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**Chinese Herbal Medicines (CHM)**

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

## Original article

# Antipyretic Effects of Liposoluble Fractions of *Viola yedoensis*

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ARTICLE INFO

*Article history*

Received: June 16, 2014

Revised: August 6, 2014

Accepted: September 9, 2014

Available online: January 12, 2015

DOI:

10.1016/S1674-6384(15)60024-7

ABSTRACT

**Objective** To clarify the antipyretic effect of the Chinese materia medica, *Violae Herba* (*Viola yedoensis*), and its active fractions by examining the effects of *V. yedoensis* extracts with differing polarities on body temperature, total white blood cell (WBC) count, WBC differential count, and total serum complement of rabbits with lipopolysaccharide (LPS)-induced fever. **Methods** The rabbits were treated with water and ethanolic extracts of *V. yedoensis*, as well as petroleum ether, ethyl acetate, and *n*-butanol fractions of the ethanolic extract at low-, mid- and high- doses. The LPS was injected via the ear vein of rabbits in model and treatment groups 30 min post-gavage. Their body temperature was measured at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 h after the LPS challenge to calculate the temperature changes and thermal response index. After the last temperature measurement, blood samples were collected to determine the blood cell counts and total serum complement ($CH_{50}$) level. **Results** Compared with the model group, body temperature was significantly lower in the low-dose ethanolic extract group, low- and mid-dose petroleum ether fraction groups, and all three ethyl acetate fraction groups. Serum $CH_{50}$ levels were lower in all treatment groups, except the ethanolic extract groups, than that in the model group, with no significant difference. *V. yedoensis* had no significant effect on the blood cells of febrile rabbits challenged with LPS for 6 h. **Conclusion** The antipyretic effects of *V. yedoensis* are strong, and its active fractions are the petroleum ether and ethyl acetate fractions of ethanolic extract.

*Key words*

anti-complement; antipyretic effect; heat-clearing; *Viola yedoensis*



## 1. Introduction

*Violae Herba* is a Chinese materia medica for clearing away heat and toxic materials. It is the dried whole plant of the perennial herb, *Viola yedoensis* Makino (Family Violaceae) (Pharmacopoeia Committee of P. R. China, 2010). It has the effects of clearing away heat, detoxicating, cooling blood, and subduing swellings. In clinical practice, it is mainly used for furunculosis, sores, swellings, ulcers, erysipelas, acute mastitis, and acute appendicitis, as well as other suppurative and infectious diseases. Modern pharmacological studies have found that *V. yedoensis* has anti-inflammatory, antibacterial,

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Fund: National Natural Science Foundation of China (81073025); Science and Technology and Commission of Shanghai Municipality (09DZ1970100 and 09DZ2270900); Scientific Research Foundation for Returned Overseas Chinese Scholars, Ministry of Education of China

anti-HIV, immunomodulating, and antihyperuricemic effects. For example, Chen et al (2008) and Kang (2012) demonstrated that the decoction and ethyl acetate (EA) fraction of the ethanolic extract of *V. yedoensis* had a strong inhibitory effect on *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and other bacteria. In an antiviral study, eight cyclotides separated from *V. yedoensis* were shown to have anti-HIV activity *in vitro* (Wang et al, 2008). However, it is worth noting that the antibacterial and antiviral activity of *V. yedoensis* is difficult to reproduce. Our previous attempts failed to demonstrate a significant antibacterial or anti-HIV activity with the water and ethanolic extracts and fractions of differing ethanolic extract polarities (data not shown). Nonetheless, *V. yedoensis* indeed had a high anti-inflammatory activity; For example, its decoction and ethanolic extract significantly inhibited xylene-induced mouse ear swelling (Chen et al, 2008). Moreover, we recently proved that a petroleum ether (PE) fraction of the ethanolic extract of *V. yedoensis* had a significant therapeutic effect on lipopolysaccharide (LPS)-induced acute lung injury in mice; The results suggested that it might reduce acute lung injury by reducing pulmonary capillary permeability and pulmonary edema and inhibiting a local inflammatory response through its anti-inflammatory mechanism (Li et al, 2012). The anti-inflammatory activity may be related to its immuno-modulating action, for example, Li and Hu (2012) reported that the anti-inflammatory effects of the water and ethanolic extracts of *V. yedoensis* might be associated with the decreased expression of TNF-α, IL-1β, and $PGE_2$. It is not yet known whether *Violae Herba* has a definite antipyretic effect as a heat-clearing drug and whether this effect is one of the mechanisms for its treatment of acute lung injury. Therefore, this study aimed to examine the antipyretic effect of *V. yedoensis* and its active substances, in order to provide a systematic exposition of the heat-clearing effect of *Violae Herba* based on the changes in white blood cell (WBC) count and complement level.

## 2. Materials and methods

### 2.1 Chemicals and reagents

Water extract (0.61%) and ethanolic extract (10.47%) of *Viola yedoensis* Makino and PE (3.07%), EA (1.30%), and *n*-butanol (BU, 0.60%) fractions of the ethanolic extract were self-made (Li et al, 2012). Aspirin (Nanjing Baijingyu Pharmaceutical Co. Ltd., China), *Escherichia coli* O111B4 LPS (ET; Sigma-Aldrich, USA), sodium pentobarbital (Sigma), dimethylsulfoxide (DMSO), sodium carboxymethyl cellulose (CMC-Na), and ELISA kit for the total complement ($CH_{50}$) of the rabbit serum (Meilian Bio-Tech Co. Ltd., Shanghai, China) were used.

### 2.2 Experimental animals

One-hundred and forty New Zealand white rabbits, male or female, weighing (2.5 ± 0.2) kg (Shengwang Laboratory Animal Co. Ltd., China; license No. SCXK (Shanghai) 2007-0007) were used. The animals were housed at room temperature of 16−26 ºC with humidity of 40%−70%.

### 2.3 Equipments

Ordinary household mercury thermometer, ADVIA 120 Hematology Analyzer with a five-part differential capability (Bayer AG, Germany), Synergy HT Multidetection Microplate Reader (BioTek, VT), and catheters for human were used.

### 2.4 Drug solution preparation

We prepared the stock solution (100 mg/mL) to be tested as follows: 2 g water and the ethanolic extracts of *V. yedoensis* and fractions of each ethanolic extract were added to 200 μL DMSO and dissolved in 20 mL water with ultrasound. The resulting solutions were transferred into tubes (5 mL each) and stored at −70 ºC. They were diluted with distilled water into low-dose (2 mg/mL), mid-dose (6 mg/mL), and high-dose (10 mg/mL) solutions just before use. The control group was given a water solution containing 0.1% DMSO (same as high-dose solution). Preparation of aspirin suspension (20 mg/mL) was performed as follows: one aspirin tablet (0.4 g) was ground, and 0.5% CMC-Na was added to achieve 20-mL volume. After mixing, 0.1% DMSO was added to the mixture. Preparation of LPS solution (0.25 μg/mL) was then performed as follows: 10 mg LPS was diluted with 50 mL nonpyrogenic saline, added to 0.2 mg/mL stock solution, and stored at 4 ºC for later use. The stock solution was ultrasonically treated for 20 min, and 40 mL normal saline was added to 50 μL stock solution and mixed just before use.

### 2.5 Selection and screening of eligible rabbits

Healthy and eligible rabbits were selected, and their temperature was measured daily for consecutive 3 d. Rabbits with the temperature of 39.6−40.0 ºC and a fluctuation of less than 0.3 ºC were finally selected for the experiment.

One-hundred and eight eligible rabbits were randomly divided into 18 groups ($n$ = 6), such as normal group, model group, positive control (aspirin) group, as well as low-, mid-, and high-dose PE fraction of *V. yedoensis* ethanolic extract groups; low-, mid-, and high-dose EA fractions groups; low-, mid-, and high-dose BU fraction groups; and low-, mid-, and high-dose *V. yedoensis* water extract groups. The low-, mid-, and high doses were set at 10, 30, and 50 mg/kg, respectively.

### 2.6 Body temperature measurement of rabbits with an LPS-induced fever

The body temperature of each rabbit was measured twice before the experiment at a 20-min interval, and the average was used as the basal body temperature before administration. The rabbits in each treatment group were ig administered with an equal volume (5 mL/kg) of corresponding medication, and