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EFFECT OF ETHANOL EXTRACT OF *SELAGINELLA DOEDERLEINII HIERON* ON THE PROLIFERATION OF NASOPHARYNGEAL CARCINOMA CNE-1 AND C666-1 CELLS

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Abstract

This paper mainly studied the effect of ethanol extract of *Selaginella doederleinii Hieron* on the proliferation of two kinds of nasopharyngeal carcinoma cell lines, CNE-1 and C666-1, and their mechanisms of action. Extract was obtained by heat reflux extraction with ethanol, and the effect of extract on the extracellular matrix adhesion of the cells, on their proliferation process, as well as on their colony-forming ability were tested using MTT assay. The results showed that the yield of dry extract was 36.4%. 2.5 g/ml extract in the high concentration group exhibited inhibitory activity that was directly proportional to the concentration on CNE-1 cells, while not exhibiting obvious proportional trend in respect with C666-1 cells. However, the inhibition rates against two types of cells can both reach between 30%~50%. Under the effect of ethanol extract of *Selaginella doederleinii Hieron*, proliferative capacities of C666-1 and CNE-1 cells were affected to some extent.

Keywords: *Selaginella doederleinii Hieron*; Ethanol extract; CNE-1; C666-1

Introduction

Nasopharyngeal carcinoma (NPC) is a common malignancy originating in the head and neck region, with especially higher incidence in South China (Jiang et al, 2011). For the treatment of NPC, combined chemotherapy and radiotherapy may have better efficacy. But cisplatin, one of the chemotherapy drugs, has greater side effects on the immune system. Therefore, we tried to isolate active drug components from Chinese medicines that have greater inhibitory effect on the two NPC cell lines (Zeng, 2011). Among the commonly used commercialized Chinese medicines, Biyan Qingdu Granule, Compound Tianxian Capsule, Yiyiren and Zhongjiefeng can decrease the G_2/M phase cell ratio, and increase the G_0/G_1 phase cell ratio, thus reducing the mitotic cells, and inhibiting the growth of NPC cells (Kang, 2008). Based on a variety of Chinese medicines that are effective on NPC, this study also conducted similar experimental study on Chinese medicine *Selaginella doederleinii Hieron*.

Materials and Methods

Preparation of crude extract (Song et al, 2011)

About 150 g of *Selaginella doederleinii Hieron* (which was identified by professor Ma FH, and the specimen placed in the lab center) was weighted, and then pulverized into powder using an electric grinder. 50 g of the powder was accurately weighed, placed in a 1000 ml round-bottomed flask, and then extracted by heat reflux extraction with ethanol. For the specific methods, powder was extracted for 45 min with 8-fold volume of 95% ethanol three times, and the resulting three ethanol extracts were combined and filtered to obtain a filtrate. After ethanol was removed by rotary evaporation, dry extract was obtained. Dry extract yield was calculated as follows: Dry extract yield = actual weight of dry extract (g) / 50g.

Preparation of drug solution

Before the experiment, the ethanol extract of *Selaginella doederleinii Hieron* was diluted into crude extract solutions of three different concentrations of 2.5 g/ml, 1.25 g /ml and 0.625g/ml with sterile saline for later use. In 96 well plates, each concentration group had 5 replicate wells. Drug dosage in each well was 10 μL, and then 90 μL of fetal calf serum medium was added to each well.

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Cells and in vitro cultivation (Wu et al, 2012; Yang et al, 2012; Zhang, 2011)

NPC CNE-1 and C666-1 cell lines, purchased from Shanghai Aiyan Biotechnology Co., Ltd., were seeded in 10% FCS-containing RPMI-1640 medium (Gibco), and cultured in an incubator set at 37 ℃, 5% CO_2, with saturated humidity. The medium was replaced every 2 days, and cells in logarithmic growth phase were collected. When the cells covered the bottom of culture flask, they were digested with trypsin, seeded in 96-well plates in a concentration of 2×10^6 cells/ml with 100 μL per well, and cultured for 48 h. The cells were observed using an inverted microscope, and then drug addition was prepared.

The total volume of each well was 200 μL. 5 parallel wells were set up for each drug concentration. After cultivation was continued for additional 12 h, 24 h, 48 h and 72 h, the supernatant was removed. 100 μL of freshly prepared MTT (5 mg/ml) solution were added to each well (4 h before the end of each culture time). After incubation at 37℃ for 4 h, cultivation was stopped. Supernatant was discarded and 200 μL of DMSO was added to each well. After the crystals were fully dissolved, absorbance was measured at 490 nm wavelength using an automatic ELISA reader; readout was set to zero by adjusting the blank control, and the cell growth inhibition rate was calculated thus: inhibition rate = (1 - average OD of the experimental group / average OD of the control group).

Determination of colony forming ability (Wang et al, 2012)

24 h after cell treatment by 2.5 g/ml ethanol extract of *Selaginella doederleinii Hieron*, cells were seeded into 35 mm diameter plates in a concentration of 450 cells / plate and cultured for 2 weeks. Then, cells were stained with Wright's Giemsa and counted under the microscope. Colony number should be greater than or equal to 50.

Results

Dry extract yield

The amount of dry extract obtained by ethanol heat reflux was 18.2 g; so, the dry extract yield was 36.4%.

Inhibition rate of ethanol extract against CNE-1 cells (Zhou et al, 2010)

It can be seen from the figure and table below that the higher the concentration of aqueous solution of the extract, the more obvious the inhibitory effect. The inhibitory effects on the cells were very good under two concentrations of 2.5 g/ml and 1.25 g /ml and were in the rising and stable two phases within 48 h. Yet, the extract still had some inhibitory effects between 48 h and 72 h. However, it is apparent from the figure that extract with a concentration of 0.625 g/ml basically had no inhibitory effect on cells.

Table 1: Inhibition rate of ethanol extract against CNE-1

Time(h)	2.5g/ml	1.25g/ml	0.625g/ml
12	40.4	23.1	5.2
24	48.7	25.8	5.5
48	52.5	26.9	4.1
72	46.2	21.6	4.2

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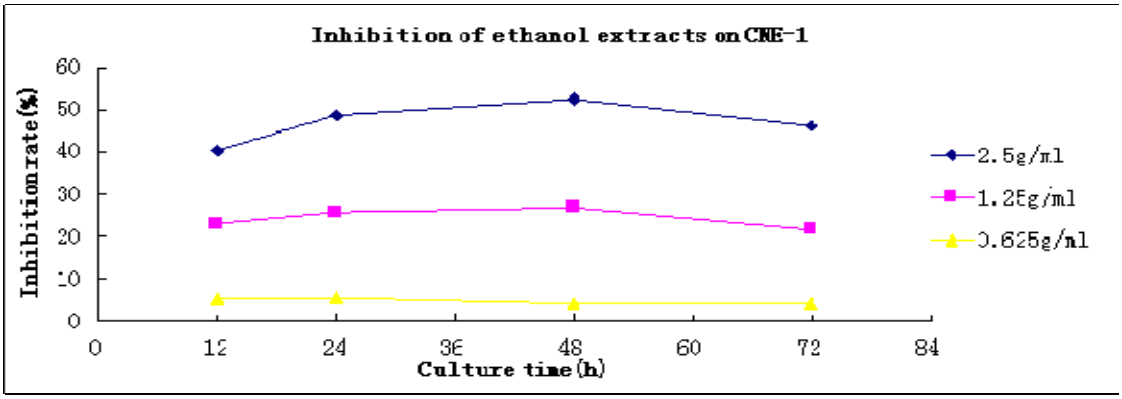

Figure 1: CNE-1 inhibition rate - culture time diagram

Inhibition rate of ethanol extract against C666-1 cells (Chen et al, 2009)

Table 2: Inhibition rate of ethanol extract against C666-1

Time(h)	2.5g/ml	1.25g/ml	0.625g/ml
12	28.4	14.3	3.2
24	35.1	10.5	3.5
48	43.6	8.8	3
72	37.2	9.1	2.8

It can be seen from figure 2 and table 2 that the concentration of aqueous solution of the extract was not obviously proportional to its inhibitory effect. The extract had a very good inhibitory effect on cells only under a concentration of 2.5 g/ml. Moreover, the inhibitory effect was in the rising and stable two phases within 48 h. Yet, the extract still had some inhibitory effect between 48 h and 72 h. Extract with a concentration of 1.25 g/ml had a very small inhibitory effect on cells, and the drug solution with a concentration of 0.625 g/ml basically had no inhibitory effect on C666-1 cells.

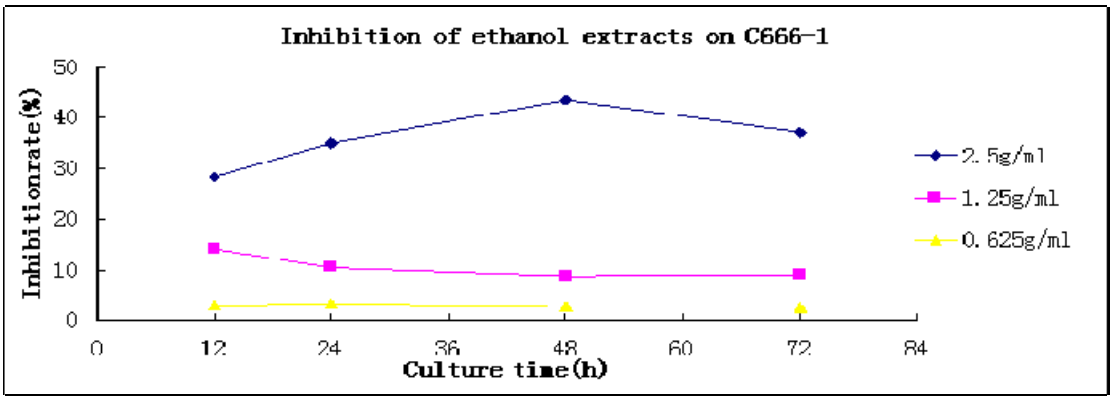

Figure 2: C666-1 inhibition rate - culture time diagram

Test of colony-forming ability

24 h after CNE-1 cell treatment by 2.5 g/ml aqueous solution of ethanol extract, 7 colonies were formed, while the CNE-1 cells in the corresponding control group (not treated by the ethanol extract) formed 85 colonies, indicating that 2.5 g/ml ethanol extract of *Selaginella doederleinii Hieron* inhibited the colony formation ability of tumor cells to some extent. In contrast, high-concentration aqueous solution of the ethanol extract had relatively weak effect on the colony formation ability of C666-1 cells. The number of colony of the high concentration experimental group was 25, while the number of colony of the control group was 92, suggesting that the ethanol extract of *Selaginella doederleinii Hieron* was not very effective on colony-forming ability of C666-1 cells when its concentration was 2.5 g/ml.

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Discussion

This study focused primarily on two types of cells in the NPC cell lines: C666-1 and CNE-1. Under the effect of Chinese medicine *Selaginella doederleinii Hieron* extract, their proliferative capacities were affected to some degree. Overall, CNE-1 cells have higher sensitivity to *Selaginella doederleinii Hieron* than the C666-1 cells, and are more greatly inhibited. We know that cancer treatment needs simultaneous radiotherapy and chemotherapy, but the xerostomia after radiotherapy for NPC seriously affects patients' quality of life (Xia et al, 1988). Chinese medicine can stimulate saliva production, thus improving xerostomia after radiotherapy for NPC (Zhang et al, 2011). Chinese medicines can not only improve the efficacy of radiotherapy to extend the life cycle and improve the quality of life but can also reduce the toxic reactions brought about by radiotherapy, such as the inflammatory responses in the body induced after radiotherapy, including the decline in leukocyte count, and the damaged immune system (Hou et al, 2009). Further studies on anticancer activity and anti-nasopharyngeal carcinoma mechanism of *Selaginella doederleinii Hieron* concerned in this study may mainly focus on studying the activity of monomer compounds extracted from Chinese medicine species and their anticancer mechanisms.

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