

## The effects of Zhikang capsule on the growth and apoptosis of breast cancer MCF-7.

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### Abstract

**Objective:** To investigate the effect of the Zhikang capsule on the growth and apoptosis of breast cancer cell line MCF-7.

**Method:** MTT method was used to detect the growth situation of normal breast cancer MCF-7 cells at 24 h, 48 h and 72 h after treatment by Zhikang capsule and the effects of Zhikang capsule on cell cycle of MCF-7 cells were detected by flow cytometry using flow cytometer. The effects of 24 h, 48 h and 72 h after treatment by Zhikang capsule on the growth of normal breast cancer MCF-7 cells were measured by flow cytometry; the effect of Zhikang capsule on apoptosis of MCF-7 cells was detected by PI single staining flow cytometry.

**Result:** Different concentrations of Zhikang capsule could effectively inhibit the growth of MCF-7 cells, and with the increase of concentration and extension of action time, the cell growth inhibition rate increased. The MCF-7 cells were effect for 72 h by the 0.5, 1, 2.0 mg/ml of the Zhikang capsule, and the proportion of G0/G1 phase cells in cell cycle was  $19.33 \pm 10.38\%$ ,  $14.12 \pm 5.37\%$  and  $26.84 \pm 2.13\%$ ; While the control group G0/G1 phase ratio was  $51.83 \pm 1.90\%$ . The apoptosis rate of the cells were  $2.09 \pm 0.74\%$ ,  $3.84 \pm 0.78\%$  and  $5.35 \pm 0.83\%$  respectively treated by the 0.5, 1, 2.0 mg/ml of the Zhikang capsule.

**Conclusion:** The Zhikang capsule could inhibit the growth of MCF-7 cells *in vitro*, and the inhibitory effect showed the time effect and dose effect relationship; and it could promote the apoptosis of breast cancer cells by blocking the cell cycle in the G2/M phase.

**Keywords:** Breast cancer, Zhikang capsule, Cell growth, Cell cycle.

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### Introduction

The Zhikang capsule was composed by 14 kinds of traditional Chinese medicines including Rhubarb, *Coptis chinensis*, pseudo-ginseng, *radix angelicae*, donkey-hide gelatin, fossil fragments, common *Bletilla* tuber, stir-baked Myrrha with vinegar, cuttle-bone, madder, *resina draconis*, liquorice, pearl, borneol. It had the function of heat clearing and hemostasis, *Qufushengji*, relieving pain, promoting tissue repair and shorten the time of wound healing. It was more common to find the skin flap necrosis, infection and subcutaneous effusion after modified radical mastectomy for breast cancer. Once the wound healing time was prolonged, it would bring unnecessary pain to patients, prolong the length of stay, increase the economic burden and delay the implementation of other treatment options. Over the years, surgeons have been actively looking for effective ways to promote wound healing. Through the literature we knew that Zhikang capsule can promote the healing of the wound, but it was rarely used in skin flap in patients with breast cancer healing reports. In this study, *in vitro* culture of breast cancer

cell line MCF-7 cells were as the research object, then we observed the effect of the Zhikang capsule on the growth of MCF-7 cells, and it provided a new treatment for breast cancer patients with wound healing.

### Materials and Methods

#### *Cell lines, experimental drugs and reagents*

MCF-7 human breast cancer cell line was presented by Cancer Research Institute of the First Affiliated Hospital of Xi'an Jiao Tong University. Zhikang capsule was purchased from Xi'an Millennium Pharmaceutical Co., Ltd. Fetal bovine serum was purchased from Sijiqing Zhejiang Tianhang Biological Technology Co., Ltd.

H-DMEM was purchased from Hyclone Company. MTT and DMSO were purchased from Sigma Company. Annexin-V-FITC/PI apoptosis detection kit was purchased from beyotime Biotechnology Research Institute.

### Detection the effect of Zhikang capsule on the growth of MCF-7 cells by MTT method

MCF-7 cells were cultured in H-DMEM culture medium containing 10% fetal bovine serum under the condition of 5% CO<sub>2</sub> and 37°C. Liquid was changed 1 time every 24 h. MCF-7 cells were digested with 0.25% trypsin solution during the logarithmic growth phase. The experimental group were respectively added 0.5, 1, 2.0 mg/ml of the Zhikang capsules 200 µl, the control group were added the same amount of culture fluid, then each hole was added MTT (5 mg/ml) 20 µl after the culture of 24 h, 48 h, 72 h. Each hole was added with 150 µl DMSO, and oscillated for 10 min, and the value was measured by the enzyme standard instrument in the 490 nm absorbance (A). The experiment was carried out 2 times, and taken the average (A) value of the 2 results. Cell growth inhibition rate=(control group A value-treatment group A value)/control group A value × 100%.

### Detection the effect of Zhikang capsule on cell cycle of MCF-7 cells by flow cytometry

MCF-7 cells were inoculated in the culture bottle, and divided into control group and 0.5, 1, 2.0 mg/ml of the capsule groups. The control group was added with an equal amount of 10% fetal calf serum containing high glucose DMEM complete medium. Cell cycle distribution was analyzed by computer software and flow cytometry.

### Detection the effect of on the apoptosis of MCF-7 cells induced by Kang capsule by Annexin-V-FITC/PI double labelled fluorescent staining

MCF-7 cells were inoculated in the culture bottle, and divided into control group and 0.5, 1, 2.0 mg/ml of the capsule groups. Each tube was added 195 µl Annexin-V-FITC binding buffer solution and 5 µl PI solution. It was detected by Flow cytometry, and the data was processed and printed by computer. The experiment was repeated 3 times.

### Statistical methods

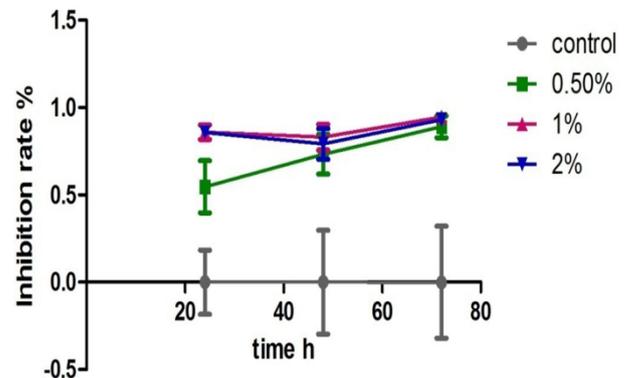
The data was processed by SPSS13.0 statistical software, and the measurement data was showed by  $\bar{x} \pm s$ . One way ANOVA was used to analyze the difference between the two groups, and the difference was statistically significant with  $P < 0.05$ .

## Results

### Inhibitory effect of Zhikang capsule on the growth of MCF-7 cells

Different concentrations of the Zhikang capsule could effectively inhibit the growth of MCF-7 cells. At the same time of action (24 h, 48 h, 72 h), with the increase of the concentration of the drug, the inhibition effect on the growth of MCF-7 cells was gradually increased ( $P < 0.05$ ) (Table 1). It was no obvious inhibitory effect on cells when the concentration of Zhikang capsule was less than 0.5 mg/ml.

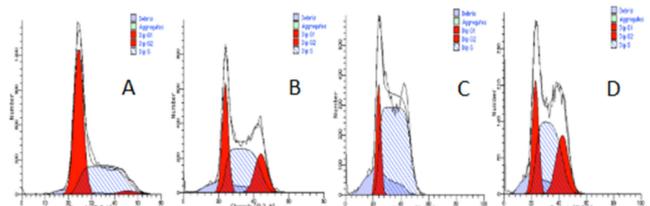
There was a small amount of living cell growth after 24 h of training when the concentration of Zhikang capsule was more than 4.0 mg/ml (Figure 1).



**Figure 1.** The effect of different concentrations of the Zhikang capsule on the growth inhibition rate of MCF-7 cells.

### Detection the effect of Zhikang capsule on cell cycle of MCF-7 cells by flow cytometry

Detection results of cell cycle showed that the cell cycle distribution of cells was changed and the cycle of MCF-7 cells was significantly blocked after treated by the concentration of 0.5, 1, 2.0 mg/ml of the Zhikang capsule. The S phase of 2.0 mg/ml high concentration group of Zhikang capsule increased from  $44.85 \pm 2\%$  to  $57.53 \pm 6.57\%$  ( $P < 0.05$ ) and the G2/M phase increased from  $3.32 \pm 2.51\%$  to  $16.66 \pm 8.51\%$  ( $P < 0.05$ ). The difference was statistically significant. The proportion of cell cycle in G0/G1 phase was  $19.33 \pm 10.38\%$ ,  $14.12 \pm 5.37\%$  and  $26.84 \pm 2.13\%$ . The proportion of G0/G1 phase in the control group was  $51.83 \pm 1.90\%$ . There were significant differences in the proportion of cell cycle of G0/G1 phase cells in different concentrations of the Zhikang capsule group compared with the control group ( $P < 0.05$ ) (Figures 2A-2D).



**Figure 2.** Detection the effect of Zhikang capsule on cell cycle of MCF-7 cells by FCM. (A) Treated with 0.1% DMSO; (B-D) Treated with Zhikang capsule (the concentration was 0.5, 1, 2.0 mg/ml).

### Detection of apoptosis of breast cancer cells by Annexin-V-FITC/PI double staining flow cytometry

After treatment 48 h by Zhikang capsule, the apoptosis rate of the cells was significantly increased.

The apoptosis rate was  $2.09 \pm 3.84\%$ ,  $0.74 \pm 0.78\%$  and  $5.35 \pm 0.83\%$  after treatment of 0.5, 1, 2.0 mg/ml Zhikang capsule for 48 h.

**Table 1.** The effect of different concentrations of the Zhikang capsule on the proliferation inhibition rate of MCF-7 cells.

Drug concentration	Cell proliferation inhibition rate (%)		
	24 h	48 h	72 h
Blank control group	0.000 ± 0.184	0.000 ± 0.297	0.000 ± 0.320
Zhikang capsule (mg/ml)			
0.5 mg/ml	0.547 ± 0.150*	0.733 ± 0.114*	0.890 ± 0.628*
1.0 mg/ml	0.859 ± 0.417*	0.830 ± 0.744*	0.945 ± 0.306*
2.0 mg/ml	0.858 ± 0.192*	0.792 ± 0.875*	0.930 ± 0.366*
4.0 mg/ml	-	-	-

\*Compared with the control group P<0.05.

## Discussion

The anti-tumor mechanism of traditional Chinese medicine has many aspects. Such as, inhibition of DNA synthesis, inhibition of tumor angiogenesis, inducing cell differentiation, promoting cell apoptosis, regulating the body's immune function, inhibiting protein tyrosine kinase (tyrosine kinase protein, PTK) activity, etc. [1-4]. The Zhikang capsule was composed by Rhubarb, *Coptis chinensis*, pseudo-ginseng, common *Bletilla* tuber and had the function of heat clearing and hemostasis, *Qufushengji*, relieving pain, promoting tissue repair and shorten the time of wound healing. It was confirmed by several experiments that *Bletilla* had antitumor activity. Aijing screened compounds extracted from *Bletilla striata* [5]. She found that the compound 1 was consistent with oleanolic acid-3-O- $\alpha$ -L-rhamnose-(1 $\rightarrow$ 2)- $\beta$ -D- glucopyranoside and had some anti-tumor activity. It made the theoretical basis of *Bletilla* anticancer activity to be more confident. After the cell cycle was detected by flow cytometry, it was found that compound 1 could block the cell cycle in the G0/G1 phase and the synthesis of DNA. At the same time, there was a significant sub-G0/G1 peak in after compound 1 acting on lung adenocarcinoma cell A549 cells for 24 h, which showed that the compound had the function of promoting the apoptosis of A549 cells. *Bletilla* also had the function of inducing apoptosis of tumor cells. Xuefen used 95% ethanol reflux to extract of *Bletilla* [6]. System solvent extraction method divided ethanol extract from *Bletilla striata* into petroleum ether layer, chloroform layer, ethyl acetate layer, butyl alcohol layer and water layer. CCK8 method was used to screen the active site of growth inhibition, and the effect of the active site on the apoptosis of mouse melanoma B16 cells was studied by fluorescence microscopy and flow cytometry analysis. The results showed that *Bletilla* chloroform and ethyl acetate layer inhibited on the growth of mouse melanoma B16 cells. The results showed that *Bletilla* chloroform layer could effectively induce B16 cell apoptosis. The results of this study showed that: the Zhikang capsule had a significant inhibitory effect on the growth of MCF-7 cells in the breast. With the increase of the concentration of the drug action and the extension of the action time, the inhibitory effect was time and concentration dependent.

The effect of promoting wound healing and hemostasis by Zhikang capsule has been widely affirmed [7,8]. Pharmacological studies showed that the main component of Zhikang capsule was rheum officinale, pseudo-ginseng and *Bletilla*. The pseudo-ginseng contained Panax Notoginseng Saponins, flavonoid glycoside and alkaloid, and had the function of hemostasis, analgesia and repair of mucous membranes. *Bletilla* contained the *Bletilla hyacinthina* gum. *Bletilla striata* polysaccharide could promote the growth of granulation tissue, capillary regeneration, and increase the wound exudate polymorphonuclear white cells and fibroblasts, and it was conducive to the wound to stop bleeding, repair and anti-infection [9-12]. Further study showed that *Bletilla striata* polysaccharide could increase the mRNA expression of macrophage inducible nitric oxide synthase, TNF- $\alpha$  and IL-1 $\beta$  and enhance the expression of these cytokines. The recent study found that the effective ingredients of *Bletilla striata* not only had the function of mechanical obstruction in the blood vessel, but also played a role in promoting blood coagulation and preventing the revascularization of the tumor [13]. It was confirmed that *Bletilla* had inhibitory effect on tumor growth of tumor bearing rabbit and *Bletilla* could inhibit tumor growth and narrow the tumor.

In conclusion, at the same time Zhikang capsule could promote the wound healing; it also might inhibit the tumor growth by blocking the cell cycle, inducing apoptosis, enhancing the expression of TNF- $\alpha$  and inhibiting tumor angiogenesis. It provided strong evidence for the application of Kang capsule in the wound healing after breast cancer surgery, but the specific molecular mechanism needed further study.

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