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# EXPERIMENTAL STUDY ON INHIBITION OF $S_{180}$ TUMOR CELLS BY *AGRIMONIA PILOSA* EXTRACT

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## Abstract

We evaluated the *in vitro* and *in vivo* inhibition of $S_{180}$ tumor cell growth of the water extract of *Agrimonia pilosa*. Inhibitory effect of *Agrimonia pilosa* on *in vitro*-cultured $S_{180}$ cell proliferation was measured by MTT assay; mice model of transplanted tumor was established, after 8 days of continuous administration, the tumors were removed, weighed, and compared with the control group, and the *in vivo* anti-tumor effect of *Agrimonia pilosa* on mouse sarcoma $S_{180}$ was compared using inhibition rate. The *in vitro* anti-tumor experiment indicated that the inhibition rate gradually increases with the increase of extract concentration and the extension of time, with $IC_{50}$ 175.64, 90.59, and 71.74 μg/ml at 24 h, 4 h, and 72 h respectively. In this study, the *in vitro* MTT assay was used to determine the inhibitory effect of *Agrimony pilosa* water extract on $S_{180}$ tumor cells, the method is simple, reliable, and practical; mice model of in vivo transplanted $S_{180}$ tumor was established, which allowed direct observation of tumor inhibitory effect, and thus found out that the water extract of *Agrimonia pilosa* has inhibitory effect on $S_{180}$ tumor cells.

**Keywords:** Agrimonia pilosa; $S_{180}$; Anti-tumor

## Introduction

*Agrimonia pilosa* is the aerial parts of the *Agrimonia pilosa Ledeb*, which belongs to the Rosaceae plant family; it is mainly used in the treatment of various hemorrhage, malaria, fatigue, etc (Xiao-dan Wu et al., 2008). In recent years, with the gradual clarification of chemical composition of *Agrimonia pilosa*, its pharmacological effects are gradually increasing (Ying Zhao et al., 2007). Main effects include anti-tumor, antibacterial, antioxidant, cardiotonic, hypoglycemic, immune enhancement, hypotensive, taenicide, analgesic, and anti-inflammatory (Yu-qun Li et al., 1999 ; Xia-hui Zou et al., 2002 ). In this paper, *in vitro* and *in vivo* anti-cancer effect of water extract of *Agrimonia pilosa* was studied, improving the experimental basis for the in-depth study of pharmacological effects of *Agrimonia pilosa*.

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## Materials and Methods

### Reagents and Apparatus

*Agrimonia pilosa* (purchased from Nepstar Drugstore); RPMI-1640 cell culture medium (GIBCO BRL); trypsin (Sigma); MTT (Sigma); fetal bovine serum (Hangzhou Shijiqing Biological Engineering Material Co., Ltd.); DMSO (Tianjin Kermel Chemical Reagent Co., Ltd.); *Agrimonia Pilosa* was differentiated as the aerial parts of the *Agrimonia pilosa* Ledeb by Henan College of Traditonal Chinese Medicine.

### Apparatus

96-well microplate (Biofil ); $CO_2$ incubator (Model CP, Japan); inverted microscope (Model CK, Olympus, Japan); Clean Bench (Sujing Group Antai Company); automatic microplate reader (Model 450, Bio-Rad, USA); Analytical balance FA2004 (Shanghai Balance Instrument Plant)

### Cancer Cell Strains

$S_{180}$ tumor cells (purchased from Cell Bank of Chinese Academy of Sciences)

### Preparation of *Agrimonia Pilosa* Water Extract

500g of dry *Agrimonia pilosa* was weighed and added with water 15 times its volume; the solution was extracted for three times using ultrasonic extraction with 1 h each; the extracts were combined, concentrated and freeze-dried; and powder was obtained for later use.

### Determination of Inhibitory Effect of the Drug on Mice $S_{180}$ Cell Growth with MTT Assay (Jing Gao et al., 2008)

Cells in the logarithmic growth phase were collected and added with 0.25% trypsin, and digested at 37 □ for 2 min, 10% fetal bovine serum RPMI1640 culture medium was added to stop the digestion, after centrifugation for 5 min, the supernatant was discarded. Fresh culture medium was added again and single cell suspension was prepared, after cell counting, the concentration of suspension was prepared as $1\times10^4$/ml. 100 μ l of the cell suspension was added into each well of the 96-well microplate, the microplate was placed at a 37 $^oC$, 5% $CO_2$ incubator and cultured for 24 h for cell adherence. Different concentrations of drugs were added in the cell culture microplate, with at least three parallel wells for each concentration; solvent control-treated cells were prepared as the control group. The cultivation was continued and 50 μl of MTT solution (1mg/ml) was added at 24 h, 48 h, and 72 h respectively. The incubation was continued for 4 more hours, and the supernatant was discarded, 200 μl of DMSO was added, and the microplate was placed and shaken on the microplate shaker. The absorbance was determined at 490 nm using a microplate reader, and the inhibition rate of tumor cells was calculated. The calculation formula is as follows:

Cell growth inhibition rate (%) = [(average value of the control group - average value of the treatment group) / average value of the control group] × 100%

The half maximal inhibitory concentration ($IC_{50}$) could be obtained by mapping of different drug concentrations and inhibition rate.

### Experiment of Transplanted $S_{180}$ Inhibitory Effect in Mice (Ying Hu et al., 2003)

KM mice weighing 18~22 g (half male half female) were inoculated intraperitoneally with murine $S_{180}$ cells. One week later, the mice were sacrificed by cervical dislocation, and the ascites were removed under sterile conditions and diluted with saline to the desired concentration. 200 μl of tumor effluent was inoculated

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subcutaneously into the right axillary of each mouse. On the next day of inoculation, the mice were randomly grouped and weighed, the *Agrimonia pilosa* extract group was divided into low, medium, and high dose groups, with doses of 10, 50, and 100 mg/kg respectively, and with cyclophosphamide as the positive control group, and normal saline the negative control group, each group consisted of 10 mice, they were intraperitoneally injected with the drug once a day, 200 µl per time, the administration continued for 8 days. 24 h after drug discontinuance, the mice were sacrificed by cervical dislocation and weighed, and the underarm tumor was removed and weighed. The tumor inhibition rate was calculated. The calculation formula is as follows:

Tumor growth inhibition rate (%) = [(average tumor weight of control group - average tumor weight of treatment group) / average tumor weight of control group] × 100%

**Determination of Mouse Peritoneal Macrophage Phagocytosis**

Another 50 mice were inoculated with tumor strains and grouped, dosing schedule was the same as the schedule prescribed in section above, in the 8th day, 1 h after last administration, each mouse was intraperitoneally injected with 1 ml of 5% (V/V) chicken red blood cell suspension, 30 min later, the mice were sacrificed by cervical dislocation, and intraperitoneally injected with 2 ml of saline, after gently rubbing the mouse abdomen for 1 min, 1 ml of peritoneal washes were drawn and dropped on the glass slide (two parallel samples), and incubated at 37 $^{o}$C for 30 min, and then rinsed with saline, dried, and fixed. After giemsa staining, 100 macrophages were observed and counted under the microscope, and phagocytic percentage and phagocytic index were calculated in accordance with the following formula:

Macrophage phagocytic percentage = swallow chicken erythrocytes / 100

Phagocytic index = 100 phagocytes engulfed by the total number of chicken erythrocytes / 100.

## Results and Discussion

The MTT colorimetry assay was used to observe the $S_{180}$ cell growth inhibition at 24 h, 48 h, and 72 h time points; and relatively obvious sarcoma cell proliferation effect was found, which was featured by dose dependent and time dependent inhibition (Tables 1 and 2). In the high concentration group (1000 µg/ml), the inhibition rate reached 69.23 % at 24 h, and with the extension of time, cell growth inhibition was markedly enhanced, after 72 h, the inhibition rate reached 76.92%. With the decrease of concentration, the inhibitory effect was also decreasing. In the medium concentration group (125 µg/ml), the inhibition rate reached 58.62 % after 48 h. And in the low concentration group (15.63 µg/ml), the inhibitory rate was 26.37 % after 72 h, which was not quite obvious. Studies by other researchers also demonstrated the existence of dose-time dependence in the inhibitory effect of *Agrimonia pilosa* on tumor cells (Wei Guo et al., 2002).

Phagocytic percentage and phagocytic index gradually increased with the increase of concentrations of *Agrimonia pilosa* extract (Table 4), which was especially significant in the high dose group ($P < 0.05$), while the positive drug cyclophosphamide had a certain inhibitory effect on the phagocytosis of peritoneal macrophages.

Animal transplantable carcinomas experimental method is a commonly used experimental method, it is easy to implement, where a large number of uniformly grown tumors can be obtained at the same time, during the administration period, inhibition effect of the drug on tumor growth can be determined through the observation of general condition of the mice, such as diet, activity, weight and mortality. After dissection, the tumor was separated, and the tumor inhibition status can be directly observed, which is irreplaceable by any other *in vivo* experiments. In the $S_{180}$ ascites dilution, the ratio with saline is generally about 1:3 or 1:4. After dilution, the solution should be placed in the sterile flat bottom Erlenmeyer flask, and immersed in an ice bath maintained at low temperatures. To ensure each laboratory mouse was inoculated with the same number of cells, the solution should be gently shaken during each extraction.

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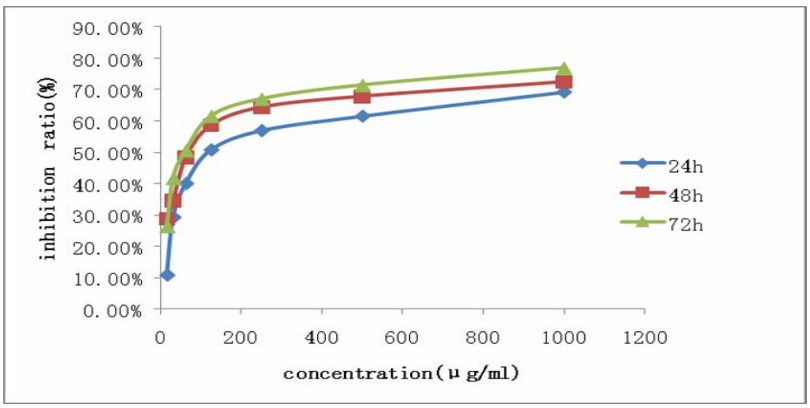


**Figure 1** Inhibition Rates of $S_{180}$ Cells by Different Concentrations of *Agrimonia Pilosa* Extracts

**Table 1** Inhibition Rates of $S_{180}$ Cells by Different Concentrations of *Agrimonia Pilosa* Extracts

| concentrations(μg/ml) | 24h（%） | 48h（%） | 72h（%） |
|---|---|---|---|
| 15.63 | 10.77 | 28.74 | 26.37 |
| 31.25 | 29.23 | 34.48 | 41.76 |
| 62.5 | 40.00 | 48.28 | 50.55 |
| 125 | 50.77 | 58.62 | 61.54 |
| 250 | 59.92 | 64.37 | 67.03 |
| 500 | 61.54 | 67.82 | 71.43 |
| 1000 | 69.23 | 72.41 | 76.92 |

**Table 2:** $IC_{50}$ of $S_{180}$ Cells by *Agrimonia Pilosa* Extract at Different Time Points

| **Time** | **24h** | **48h** | **72h** |
|---|---|---|---|
| $IC_{50}$(μg/ml) | 175.64 | 90.59 | 71.74 |

It can be seen from Figure 1 that the inhibitory effect of the drug gradually increased with the gradual increase of concentrations, and the extension of time.

**Table 3:** Effect of *Agrimonia Pilosa* Extract on Body Weight and Tumor Weight of $S_{180}$ Inoculated Mice ($\bar{x}$ ± s, n = 10)

| **Group** | **Dosage (mg/kg)** | **Body weight (g) Before administration** | **After administration** | **Tumor weight (g)** | **Average inhibition rate (%)** |
|---|---|---|---|---|---|
| Low dose group | 10 | 20.36±1.56 | 25.63±1.02 | 1.87±0.35 | 25.50* |
| Medium dose group | 50 | 21.42±0.73 | 26.19±0.85 | 1.36±0.28 * | 45.81* |
| High dose group | 100 | 19.98±2.41 | 24.04±1.37 | 0.81±0.31 * | 67.73** |
| Positive control group | 100 | 22.16±1.81 | 25.15±1.19 | 0.46±0.25 * | 81.67** |
| Negative control group | - | 21.37±0.92 | 27.01±0.92 | 2.51±0.41 | - |

Note: comparison with the negative control group: * p <0.05, ** p <0.01

**Table 4:** Effect of *Agrimonia Pilosa* Extract on Phagocytic Percentage and Index of $S_{180}$ Transplanted Mice ($\bar{x}$ ± s, n = 10)

| **Group** | **n** | **Phagocytic percentage（%）** | **Phagocytic index（%）** |
|---|---|---|---|
| Low dose group | 10 | 30.3±5.5 | 1.12±0.35 |
| Medium dose group | 10 | 41.4±8.7 | 1.17±0.28 |
| High dose group | 10 | 52.9±6.4 * | 1.28±0.31 * |
| Positive control group | 10 | 25.1±3.9 | 0.86±0.25 |
| Negative control group | 10 | 28. 7±4.9 | 0.91±0.41 |

Note: comparison with the negative control group: * p <0.05

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In this experimental study, evident inhibitory effect of *Agrimonia pilosa* water extract on $S_{180}$ cells and its dose-time dependence were found with the MTT assay. *In vivo* mice transplanted $S_{180}$ model experiment the tumor inhibition effect can be observed directly, and thus found out that the inhibitory effect of *Agrimonia pilosa* on the growth of $S_{180}$ tumor cells exists both *in vitro* and *in vivo*, which was consistent with the reported literature (Jing Yuan et al., 2000 ; Kai-min Gao et al., 2000).

This experimental study found that the water extract of *Agrimonia pilosa* can enhance the immune function of $S_{180}$ transplanted mice, and promote the phagocytic activity of macrophages, and such immune function enhancement showed a dose dependent trend. Moreover, the effects of *Agrimonia Pilosa* extracts on improving organism immunity are primarily proved, of which the exact action mechanism should be further evaluated.

## Acknowledgement

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## References

1. Xiao-dan Wu, Zhe-xiong Jin. Research Progress on Agrimonia Pilosa Ledeb , 2008:297-304.
2. Ying Zhao, Chun-fang Zhao, Jin-ping Liu et al. Study on Chemical Composition and Biological Activity of Agrimonia Pilosa Ledeb. Special Wild Economic Animal and Plant Research, 2007, 257-61.
3. Yu-qun Li. Research Progress on Anti-Tumor Effect of Agrimonia Pilosa Ledeb. China Pharmaceuticals, 1999, 8 (11): 60.
4. Xia-hui Zou. Experimental Study on Anticancer Effect of Agrimonia Pilosa Ledeb on Hepatocellular Carcinoma SMMC-7721 Cells. Acta Academiae Medicinae Jiangxi, 2002.
5. Jing Gao. Study on Effective Fractions of Agrimonia Pilosa Ledeb Compound and Its Anti-tumor Effect. Peking Union Medical College, Chinese Academy of Medical Sciences, 2008.
6. Ying Hu, Zeng-zhen Wang, Fan-bo Zeng et al. Study on Inhibitory Effect of Allium Fistulosum on Growth of Transplanted S180 Tumors in Mice. Cancer Prevention and Treatment Research, 2003, 30 (5): 393-394.
7. Wei Guo, Ze-zhen Zhao, Bao-en Dan et al. Experimental Report on Anti-Mutagenic and Anti-Tumor Activity of Six Kinds of Chinese Herbal Medicine. Carcinogenesis, Teratogenesis and Mutagenesis, 2002, 14 (2): 94-98.
8. Jing Yuan, Yuan-xun Wang, Zheng-ming Hou et al. In Vitro Inhibitory Effect of Agrimonnin on Human Tumor Cells. Chinese Journal of Traditional Medical Science and Technology, 2000, 7 (6): 378-379.
9. Kai-min Gao, Ling Zhou, Jin-ying Chen et al. In Vitro Induction of Apoptosis of HL-60 Cells by Agrimony Decoction. Journal of Chinese Medicinal Materials, 2000, 23 (9): 561-562.