The Effect of Coixen Ester on HeLa Cell Proliferation Inhibition

After the log phase HeLa cells were treated with coixen ester for 24 hours, the MTT method was used to detect the effect of coixen ester on the proliferation of HeLa cells. The results showed that compared with the negative control group, with the increase of the drug concentration in the Coixenolide group, the drug's growth inhibition rate on HeLa cells also increased, and the final concentration of 150μg/ml Coixenolide on HeLa cells was up to (67.28±3.56) %; therefore, Coixen ester has a significant inhibitory effect on the proliferation of HeLa cells. Table 1 shows the effect of coixen ester in different concentration groups on the proliferation of HeLa cells.

Table 1.
The effect of coixen ester on the proliferation of hela cells

Group	Concentration(MG/Ml)	OD Value	Inhibition Rate (%)
Control Group	0	1.17±0.08	/
Dose 1	25	1.01±0.05	13.17±5.09
Dose 2	50	0.74±0.04	31.41±6.08
Dose 3	75	0.57±0.04	48.55±4.77
Dose 4	100	0.45±0.03	60.59±5.21
Dose 5	150	0.37±0.02	67.28±3.56

The Effect of Coixen Ester on HeLa cell Activity

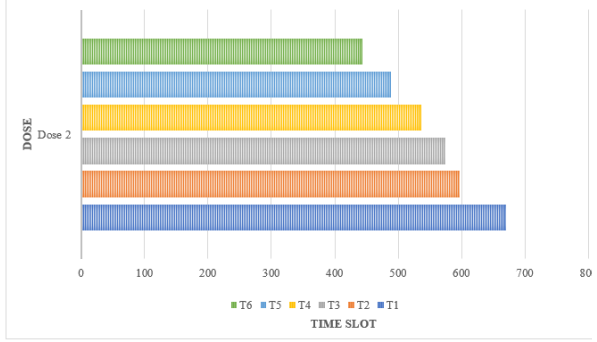
Observe the number of apoptosis in different time periods under the same concentration of Coixen ester in different experimental groups. The test process is 24 hours in total, divided equally into six time periods T1, T2, T2, T3, T4, T5, and T6 for observation. Under the action of 25μg/ml coixen ester, the number of cells in six time periods is shown in Figure 2.

Under the action of a concentration of 50μg/ml coixen ester, the number of cells in six time periods is shown in Figure 3.

Comparing the changes of cell number under the action of 25μg/ml and 50μg/ml concentration of coixen ester, it can be found that at 25μg/ml and 50μg/ml concentration, the inhibitory effect of coixen ester on HeLa cells is not obvious. In order to observe more clearly, directly compare the number of cells at a concentration of 25μg/ml and a concentration of 150μg/ml and Figure 4 can be obtained.

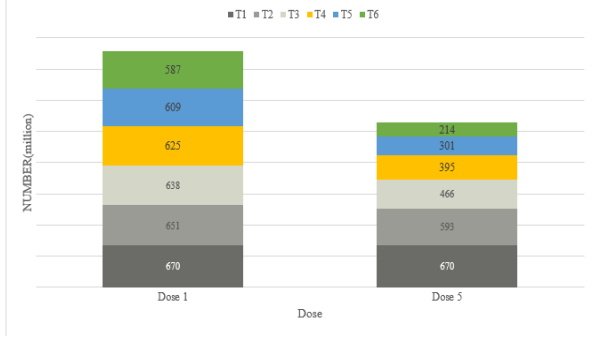
After coixene ester intervenes in cervical cancer HeLa cells, trypan blue staining was used to detect the changes in cell viability. The results showed that the HeLa

Figure 3.
The number of cells in each time period at a concentration of 50μg/ml



cell viability in the negative control group did not change due to changes in time, and remained basically unchanged; the effect of coixene ester after 24h, the cell viability of each dose group decreased, and the HeLa cell viability of the high-dose group decreased to 0.38±0.07. Compared with the

Figure 4.
The number of cells in each time period at a concentration of 25μg/ml and a concentration of 150μg/ml



negative control group, the low-dose, middle-dose and high-dose groups had a higher degree of cell activity reduction after 24 hours treatment than the 24 hours treatment group. In short, Coixen ester has a significant inhibitory effect on the activity of HeLa cells.

CONCLUSIONS

In this study, in vitro experiments were conducted to study the effects of coixen ester on cervical cancer and its mechanism, focusing on exploring the effects

of coixen ester on the proliferation of HeLa cells from two aspects: cell activity and cell proliferation inhibition. The research conclusions are as follows: (1) 150μg/ml at this concentration, the inhibitory ability of coixen ester on cervical cancer HeLa cells is stronger; and at this concentration, the coixen ester has a certain effect in inhibiting the proliferation of cervical cancer HeLa cells. (2) Trypan blue staining method to detect the viability of HeLa cells. After coixene ester acts on HeLa cells, the activity of HeLa cells decreases to about 0.35 compared with the negative control group. Coixene ester can effectively inhibit the activity of HeLa cells and has Time-dose dependent. (3) The MTT method detects the effect of the complex on the growth inhibition of HeLa cells. The growth inhibition rate of HeLa cells increases with the increase of drug action time and concentration, which is consistent with the results of the HeLa cell viability detection test. Therefore, this study believes that coixen ester has an anti-cervical cancer effect, which not only inhibits the proliferation of HeLa cells, but also promotes the transmission of apoptotic signals through the mitochondrial pathway and death receptor pathway, and ultimately activates the Caspase cascade and cell development Apoptosis. This study conducted a preliminary exploration of the mechanism of coixen ester against cervical cancer, and provided a scientific basis for screening effective anti-tumor active ingredients in traditional Chinese medicine.

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