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Effects of *Hydrocotyle sibthorpioides* extract on transplanted tumors and immune function in mice

Farong Yu[a], Fahong Yu[b,*], P.M. McGuire[b], R. Li[c], R. Wang[c,**]

[a]*Gansu Institute of Political Science and Law, Lanzhou 730070, China*
[b]*Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610, USA*
[c]*School of Life Sciences, Lanzhou University, Lanzhou 730000, China*

Abstract

This paper describes the effects of an ethanolic extract of *Hydrocotyle sibthorpioides* on transplanted tumors and immunologic function in mice. When the *H. sibthorpioides* extract was administered orally at a dose of 1.5 or 3.0 g/kg body wt./day for 10 days, the inhibition rates for murine hepatic carcinoma clone (Hep), sarcoma 180 crocker clone (S_{180}), and uterine cervical carcinoma clone (U_{14}) were significantly enhanced. The antitumor activity of *H. sibthorpioides* is comparable to that of the common antitumor agent 5-fluorouracil. Also, our results indicate that the *H. sibthorpioides* extract promoted the thymus and spleen indices, and humoral immunity of mice. These observations demonstrated that *H. sibthorpioides* exerted a potent inhibitory effect on the growth of tumors, in addition to mediating immunomodulatory effects in mice.

Keywords: *Hydrocotyle sibthorpioides*; Antitumor effect; Tumors; Immunological function; Mice

Introduction

The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs in cases where synthetic medicine could not relieve patients who suffer from hard-to-cure illnesses. The shining pennywort or maritima, *Hydrocotyle sibthorpioides*, is a widespread and uncultivated perennial herb which holds an important place in Chinese herbal medicine. Many studies have confirmed the usefulness of *H. sibthorpioides* in the treatment of human diseases. For example, *H. sibthorpioides* is recommended in the treatment of psoriasis (Li, 1986), rheumatalgia, dysentery, whooping cough, jaundice, and hepatitis B virus infections (Li, 2000). It also plays a significant role in traditional Chinese medicine for treating a myriad of mankind's afflictions including herpes zoster (Wang, 2000), kidney stones, and liver cancer. However, no studies to date have been able to demonstrate the antitumor effect of *H. sibthorpioides* in transplanted tumors. The effect on immunologic function is also not fully understood and the pharmacological data for this herb are incomplete.

Hepatic carcinoma (Hep), sarcoma (S_{180}), and uterine cervical carcinoma (U_{14}) are highly malignant tumors in mice. 5-Fluorouracil (5-FU) is one of the many chemotherapeutic agents used to treat a variety of tumors by intravenous or oral administration in mice and rats (Ferguson, 1980; Habs et al., 1981; Sumie et al., 2003). Its antitumor effect is superior to that of any other single agent in the treatment of sarcoma (Bertino et al., 1977; Ikenaka et al., 1979; Iigo et al., 1983), liver and colorectal cancers (Ensminger et al., 1978; Rougier et al., 1992;

*Corresponding author. Tel.: 352 371 4749; fax: 352 392 1445.
**Also corresponding author.
E-mail addresses: fyu@ufl.edu (F. Yu), wangrui@lzu.edu.cn (R. Wang).

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Tomlinson et al., 2002), and carcinomas of the colon, rectum, and uterine cervix (Hidalgo et al., 2003; Thigpen et al., 1995; Maneo et al., 1999; Smalley et al., 1978).

To study the antitumor activity of *H. sibthorpioides,* Hep, S_{180}, and U_{14} were implanted into BALB/c mice. The tumor-bearing mice were administered the *H. sibthorpioides* extract and the antitumor effect was compared with that of 5-fluorouracil. Also, using BALB/c mice as an experimental model, we assessed the immunomodulatory effects of the *H. sibthorpioides* extract, as measured by the index of blood clearance, 50% complement hemolytic activity, and spleen and thymic indices.

Materials and methods

Plant material

H. sibthorpioides was collected between summer and autumn and the voucher specimen was retained in our laboratory for future reference. The entire plant was shredded and dried at room temperature prior to extraction. The extract was purified by high-performance liquid chromatography (HPLC) method and the chemical constituents were identified using gas chromatography–mass spectrometry (GC–MS).

Preparation of extraction

Air-dried *H. sibthorpioides* was extracted using 75% ethanol. The protocol for extraction is as follows:

1. The shredded *H. sibthorpioides* was soaked in distilled water and incubated at 40–50 °C for 120 min. The ratio of plant material to water was 1:5.
2. The mixture was extracted at 100 °C for 90 min and the extraction was transferred to a glass container by decanting.
3. The plant residue was decocted further at 100 °C for 60 min after adding the same amount of distilled water as in step 1. The supernatant was transferred to the same glass container.
4. After adding 75% ethanol slowly to the filtered decoction, the mixture was incubated at room temperature for 12 h. To be efficient, it is essential that the residue and ethanol are mixed thoroughly.
5. The mixture was filtered and transferred to a new glass container, and the residue was extracted further by repeating step 4. A second extraction will increase yields of the extract by 8%.
6. The extract was concentrated under reduced temperature and pressure and decanted in a vacuum desiccator. The extract was measured by HPLC using Spherisorb C18 column (250×4.6 mm) with the gradient elution (acetonitrile–H_2O (50:50)) and a wavelength of 225 nm; the final extraction yield is 32.6%. The chemical constituents were identified by GC–MS. The extract was diluted with distilled water into different doses for the experiment.

Animals

Two hundred-seventy BALB/c mice weighing 20–22 g each were obtained from the Laboratory Animal Center of the Gansu Academy of Medical Science, China, and were housed in groups in stainless steel cages (20 cm high, 25 cm wide, and 34 cm deep). Room temperature was controlled at 20 ± 3 °C with a 12-h light–dark cycle. Food and water were offered ad libitum. Prior to the experiment, all animals were maintained for an acclimatization period of 7 days under laboratory conditions.

Measurement of the inhibition rate in transplanted tumors

One hundred-fifty BALB/c mice were divided randomly into five groups of 30 animals each. In each group, 10 animals each were inoculated with the hepatic carcinoma (Hep), the sarcoma 180 crocker (S_{180}), or the uterine cervical carcinoma 14 (U_{14}) tumor-derived cell lines, which were provided by the Chinese Academy of Medical Sciences (Beijing). All clones were implanted into the dorsal subcutaneous tissue at a dose of 1×10^7 cells in 0.1–0.2 ml for each animal. On the 25th day, the control group (group I) was treated with normal saline (450 mg/kg body wt/day). Groups II, III, and IV, the three treated groups, were administered the *H. sibthorpioides* extract orally at dosages of 1.0, 1.5, and 3.0 g/kg body wt/day, respectively. Group V (the chemotherapy group) was treated with the standard anticancer compound 5-fluorouracil (5-FU, 100 mg/kg body wt/day) by intraperitoneal injection. All treatments were terminated after a total duration of 10 days. The inoculated tumors were then retrieved and weighed. The tumor inhibition rates were calculated using the following equation (Liu et al., 1999):

Tumor inhibition rate
= [(Average tumor weight of the control group (group I)
− Average tumor weight of the treated group)/
Average tumor weight of the control group (group I)]
× 100%.

Measurements of phagocytosis by the reticuloendothelial system, blood hemolysin, and the weights of immune organs

One hundred-twenty BALB/c mice were divided into four groups of 30 animals each. The control group (group I) was treated with normal saline at 450 mg/kg