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INHIBITORY EFFECT OF SAPONINS AND POLYSACCHARIDES FROM *RADIX RANUNCULI TERNATI* ON HUMAN GASTRIC CANCER BGC823 CELLS

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Abstract

The effects of different *Radix ranunculi ternati* extracts on human gastric cancer BGC823 cells were investigated, different methods were used to extract the saponins and polysaccharides from *Radix ranunculi ternati*, and MTT assay and colony formation assay were used to observe the effects of saponins and polysaccharides from *Radix ranunculi ternati* on in-vitro cultured human gastric cancer BGC823 cells. The results found that the saponins and polysaccharides from *Radix Ranunculi Ternati* had certain effects on both the growth and colony formation of human gastric cancer BGC823 cells, while improving the immune function of normal mice, of which saponins had more significant effects than polysaccharides.

Key words: *Radix Ranunculi Ternati*, saponin, polysaccharide, human gastric cancer BGC823 cells

Introduction

Radix ranunculi ternati is the root tuber of *Ranunculus ternatus Thunb.*, which belongs to the Ranunculaceae family. It is named for its fleshy, clustered, fusiform root tuber, yellowish-brown rind, and cat claw like shape. It is sweet and pungent in taste, warm in nature; it enters the liver and lung meridians; and has the effects of resolving phlegm, dissipating nodulation, removing toxicity and subsiding swelling; it is mainly used in the treatment of scrofula, phlegm nodule, furunculosis, snake and insect bites, and other diseases (Chinese Pharmacopoeia Commission, 2010; Yin et al., 1993). It is reported in the literature that the *Radix Ranunculi Ternati* has a good effect on malignancies such as breast cancer (Yin et al., 2008).; it also has a certain effect on colon cancer (Zhou et al., 2009). *Radix Ranunculi Ternati* has complex chemical composition; its main constituents include saccharides, organic acids, sterols and esters, volatile oils, amino acids, flavonoids and glycosides, alkaloid glycosides, etc.

Materials and Methods

Medicinal Materials

Radix Ranunculi Ternati, (voucher No km-2012-67) grown in Xinyang, Henan, purchased from Xinxiang Pharmaceutical Company.

Instruments

Olympus inverted microscope (OLYMPUS, Japan), low temperature refrigerated centrifuge (Eppendorf, Germany), clean bench (Suzhou Purification Equipment Co., Ltd.), CO_2 incubator (SANYO, Japan), continuous wavelength microplate reader (Bio-RAD).

Reagents

RPMI1640 medium (GIBCO, USA), 0.25% trypsin (Sigma, USA), MTT (Sigma, USA), fetal bovine serum

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(Hangzhou Sijiqing Biological Engineering Materials Co., Ltd.), other reagents were all of analytical grade.

Cell Lines

Human gastric cancer cells BGC823 were provided by the Affiliated Hospital of Xinxiang Medical University.

Preparation of saponin extract from *Radix ranunculi ternati*

Referring to the methods in (Wang et al., 2004; Liu et al., 2007), 100 g of dried *Radix ranunculi ternati* was crushed into coarse powders, put in a Soxhlet extractor, and extracted with 95% ethanol for 6 h, ethanol was recovered from the alcohol extract, and the resulting solution was extracted twice with diethyl ether, and then extracted three times with water saturated n-butanol, the n-butanol extract was extracted twice with 0.1 mol/L sodium hydroxide, n-butanol solution was recovered, followed by drying, and *Radix Ranunculi Ternati* total saponins were obtained. The total saponins were dissolved in water, and prepared as 1 g crude drug/mL, then filtration sterilized, and stored at 4℃ for later use.

Preparation of polysaccharide extract from *Radix ranunculi ternate*

Referring to the method in (Twentyman, 1987), 100 g of dried *Radix ranunculi ternati* was crushed, and ultrasonically extracted three times with ethanol as the solvent, and with the extraction time of 20 min each, ultrasonic power of 300 w, then the filtrates were combined, and ethanol was recovered, followed by drying. The polysaccharides were dissolved in water, and prepared as 1 g crude drug/mL, then filtration sterilized, and stored at 4℃ for later use.

Determination of cell proliferation inhibition rate by MTT assay (Svegliati et al., 1999)

Poorly differentiated human gastric adenocarcinoma BGC823 cells in the logarithmic growth phase were collected, and diluted to a concentration of about 5×10^4/ml with culture medium, seeded in 96-well plate, and cultured for 24 h, then the culture medium was discarded, and the drug-containing medium was added (triplicate wells for each concentration group), meanwhile, blank control group which was added with complete medium only was set up, after placing in a 37℃, 5% CO_2, saturated humidity incubator and incubated for 48 h, each well was added with 20 μL of MTT solution, the incubation was continued for another 4 h, then the supernatant was discarded, each well was added with 150 μL of DMSO, and shaken with a micro mixer for 10 min, after the crystals were fully dissolved, OD values were measured at a wavelength of 570 nm using a microplate reader, and the tumor cell proliferation inhibition rate was calculated according to the following formula:

Inhibition rate (%) = (1 - OD value of experimental group / OD value of control group) × 100%

Colony forming assay (Zhang., 2004; Zhang et al., 2005)

Poorly differentiated human gastric adenocarcinoma BGC823 cells in the logarithmic growth phase were collected, and seeded in 24-well plates at the density of 100 living cells/well, cultured for 24 h, then added with different concentrations of drugs (triplicate wells for each concentration group), meanwhile, blank control group which was added with complete medium only was set up. After the cells were incubated in a 37℃, 5% CO_2, saturated humidity incubator for 10 d, the supernatant was discarded, then the cells were fixed in 95% ethanol, and crystal violet stained. Colonies containing more than 50 cells were counted under a microscope, and colony formation inhibition rate was calculated according to the following formula: Colony formation inhibition rate (%) = (1 - CFU number of experimental group / CFU number of control group) × 100%

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Effect of saponins and polysaccharides from *Radix Ranunculi Ternati* on immune function of normal mice (Pang et al., 2001)

The mice were randomly divided into blank control group, saponin group and polysaccharide group, with 10 mice in each group, after continuous intragastric administration for 14 d, immune function was determined.

Lymphocyte transformation test

Spleen cells were collected under sterile conditions, minced and ground, centrifuged at 1000 r/min twice for 10 min each time, then cell concentration was adjusted to 1.5×10^6/ml with culture medium, triplicate wells were set up for each experimental group, with the final volume for each well 100 µl, 100 µl of saponins and polysaccharides were added, while in the blank control group, only the culture medium was added, after culturing for 72 h, OD value was measured at 540nm, and stimulation index (SI) was calculated: SI = OD value of test well or negative control well / OD value of blank control well.
Spleen index: Spleen index = spleen mass (mg) / body mass (g).

Determination of natural killer (NK) cell activity (Zhou et al., 1995)

The volume fraction of spleen cells was 1.5×10^6/ml, and the volume fraction of the target cell BGC823 5×10^5/ml, control wells were 0.1 ml of effector cells and 0.1 ml of culture medium; 0.1 ml of target cell solution and 0.1 ml of culture medium respectively. Experimental wells were 0.1 ml of effector cell solution and 0.1 ml of target cell solution, after incubating at 37℃ for 2 h, the effector-target reaction was stopped, followed by centrifugation at 1500 r/min for 5 min, after that, 100 µL of supernatant was added to each well of 96-well plate, preheated at 37℃, and 100 µL of substrate solution was added to each well, allowed to stand at room temperature for 10 min, then 30 µL of 0.1 mol/L citric acid was added to stop the enzymatic reaction. OD value at 570 nm was measured, and NK cell activity was calculated: NK cell activity (%) = [1 - OD value of experimental well / (effector cell + culture medium) OD + (target cell + culture medium) OD)] × 100.

Results

Effects of different concentrations of saponin and polysaccharide extracts from *Radix Ranunculi Ternati* on human gastric cancer BGC823 cells

The results showed that the five dose groups of saponins and polysaccharides all had inhibitory actions on the growth of human gastric cancer BGC823 cells. The *Radix ranunculi ternati* saponins significantly inhibited human gastric cancer BGC823 cells, and the effect was enhanced with the increase of concentration, which was in a concentration-dependent manner. The polysaccharide dose group, although exhibited significant difference when compared with the control group, had a less effective tumor inhibitory effect than saponins.

Effects of saponins and polysaccharides from *Radix Ranunculi Ternati* on colony formation of human gastric cancer BGC823 cells

Under different concentrations, the experimental groups all showed statistically significant differences ($P<0.05$) when compared with the control group. The effect of *Radix Ranunculi Ternati* polysaccharides on colony formation of human

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gastric cancer BGC823 cells was concentration dependent, with the increase of concentration, colony formation inhibition rates were 32.64%, 55.62%, and 100% respectively. No colony formation was observed for the *Radix Ranunculi Ternati* saponins within the concentration range; visual analysis showed that the *Radix Ranunculi Ternati* saponins had stronger colony formation inhibitory effect on human gastric cancer BGC823 cells than the *Radix Ranunculi Ternati* polysaccharides.

Table 1: Effects of *Radix Ranunculi Ternati* saponins and polysaccharides on inhibition of human gastric cancer BGC823 cells (%)

Drug concentration (μg/ml)	Inhibition rate (%)	
	Saponins	Polysaccharides
6.25	24.52**	3.75*
12.5	33.76**	5.12*
25	49.11**	14.98**
50	68.65**	30.47**
100	92.53**	53.68**

Comparison with the control group, *P<0.05, **P<0.01

Table 2: Effects of saponins and polysaccharides from *Radix Ranunculi Ternati* on colony formation of human gastric cancer BGC823 cells ($\bar{X}$ ±S, n=3)

Group	Concentration (μg/mL)	CFU number	Colony formation inhibition rate (%)
Blank control group		89.24±1.63	
Saponin group	12.5	0**	100
	25	0**	100
	50	0**	100
Polysaccharide group	12.5	60.12±2.45**	32.64
	25	39.54±1.23**	55.62
	50	0**	100

Comparison with the blank control group, *P<0.05, **P<0.01

Effects on immune function of normal mice

It can be seen from the results that the different concentrations of *Radix ranunculi ternati* saponins and polysaccharides can all significantly improve the spleen index and tumor cell killing rate of NK cells in normal mice. Of which the *Radix ranunculi ternati* saponins had a more pronounced effect, it exerts its anti-tumor effect by improving immune function, thus promoting the growth of normal cells.

Discussion

Many literatures have reported the anti-tumor effect of *Radix Ranunculi Ternati*. *Radix Ranunculi Ternati* is also clinically applied to treat tumors, with respect to the anti-tumor mechanism of *Radix Ranunculi Ternati*, literature has found that the *Radix Ranunculi Ternati* can induce the production of tumor necrosis factor (TNF), after taking it, the body's own TNF secretion levels can be elevated, thereby treating and immunizing against the tumors (Nicotera et al., 1998). Another

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study has found that the anti-tumor effect of *Radix Ranunculi Ternati* may be associated with the increase of Ca^{2+} (Szende et al., 1990), elevated intracellular calcium plays a decisive role in apoptosis (Szende et al., 1990), and in experiments, after the cells were affected by the *Radix Ranunculi Ternati* saponins, Ca^{2+} was significantly increased.

Table 3: Effects of *Radix ranunculi ternati* on immune function of normal mice ($\bar{X} \pm S$)

Group	Dose (g crude drug/kg)	Spleen index	SI	NK cell killing rate (%)
Blank control group		4.3±0.8	1.21±0.14	29.20±4.84
Radix Ranunculi Ternati saponins	3	4.9±1.3	1.29±0.21	40.58±6.57
	6	5.1±0.9	1.29±0.21	46.86±5.88
	12	5.6±1.2	1.29±0.21	57.62±6.81
Radix Ranunculi Ternati polysaccharides	3	4.3±1.2	1.20±0.27	31.57±6.48
	6	4.5±0.4	1.20±0.27	33.82±5.72
	12	5.0±0.7	1.20±0.27	38.17±4.07

Radix ranunculi ternati contains relatively high amount of saponins and polysaccharides, which are complex in composition. Many studies have shown that saponins and polysaccharides both good pharmacological activities. This study found that the saponins and polysaccharides from *Radix Ranunculi Ternati* had significant *in vitro* anti-tumor effects on human gastric cancer BGC823 cells, and could improve the immune function of normal mice, however, their exact anti-tumor constituents still need further analysis.

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