Journal of Ethnopharmacology 131 (2010) 220–223

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

# The *in vivo* expectorant and antitussive activity of extract and fractions from *Reineckia carnea*

Na Han[a], Chunling Chang[b], Yichun Wang[a], Ting Huang[a], Zhihui Liu[a], Jun Yin[a,*]

[a] *Department of Pharmacognosy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China*
[b] *Shenyang Jingcheng Pharmaceutical Company, Shenyang, China*

ARTICLE INFO

*Article history:*
Received 25 January 2010
Received in revised form 2 June 2010
Accepted 4 June 2010
Available online 18 June 2010

*Keywords:*
*Reineckia carnea*
Expectorant
Antitussive
Cough incubation period
Effective fraction

ABSTRACT

*Aim of study:* To evaluate the potential expectorant and antitussive activity of a traditional Chinese medicine.
*Materials and methods:* The water extract and four fractions of the aerial part of Reineckia carnea were orally administrated to coughing mice induced by ammonium hydroxide and mice injected with phenol red, respectively, to investigate their medification effect on coughing and mucus scretion.
*Results and discussion:* 90% Ethanol fraction significantly lengthened the latent period of cough and decreased cough frequency caused by ammonium hydroxide at the dose of 0.372 g/kg ($p < 0.05$). Sixty percent ethanol fraction reduced the cough frequency as well as the mucus secretion from mouse tracheas obviously at the dose of 0.570 g/kg ($p < 0.05$) by measuring the tracheal output of phenol red in mice. The medication effects in multiple doses of the active fractions were then performed and it has been proved that the 60% ethanol and 90% ethanol fraction were curatively effective on expectoration and coughing, respectively both at the high and middle dose, which supplied proofs for the further research on chemical constituents in both of two effective fractions.


## 1. Introduction

Coughing is a main symptom of respiratory illness with a worldwide high incidence, which would cost the patients 3 billion dollars per year just in America. Normally, coughing is a natural protective reflex helping people get rid of fremde stoffes or airway secretions from respiratory tract. However, the pathological coughing which was mainly caused by pathogen invasion of respiratory passages would bring lots of troubles like laryngalgia, dumb and so on. The mechanism that medulla oblongata controlled a coughing reflex was often utilized to develop antitussive drugs such as codeine and dextromethorphan, which nonetheless are limited in using extension due to the multiple adverse effects. Another issue is that most of the drugs are effective on tussiculation but powerless to the patients with superfluous phlegm production, even contrarily aggravate coughing or threaten the patients' life. Thus it is necessary to develop a drug which owns not only antitussive but also expectorant activity as well as less adverse effects on bodies.

In traditional Chinese medicine, coughing and expectoration were tightly related with lung disease such as lung affection, heat and dryness. Some natural materials, which always had the function of removing the heat from the lung or moistening the lung, were specialized as drugs of resolving phlegm, relieving cough and asthma.

As one of such herbs, *Reineckia carnea*, whose aerial part is used for preventing cough, eliminating phlegm, rheumatism disease treatment and hepatitis (Zhang et al., 2003), was firstly documented in a traditional Chinese herbal *Bencao Shiyi* in Tang dynasty. *Reineckia carnea* is a famous natural medicinal drug in Miao Minority in the southern China and mainly distributed in Guizhou and Yunnan province. It is also a primary ingredient in many famous Chinese formulas such as Kesuting Syrup and Jiangxiangcao Troche, which used for coughing and excessive phlegm patients with perfect curing efficacy, showing that the aerial part of *Reineckia carnea* may contain some effective compounds that would develop into ideal antitussive drug.

On clinical application, the aerial part of *Reineckia carnea* is often decocted with water for antitussive and expectorant usage. Even though it has been reported that the active compounds may be saponins (Zhang et al., 2006), the researchers have not given sufficient proof and illuminated the specific substances. In our research, the aqueous extract of aerial part of *Reineckia carnea* was subjected to the chromatographic separation on Diaion-101 macroporous desorption resin column to give water, 30%, 60% and 90% ethanol–water fractions successively, and antitussive effects and expectorant activity of each fraction were investigated, respectively for further research on chemical constituents.

* Corresponding author. Tel.: +86 24 23986491; fax: +86 24 23986460.
*E-mail address:* yinjun2002@yahoo.com (J. Yin).

0378-8741/$ – see front matter. 
doi:10.1016/j.jep.2010.06.024

## 2. Materials and methods

### 2.1. Extraction and isolation

Aerial parts of *Reineckia carnea* were collected from Guizhou province in China and authenticated by Professor Jun Yin in pharmacognosy department in Shenyang pharmaceutical university. A voucher specimen coded SPU 2180 was deposited in herbarium of the university. Crude drugs were refluxed with water for two times, each for 1.5 h. Then the aqueous extract was stand for 12 h and the supernatant was centrifuged at 3000 rpm for 15 min. The centrifuged supernatant was concentrated under reduced pressure and subjected to chromatographic separation on D101 macroporous adsorption resin column, eluted with water, 30%, 60% and 90% ethanol–$H_2O$ to give four fractions.

### 2.2. Animals

Kunming mice of either sex (18–22 g) for antitussive and expectorant experiments were purchased from animal experiment centre of Shenyang Pharmaceutical University (Shenyang, China). All animals were housed in pathogen-free conditions in accordance with the principles approved by the animal ethical committee of Shenyang Pharmaceutical University and allowed free access to sterilized food and water.

### 2.3. Antitussive activity of extract and fractions

After 3 days of adaption, the mice were exposed to a desiccator with ammonium hydroxide (0.2 ml) cotton ball to record the incubation time and cough frequency. Mice with latent period less than 1 min and the cough frequency more than three times in 1 min were chosen to be eligible animals. After 1 week, 84 eligible mice were divided into different groups (12/group) randomly, including control, positive control and delivery groups. Mice in positive control were given *i.g.* 0.5% pentoxyverine (0.8 g/kg), and mice in delivery groups were given fractions or the aqueous extract, respectively. The administration dose of extract and fractions were designed to be 2.716 g/kg (water extract), 1.052 g/kg (water fraction), 0.722 g/kg (30% ethanol fraction), 0.570 g/kg (60% ethanol fraction) and 0.372 g/kg (90% ethanol–water fraction), which were calculated by coefficient commutation of somatotypes according to the clinical dose of this plant and yield of extract and fractions (clinical dose: 0.34 g/kg, yields: water extract 38.7%; 30% ethanol extract 26.6%; 60% ethanol extract 21.0%, 90% ethanol extract 13.7%). The administration had been lasted for 5 days and the mice were exposed to a desiccator with ammonium hydroxide (0.2 ml) cotton ball after 30 min of the last administration and the cough incubation period was recorded. After 1 min, the mice were taken out from the desiccator and placed in a beaker and the frequency of cough within 2 min was observed and recorded (Xu et al., 2005).

### 2.4. Antitussive activity of 90% ethanol fraction

In order to investigate the activity of 90% ethanol fraction, medication with multiple doses were applied to coughing mice models using the same method as above. The eligible mice were divided into five groups: control, high dose group of 0.744 g/kg, middle dose group of 0.372 g/kg, low dose group of 0.186 g/kg and positive control. The administration lasted for 5 days and after that the mice were put into a desiccator with ammonium hydroxide cotton ball for recording the incubation time. One minute later, the mice were taken out and the coughing times were counted within 2 min.

### 2.5. Expectorant activity of extract and fractions

After 1 week of adaption, Kunming mice were divided into different groups (12/group) randomly, including control, positive control and delivery groups. Mice in positive control were given *i.g.* 0.5% ammonium chloride (1 g/kg), and mice in delivery groups were given fractions or the aqueous extract, respectively. The doses of extract and fractions were as same as those in antitussive experiment. The administration was last for 5 days; mice were injected with 5% phenolsulphonphthalein after 30 min of the last administration. Forty five minutes later, they were dissected to taken out trachea and bronchio parts and then 5% $NaHCO_3$ was used to flush the inside of these parts three times, 0.5 ml for each time. Collected all the flushed water and centrifuged at 3000 rpm for 15 min and absorbance of the centrifuged supernatant was measured at the wavelength of 546 nm using 752N UV–vis spectrophotometer produced in Precision Scientific Instrument Limited Company in Shanghai of China (Xu et al., 2005).

### 2.6. Expectorant activity of 60% ethanol fraction

The expectorant activity of 60% ethanol fraction in multiple doses was studied using the same method of Xu et al. (2005). Briefly, mice were separated into five groups: control, high dose group of 1.140 g/kg, middle dose group of 0.570 g/kg, low dose group of 0.285 g/kg and positive control group. The delivery methods were as same as those in expectorant activity test of extract and fractions. After 30 min of the last administration, mice were injected with 5% phenolsulphonphthalein and later were dissected to taken out trachea and bronchio parts. Five percent $NaHCO_3$ was used to flush the inside of these parts three times, 0.5 ml for each time, which were collected and centrifuged to measured the absorbance of supernatant at the wavelength of 546 nm.

### 2.7. Statistical analysis

The data of incubation period and coughing times are expressed as mean ± SE and the absorption is as mean ± SD. Statistical significance between groups was determined using one-way analysis of variance followed by LSD test in the condition of variance homogeneity and Dunnett's T3 test in variance heterogeneity. The significance of difference was considered to be obvious as $p < 0.05$.

## 3. Result and discussion

The antitussive animal models could be established by mechanical stimulus, electrical stimulus and chemical stimulus. In our experiments, the method with chemical stimulus by ammonium hydroxide was applied due to the simple procedure that omitted of anesthetization and usually used in new drug development for traditional Chinese medicines. Therefore, the antitussive activity of the extract and four fractions of *Reineckia carnea* were demonstrated in *in vivo* experiment by prolonging the incubation time, and reducing coughing times in 2 min. The cough incubation period, which is time interval from exposition to ammonia hydroxide to start coughing, exhibited the potential of the drug on delaying cough. The longer cough incubation period showed stronger effect of the drug on relieving cough and the less cough times exhibited its stronger antitussive effect. As shown in Fig. 1, even though it was clinically used for thousands of years, the water extract did not show excellent activity in mice as we expected, which may be due to the species difference between human and mouse. However, 90% ethanol fraction significantly prolonged cough incubation time as well as reduced coughing times of mice, implying that *Reineckia carnea* was actually curative for reducing coughing but the traditional medication