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EXPERIMENTAL STUDY

Effect of Fanbaicao (*Herba Potentillae Discoloris*) oil on the expression of p21 and CDK4 in HepG2 cells

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Abstract

OBJECTIVE: To research the anti-cancer mechanism of the Traditional Chinese Medicine Fanbaicao (*Herba Potentillae Discoloris*) oil in the human hepatoma cell line HepG2.

METHODS: Gas chromatography was used to analyze the components of Fanbaicao (*Herba Potentillae Discoloris*). We tested the inhibitory effect of Fanbaicao (*Herba Potentillae Discoloris*) oil on the human hepatoma cell line HepG2 in vitro using 3-(4, 5-Dimet hylt hiazol-2-yl)-2,5-dip henyltetrazolium bromide assays. Fluorescence activating cell sorter analysis was used to examine the levels of apoptosis, and western blot and immunofluorescence were used to detect the expression of p21, p-p21 and CDK4 proteins.

RESULTS: Fanbaicao (*Herba Potentillae Discoloris*) oil contains 45 ingredients, and L-ascorbic acid 2, 6-bispalmitate was the main component and accounted for 44.96% of total drive-off peak area. Other components included (Z)-14-met hyl-8-exadecenal-acetal (8.56%), phytol (7.74%) and lauric acid (6.31%). Fanbaicao (*Herba Potentillae Discoloris*) oil treatment reduced the proliferation of HepG2 cells and the half growth inhibition concentration (IC_{50}) was 2.03 mg/mL. Furthermore, we also observed significantly increased HepG2 cell apoptosis in a dose-dependent manner ($P < 0.05$). Fanbaicao (*Herba Potentillae Discoloris*) oil significantly increased the expression of p21 and p-p21 and significantly decreased the expression of CDK4 in HepG2 cells compared with controls ($P < 0.01$).

CONCLUSION: Our results showed that Fanbaicao (*Herba Potentillae Discoloris*) oil has anti-cancer activities in HepG2 cells, which is probably related to the upregulation of p21 and p-p21 and downregulation of CDK4 expression.

Key words: Potentilla Discolor; Carcinoma, Hepatocellular; Hep G2 Cells; CDKN1A protein, human

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common

primary malignancy of the liver. It is the sixth most common malignancy worldwide and the third cause of cancer-related mortality.[1,2] Despite some progress in the treatment of cancers, existing therapies are limited in their ability to cure malignancies and to prevent metastases and relapses.

Traditional Chinese herbs have been widely used to treat cancer in China.[3-5] Potentilla is used as a traditional herbal medicine for various medical purposes, such as treatment of diarrhea, hepatitis, rheumatism, and scabies.[6,7] Modern pharmacological studies have confirmed the traditional use of Potentilla species and their extracts from aerial and/or underground parts as a therapy for inflammation, colitis ulcerosa, certain forms of cancer, viral and microbial infections, impaired immune system and diabetes mellitus.[8-11] Most of the biological effects of Potentilla species can be explained by the high amount of condensed and hydrolysable tannins present in the aerial and the underground parts that contribute to the antiviral and antimicrobial activities, hepatoprotective, and anti-inflammatory effects.[7,12]

About ingredient analysis of Fanbaicao oil and effect of anti-cancer,we don't found any report. In this study we isolated Fanbaicao (*Herba Potentillae Discoloris*) oil (Table 1) from Potentilla anserina L. and evaluated its toxicity and inhibitory activity as well as its effect on apoptosis in liver cancer cells *in vitro*.

MATERIALS AND METHODS

Fanbaicao (Herba Potentillae Discoloris) oil extraction

Fanbaicao (*Herba Potentillae Discoloris*) oil can be extracted by steam distillation. Fanbaicao (*Herba Potentillae Discoloris*) (100 g, Tongren Tang, Beijing, China) was cut into pieces (less than 2 cm); the pieces were placed into a 1000 mL flask with 1000 mL distilled water and soaked for 1 h. The flask was placed in a steam distillation device to extract Fanbaicao (*Herba Potentillae Discoloris*) oil for 24 h. The upper layer of oil was removed. The extract was dissolved in an hydrous ether and dehydrated by an hydrous sodium sulfate. Finally, a yellow liquid was achieved, sealed and stored in the freezer.

Gas chromatograp hy-mass spectrometry

Fanbaicao (*Herba Potentillae Discoloris*) oil components were analyzed by gas chromatograp hy-mass spectrometry (GC-MS, Agilent Tec hnologies, Palo Alto, CA, USA). Chromatograp hicseparation was achieved on a Rtx-5MS capillary column (30 mm × 0.25 mm, a film thickness of 0.25 mm, Agilent Technologies). The program was as follows: inlet temperature of 280 ℃; split stream sampling with split ratio of 1∶100 (gas carrier He); split size of 1.0 μL; linear velocity control; temperature program: 60-280 ℃ at 10 ℃/min; ion source temperature: 200 ℃; interface temperature: 280 ℃. The sample was diluted 100 times with ethyl acetate, and then mixing.

Cells and cell culture

The HepG2 cell line was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and preserved by College of Basic Medical Sciences of Jamusi University in China. HepG2 cells were cultured in 10% RPMI 1640 cell culture medium (Invitrogen Corp., Carlsbad, CA, USA) containing 10% fetal calf serum (FCS, Invitrogen Corp., Carlsbad, CA, USA), penicillin (100 units/mL, Invitrogen Corp., Carlsbad, CA, USA) and streptomycin (100 μg/mL, Invitrogen Corp., Carlsbad, CA, USA). The cells were cultured and passaged by conventional methods.

Drug preparation

Fanbaicao (*Herba Potentillae Discoloris*) oil was first diluted to 100 mg/mL concentration and then diluted to 50 mg/mL concentration with 10% cell culture medium containing 10% FCS. The finaldiluent was sterilized by filtration with a 0.22-μm Millipore filter (Millipore Corp., Billerica, MA, USA) and stored at 4 ℃. For experiments, the different concentrations of Fanbaicao (*Herba Potentillae Discoloris*) oil were prepared with medium containing 10% FCS.

3-(4,5-dimet hylt hiazol-2-yl)-2,5-dip henyltetrazolium bromide (MTT) assays

HepG2 cells were collected and resuspended at a concentration of 5×10^8 cells/L. Cells were seeded in 96-well plates (200 μL/well) for 24 h, the medium was removed, and various concentrations of Fanbaicao (*Herba Potentillae Discoloris*) oil were added. After 48 h, MTT (5 mg/mL, Sigma Corp., St. Louis, MO, USA) was added and cells were cultured for 4 h. FCS was removed, dimethyl sulfoxide was added and OD value was obtained (A492). The experiment was repeated three times.

Cell surface staining and flow cytometry

To assess the rate of cell apoptosis, treated HepG2 cells were double-stained with fluorescein isothiocyanate (FITC)-conjugated anti-Annexin V (Becton, Dickinson and Company, Franklin, NJ, USA) and propidium iodide (PI, Becton, Dickinson and Company, Franklin, NJ, USA). After hing cells twice with phosphate-buffered saline (PBS) containing 1% FCS and resuspending cells in 300 μL PBS, the cells were analyzed in a FACSCalibur Flow Cytometer (Becton, Dickinson and Company, Franklin, NJ, USA) with Cell Quest software. Viable cells were gated by forward and side scattering.

Immunofluorescence assay

Cells were stained with primary rabbit-anti-p21 polyclonal antibody (BAB) (Invitrogen, Corp., Carlsbad, CA, USA) or rabbit-anti-CDK4 BAB (Invitrogen