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## STUDY ON THE INHIBITORY EFFECT OF *DRYNARIA FORTUNEI* EXTRACT ON HUMAN MYELOMA SP20 CELLS

Baofeng Li<sup>1\*</sup>, Tingting Duan<sup>2</sup>, Lei Fan<sup>3</sup>

<sup>1</sup>Orthopaedics Department, The Affiliated hospital of Shandong University of Traditional Chinese Medicine, 250014, Ji Nan, China, <sup>2</sup>Health Care Department, The Affiliated hospital of Shandong University of Traditional Chinese Medicine, 250014, Ji Nan, China, <sup>3</sup>Institute of Information Management, Shandong University of Traditional Chinese Medicine, 250255, Ji Nan, China

\* E-mail: [libaofeng777@yeah.net](mailto:libaofeng777@yeah.net)

### Abstract

The objective of the study was to investigate the inhibitory effect of *Drynaria fortunei* extract on human myeloma SP20 cells. Three different total extracts of *Drynaria fortunei* were obtained by reflux extraction method using different organic solvents including ethanol, methanol and petroleum ether. Their anticancer activities on SP20 cells were tested, and the maximum inhibition rate was obtained. The inhibitory effects on tumour cells at 12 h, 24 h, 36 h and 48 h were tested, and the inhibition curves at different time periods were plotted. The results showed that the methanol and ethanol extracts have similar inhibition rates at 24 h, which are around 55%. On the other hand, the maximum inhibition rate of petroleum ether extract is only 36% within 24h. Moreover, within the time periods of 36 h and 48 h, its inhibition rates are all below 10%.

**Keywords:** *Drynaria fortunei*, Flavonoid compounds, Mouse SP2/0 tumour, Inhibitory.

### Introduction

Flavonoid compounds are the main active constituents of *Drynaria fortunei* medicinal herb, which biological activities include the promotion of fracture healing, prevention and treatment of osteoporosis, antioxidation, blood lipid regulation, anti-inflammation, and analgesia (Liu et al., 2001; Suna et al., 2002; Liu et al., 2012). However, the anticancer activities of *Drynaria fortunei* and related extracts have not been widely studied. Therefore, this paper chooses myeloma SP20 cells to perform anticancer activity analysis. Because of its good biological activities, its total flavonoids can significantly improve the bone density in ovariectomised rats, inhibit TNFA, IL-6 levels in serum, and promote IL-4 secretion (Xie et al., 2004). It is of great referential significance for the treatment of osteoporosis. And a study has demonstrated the presence of relatively highly active substances in the aqueous and ethanol extracts of *Drynaria fortunei* which contribute to bone cell proliferation, differentiation and calcification (Tang et al., 2004). In China, the survival of patients with bone metastases has been prolonged using the kidney and spleen nourishing Chinese medicines, and *Drynaria fortunei* is one of those Chinese medicines. Therefore, it is reasonable to study the inhibitory activity of *Drynaria fortunei* on myeloma SP20 cells (Chen, 2004).

### Materials and methods

#### Materials

Chinese medicine *Drynaria fortunei* was purchased from Xiamen Traditional Chinese Medicine Co., Ltd., and was identified by Professor Daren Cheng of Shandong University of Traditional Chinese Medicine. The specimen was placed in

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the Chinese herb lab of the university. Methanol, ethanol and petroleum ether were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Mouse SP2/0 tumour cell lines were provided by Shandong University of Traditional Chinese Medicine. DMEM, RPMI 1640, HEPES and MTF were purchased from Sigma. Annexin-V-FITC apoptosis detection kit was the product of Biosea Biotechnology Co., Ltd. Ultrapure water was produced by Milli-Q ultrapure water system.

#### **Cell cultivation** (Li et al., 2008)

The cells were cultured with RPMI 1640 medium containing 10% FBS. The medium was added with 2.2 g/L NaHCO<sub>3</sub>, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were then subcultured in an incubator at 37 °C with 5% CO<sub>2</sub>. The experiment was performed when the cells were in the logarithmic growth phase.

#### **Preparation of extracts** (Jia, 2012)

*Drynaria fortunei* was crushed into coarse powder, and then weighed in triplicate. The weight of each portion of coarse powder was 3 g. The coarse powders were added with 150 ml of ethanol, methanol and petroleum ether separately, and reflux extracted four times respectively. Each extraction lasted 0.5 h. The organic solvents were evaporated from the resulting products using a rotary evaporator, and the remaining was further dried in a vacuum dryer. The resulting extracts were dissolved in DMSO and water. DMSO was not used for samples that can be directly dissolved in water.

#### **Determination of anticancer activity by MTT assay**

0.5 g of the extracts was weighed separately. The methanol and ethanol extracts were dissolved in water, while the petroleum ether extract was dissolved in DMSO, and uniformly prepared as 3.5 mg/ml solutions. Cells in the logarithmic growth phase were taken, counted and seeded in a density of 2 X 10<sup>5</sup>/ml. The anticancer activities of three extract solutions on human myeloma SP20 cells were determined simultaneously using the MTT assay. Each sample was repeated in quadruplicate. The inhibition rates of samples against cancer cells were determined at 12 h, 24 h, 36 h and 48 h respectively, and dynamic inhibition curves were plotted according to the inhibition rates.

## **Results**

#### **Comparison of weight of three extracts**

By reflux extraction method, the amount of dry powder obtained with methanol solvent is 2.8 g, the amount of dry powder obtained with ethanol solvent is 2.5 g, and the amount of dry powder obtained with petroleum ether solvent 1.4 g.

#### **Results for the determination of anticancer activity by MTT assay**

##### **Anticancer activity of different extracts**

As can be seen from the Table 1, the methanol extract reaches the maximum inhibition rate of 54.39% at 24 h and 3.5 mg/mL. Ethanol extract also reaches maximum inhibition rate at 24 h and 3.5 mg/ml, which is 55.91 %. The inhibition rates of the two are relatively close, while the inhibition rate of petroleum ether extract reaches a maximum of 36.11% at 24 h, and at 36 h and 48 h. Its inhibition rates are both below 10%, which are significantly weakened.

**Table 1:** Anticancer activity of different extracts

| Time-concentration-inhibition rate of methanol extract        |                                  |        |        |        |
|---|----------------------------------|--------|--------|--------|
|   | Different concentrations (mg/ml) |        |        |        |
| Time (h)  | 3.5                              | 1.75   | 0.863  | 0.432  |
| 12  | 42.64%                           | 33.28% | 21.47% | 7.67%  |
| 24  | 54.39%                           | 41.96% | 29.09% | 22.37% |
| 36  | 38.80%                           | 31.28% | 6.67%  | 4.62%  |
| 48  | 20.68%                           | 13.47% | 4.93%  | 2.85%  |
| Time-concentration-inhibition rate of ethanol extract         |                                  |        |        |        |
|   | Different concentrations (mg/ml) |        |        |        |
| Time (h)  | 3.5                              | 1.75   | 0.863  | 0.432  |
| 12  | 49.61%                           | 33.59% | 27.06% | 15.86% |
| 24  | 55.91%                           | 37.02% | 32.72% | 24.27% |
| 36  | 45.38%                           | 37.70% | 28.10% | 8.73%  |
| 48  | 17.03%                           | 10.07% | 3.66%  | 2.01%  |
| Time-concentration-inhibition rate of petroleum ether extract |                                  |        |        |        |
|   | Different concentrations (mg/ml) |        |        |        |
| Time (h)  | 3.5                              | 1.75   | 0.863  | 0.432  |
| 12  | 30.67%                           | 28.22% | 11.66% | 6.29%  |
| 24  | 36.11%                           | 33.04% | 24.85% | 9.21%  |
| 36  | 9.91%                            | 4.96%  | 0.68%  | 0.85%  |
| 48  | 1.52%                            | 2.28%  | 0.76%  | 0.57%  |

**Dynamic inhibition curves of *Drynaria fortunei* methanol, ethanol and petroleum ether extracts against SP20 cells**

As can be seen from Figures 1, 2 and 3, inhibition rates against SP20 cells are higher at 12 h and 24 h, where the minimum inhibition rate is 7.64% and the maximum inhibition rate is 54.39%. Overall, the inhibition rates are relatively evident between 12~36 h. But inhibitory effects of methanol and ethanol extracts at 12~36 h are stronger than petroleum ether extract. Moreover, the maximum inhibition rate of petroleum ether extract is only about 35%. Obviously, methanol and ethanol are preferable organic solvents for the extraction of *Drynaria fortunei*.

**Discussion**

This experiment studies the inhibitory effects of methanol extract, ethanol extract and petroleum ether extract of *Drynaria fortunei* on myeloma SP20 cells separately, and analyses the changes in their inhibition rates at different time periods. The results find that the inhibition rates of methanol and ethanol extracts reach similar values at 24 h, which are approximately about 55%, while the maximum inhibition rate of petroleum ether extract, within the range of the maximum concentration and optimal time, is only 36%. Moreover, the inhibition rates of petroleum ether extract within two time periods of 36 h and 48 h are all below 10%, apparently very low. Therefore, petroleum ether should not be selected as the extraction solvent for *Drynaria fortunei*, whereas the methanol and ethanol are the preferred organic solvents.

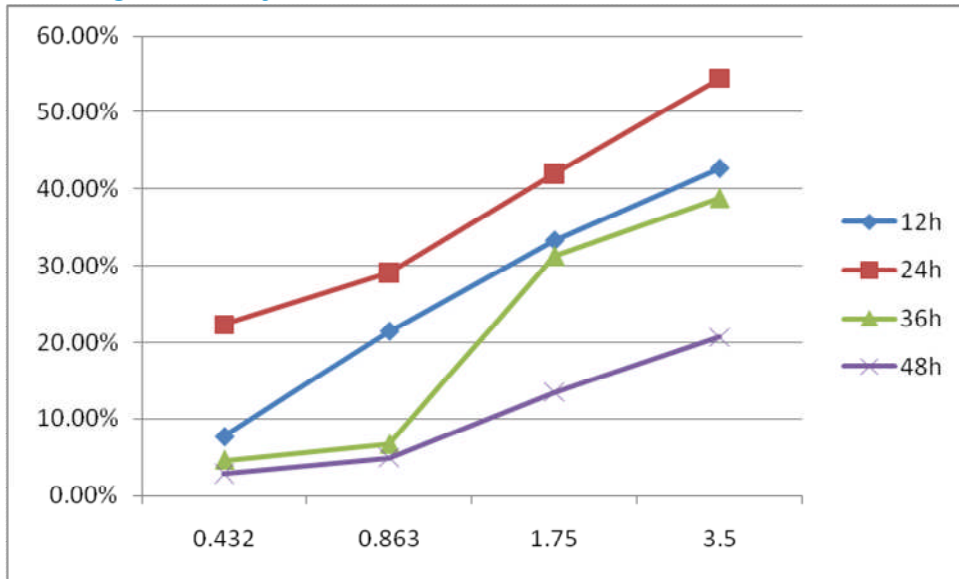


Figure 1: Dynamic inhibition curve of *Drynaria fortunei* methanol extract against SP20 cells

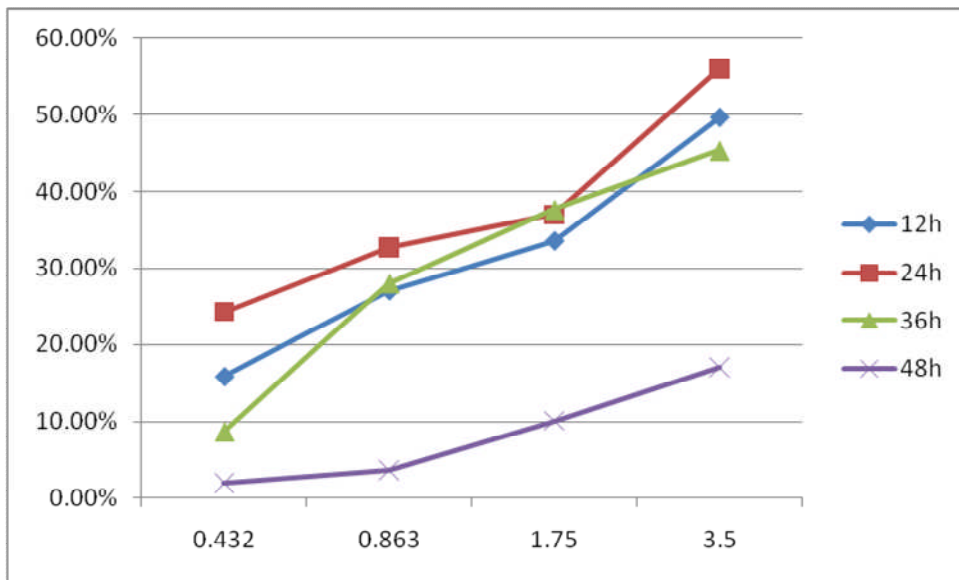


Figure 2: Dynamic inhibition curve of *Drynaria fortunei* ethanol extract against SP20 cells

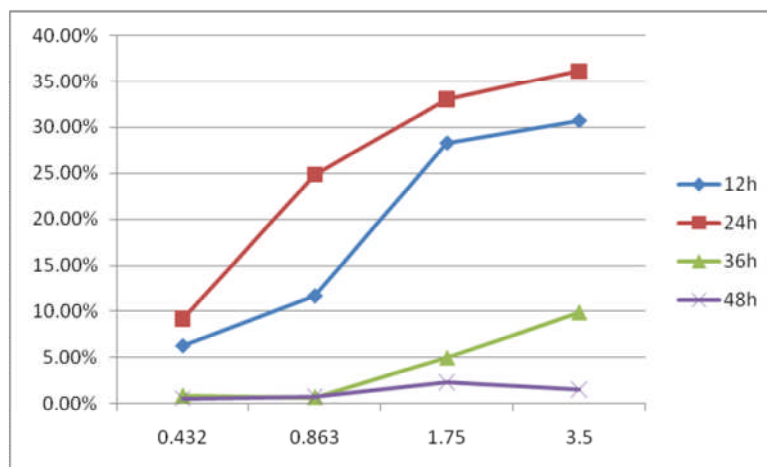


Figure 3: Dynamic inhibition curve of *Drynaria fortunei* petroleum ether extract against SP20 cells

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At present, majority of researches on *Drynaria fortunei* are focused on its liver and kidney nourishing and muscle- and bone-strengthening effects, prevention of adverse reactions induced by streptomycin and kalamycin, hypolipidemic effects, cardiogenic effects, increase of resistance to hypoxia in mice, analgesia and sedation, and also the reduction of platelet aggregation function in rabbits (Chen & Guan, 2006). A study reported the changes in the urinary metabolite spectrum in glucocorticoid-induced osteoporotic rats by UPLC-MS/MS and found that *Drynaria fortunei* ethanol extract had a relatively obvious intervention effect on it (Zhang et al., 2012). The total flavonoids from *Drynaria fortunei* can improve the osteoblast differentiation and mineralization in relation to advanced glycation end-products. The mechanism may be related to the increase of phosphorylation of p38 and ERK1/2 signalling proteins (Jin et al., 2012). In an experiment exploring the effects of *Drynaria fortunei* on serum interleukin-2 (IL-2), peripheral blood T cell subsets, brain cell apoptosis and Caspase-3 expression in severe craniocerebral injury rats, the results show that after taking *Drynaria fortunei*, serum IL-2 level decline is significantly improved and apoptosis is significantly reduced in severe craniocerebral injury rats at 24 h after injury, without affecting Caspase-3 expression, indicating that *Drynaria fortunei* has an inhibitory effect on early apoptosis in severe craniocerebral injury in rats. This effect may be related to its ability to fight against the decline in serum IL-2 content (Pan et al., 2012).

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