

A STUDY ON THE INHIBITORY EFFECT OF *RADIX SEMIAQUILEGIAE* EXTRACT ON
HUMAN HEPATOMA HEPG-2 AND SMMC-7721 CELLS

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Abstract

The main objective of this paper was to investigate the extraction process of ethanol extract of *Radix Semiaquilegiae*, as well as its inhibitory activity on human hepatoma HepG-2 and SMMC-7721 cells, and to compare the inhibitory effects of different concentrations of ethanol extracts against these two hepatoma cells. Ethanol reflux extraction and ultrasound-assisted extraction with ethanol at room temperature were used in the extraction process, and MTT assay was mainly used in the activity experiment to perform in-vitro anti HepG-2 and SMMC-7721 cell activity screening of ethanol extract, and to calculate the cell inhibition rates of the extracts. The results showed that among the two types of extracts, ethanol reflux extract had more superior antitumour activity to that of the ultrasonic extract, but all of the extracts obtained had certain anti-cancer activities, and the anti-proliferative activity increased with the increase of concentration.

Keywords: *Semiaquilegia adoxoides* (DC.), inhibitory, HepG-2, SMMC-7721

Introduction

Radix Semiaquilegiae is the dried root tuber of *Semiaquilegia adoxoides* (DC.) *makino*. (Ranunculaceae), which is a traditional Chinese herbal medicine recorded in the Chinese Pharmacopoeia (2000 edition). It is grown under hills or low mountain forests, bushes, ditches and other dark damp places, and is mainly distributed in places such as Hubei, Hunan, and Jiangsu. Its English name is *Muskroot-like Semiaquilegia Root*; the Latin name is *Radix Semiaquilegiae*. Modern pharmacological studies have shown that *Radix Semiaquilegiae* has a strong anti-cancer activity (Guan Pin, 2010; Ye Juan, 2007). The compounds that have so far been isolated from *Radix Semiaquilegiae* mainly include saccharides, glycosides, alcohol acids, sterols, and alkaloids (Lan Hua-ying, 2006; Niu Feng, 2006; Su Yan-fang, et al, 2006; Zhang Zhen-xia, 2004), of which alkaloids include thalifendine, 5,6-dihydro-9, 10-dihydroxy-Benzo[g]-1, 3-benzodioxolo-[5,6-a] quinolizinium and berberrubine. It is used in the folk medicine for the treatment of bloating, sore throat, snakebite and other symptoms. Clinically, it is mainly used in the treatment of acute soft tissue injuries, bone and joint tuberculosis, and other diseases. Chinese medicine compound preparations containing *Radix Semiaquilegiae* are mainly used in the treatment of acute mastitis, uterine fibroids, primary liver cancer, stomach cancer, etc.

Materials and Methods

The main materials used for the study include human hepatoma HepG-2 and SMMC-7721 cells, presented by the Department of Pharmacology of China Medical University; Chinese medicine *Radix Semiaquilegiae* decoction pieces, Henan Guoyao Medicine Co., Ltd.; foetal bovine serum, Hangzhou Sijiqing Bioengineering Co., Ltd.; foetal calf serum (GIBCO, USA); double antibiotics (penicillin, streptomycin, purchased from Gibco, USA); trypsin (purchased from Amresco, USA); MTT, DMSO, Sigma; RPMI 1640 medium, Gibco; Thermo microplate reader, Thermo, USA; SANYO CO₂ incubator, SANYO, Japan; KH5200DE ultrasonic cleaner, Kunshan Hechuang Ultrasonic Co., Ltd.; CKX31 inverted microscope, Olympus,

Japan. The specimen (AT-2013-14) was identified by Professor Sun QH, and was placed in the lab centre.

Extraction process (Yang Yang, 2011)

Radix Semiaquilegiae crude drug was crushed using grinder. It was passed through a 20 mesh sieve. 30 g of powder were weighed in duplicate, of which one replicate was placed in a 200 ml round-bottomed flask, added with 80 ml of ethanol, and heat reflux extracted for 12 h. Another replicate was placed in a 200 ml beaker, added with 80 ml of ethanol, and ultrasonically extracted for 12 h. After then, solvents were both removed by reduced pressure, and extracts were both obtained by freeze drying. Extract obtained by reflux method was served as group A extract, and extract obtained by ultrasonic method was served as group B extract.

Drug solution preparation

10 g of group A extract was weighed, dissolved in sterile distilled water, formulated into 2 g/mL, and filtered with a 0.22 µm membrane filter. Each of the sample solution was added into three wells. The concentrations were 2 g/ml, 1 g/ml, and 0.5 g/ml respectively. 4 replicate wells were set up. Handling of group B extract was the same as group A.

Cell culturing (Xie Bin, et al, 2012; Zeng Xiao-li, et al, 2004)

Human hepatoma HepG-2 cells were cultured in RPMI-1640 culture medium of pH about 7.4 containing 10% inactivated foetal bovine serum, 100 U/ml penicillin, and 100 µg/mL streptomycin, and incubated in a 37 °C, 5% CO₂ incubator, flasks were split once every 3 days, and cells at logarithmic growth phase were collected for the experiment (cells at about 7 days from the first day of thawing).

Human hepatoma SMMC-7721 cells were placed in DMEM medium containing 10% foetal calf serum, and incubated in an incubator set at 37 °C, 5% CO₂ with saturated humidity. The medium was replaced every 2 d. After culture flasks were covered with a single layer of cells, cells were digested with 0.25% trypsin. 2~3 min later when cells rounded up and shrank under the microscope, cells were blown with a pipette so that they can be suspended in the liquid. 10% FCS was added to stop digestion. Flasks were split, and 10% FCS was added for culturing.

Before the experiment, the logarithmic growth phases of HepG-2 and SMMC-7721 cells were collected, digested with 0.25% trypsin, repeatedly blown to form a uniform suspension, and seeded in 96-well plates with a concentration of 5×10^4 cells/mL (SMMC-7721) or seeded in 96-well plates with a concentration of 10^4 cells/mL (HepG-2). Each well contained 100 µL of culture medium. After waiting overnight for cell adhesion, the supernatant was discarded.

Activity Analysis

First, according to the literature, 5×10^4 cells/mL were selected as the inoculum concentration. OD values at 12 h, 24 h, 36 h and 48 h were determined, and HepG-2 and SMMC-7721 cell growth curves were plotted.

96-well plates were divided into several groups; namely, the distilled water group, positive control group, and sample group. 4 replicate wells were set up. The prepared samples were added to the culture plate with adherent cells, and cultured for 24 h, after which MTT, DMSO were added. Meanwhile, OD values at 12 h, 24 h, 36 h and 48 h were measured, and cell plates were prepared separately to test the OD values respectively. Then, a microplate reader was used to obtain the OD values of different groups at different time periods.

Results

As can be seen from Figure 1, in the first four days, the number of cells changed very little. Cells began to proliferate from the 5th day, and proliferation was fast. Therefore, when selecting the logarithmic growth phase cells, cells on the 8th day should be used for the activity experiment.

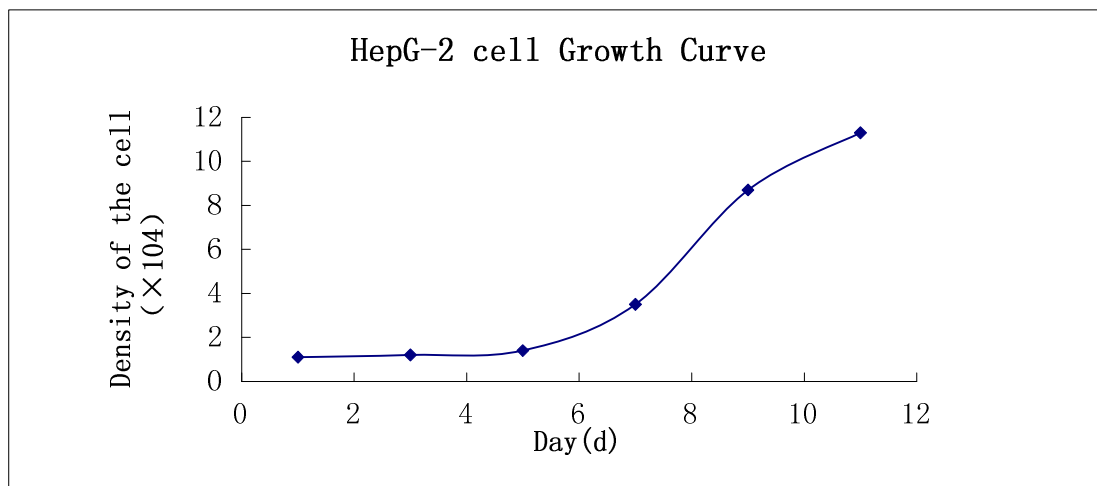


Figure 1: HepG-2 hepatoma cell growth curve

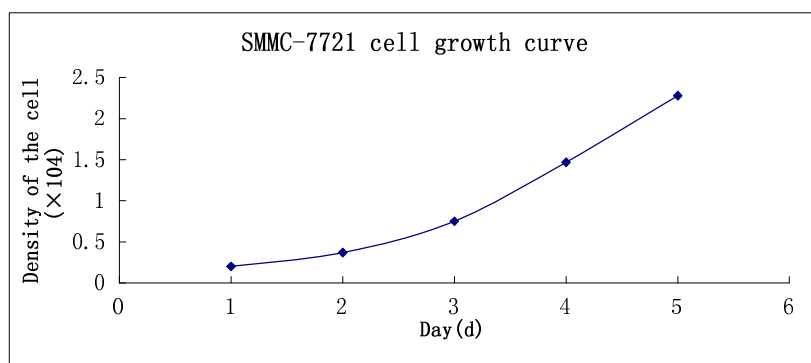


Figure 2: SMMC-7721 hepatoma cell growth curve

As can be seen from Figure 2, cell proliferation rate was relatively stable, and on about 3.5th day, cell proliferation was the fastest. So, when selecting the logarithmic growth phase cells, cells on the 3.5th day from thawing should be used for the activity experiment.

Inhibition rates of group A samples against HepG-2 and SMMC-7721 cells at different times

Table 1: Cell inhibition rate of group A samples

Group A samples Concentration (g/ml)	Cell growth inhibition rate %	
	HepG-2	SMMC-7721
8	75.4	76.2
4	62.1	71.9
2	56.3	69.2
1	21.5	35.2
0.5	15.6	28.5

The data in Table 1 indicate that the reflux extract had very good inhibition rates against both types of cells. Crude extract obtained by heat reflux extraction had slightly higher inhibition rate against SMMC-7721 cells than against HepG-2 cells. The calculation showed that the IC50 value of group A samples against HepG-2 cells produced 2.46 g/ml, and IC50 against SMMC-7721 cells produced 1.43 g/ml.

Inhibition rates of group B samples against HepG-2 and SMMC-7721 cells at different times

Table 2: Cell inhibition rate of group B samples

Group B samples Concentration (g/ml)	Cell growth inhibition rate %	
	HepG-2	SMMC-7721
8	68.2	69.8
4	54.7	61.6
2	43.9	53.2
1	15.7	22.6
0.5	2.4	1.3

The data in Table 2 indicate that the reflux extract had very good inhibition rates against both types of cells. Crude extract obtained by ultrasonic extraction had slightly decreased inhibition rates against two types of cells compared with the reflux extract. But it still had certain inhibitory activities, and the inhibitory effect was especially obvious when concentration was 2 g/ml. The calculation showed that the IC50 value of group B samples against HepG-2 cells was 3.64 g/ml; IC50 against SMMC-7721 cells was 3.21 g/ml.

Discussion

Radix Semiaquilegiae is a kind of Chinese medicine which belongs to the genus Ranunculaceae, family Ranunculaceae. Research on *Radix Semiaquilegiae* has just started at home and abroad, and has been limited to a small number of natural product isolation. Studies on many of its pharmacological activities are still not complete. Moreover, the contents of this research are relatively new, providing valuable reference material for future pharmacodynamic experimental study of *Radix Semiaquilegiae*, especially for anti-hepatoma experiments.

In this study, two different extraction processes were used to obtain ethanol extract of *Radix Semiaquilegiae*, and two types of cells — human hepatoma HepG-2 and SMMC-7721 cells – were selected as experimental subjects to screen anti-hepatoma activity of ethanol extracts. Analyses of results show that the crude extracts have very good anti-tumour activity against both types of hepatoma cells, and that the anti-cancer effect is more obvious with the increase of concentration. Crude extract obtained by heat reflux extraction has a significantly higher inhibitory effect on SMMC-7721 than on HepG-2, while the crude extract obtained by ultrasonic extraction has relatively close inhibitory effects on both cells. In addition to the anti-hepatoma effect, *Radix Semiaquilegiae* also has good inhibitory effects on mouse lung adenocarcinoma LA795 cell lines, and colon cancer HT-29 cells. Monomers further isolated from ethanol extract of *Radix Semiaquilegiae* by domestic researchers include p-hydroxybenzoic acid, p-hydroxyphenylacetic acid, p-hydroxyphenethyl alcohol, p-hydroxymethoxybenzoic acid, etc. Saccharides mainly include glucose, galactose, mannose, arabinose, fucose, fructose, etc., as well as glycosides, esters, and alkaloids. Currently, there are three alkaloids found in *Radix Semiaquilegiae*, they are as follows: thalifendine, 5, 6-dihydro-9, 10-dihydroxy-Benzo[g]-1, 3-benzodioxolo-[5,6-a] quinolizinium and berberrubine, which have anti-tumour effects. The anti-tumour mechanism may be related to the signal transduction pathway. Multiple studies have indicated that many drugs with anti-tumour activities are mostly alkaloids (Shuli Man, et al, 2012). Therefore, it

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is likely that the anti-tumour action of extracts obtained by us is exerted by the above three alkaloids. Further isolation, identification and activity test are needed to prove exactly which one or several alkaloids work(s).

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