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Antitussive Effect of *Fritillaria unibracteata* Hsiao et K. C. Hsia.

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Abstract - This experiment was conducted to clarify the antitussive effect of *Fritillaria unibracteata* Hsiao et K. C. Hsia. The antitussive effect in ethanol (75, 50, 30%) extracts from *F. unibracteata* was relatively high. The ethanol extracts of 20, 40 mg/kg concentrations were especially good antitussive effect. The ethanol extracts of *F. unibracteata* showed lower antitussive activities of 20~30% than sodium chloride injection used as a blank space control.

Key words - *Fritillaria unibracteata* Hsiao et K. C. Hsia, Antitussive effect

Introduction

The *Fritillaria* in Liliaceae is a major source of Chinese medicine. It exist about 130 species all over the world, and mainly distributed in the Northern Hemisphere temperate zone (Lis *et al*., 2001; Hua *et al*., 2003; Lin *et al*., 2001). Its pharmacodynamic is the antitussive effects, expectorant effects, asthma, anti-bacterial effects, sedation, analgesia, cardiovascular function (Hua *et al*., 2005), anti-ulcer effect (Cao *et al*., 2009), anti-platelet aggregation (Xiao *et al*., 2007) and anti-tumor effect (Wang *et al*., 2002).

In recent years, researches on cough medicine are paid closely attention, and the pathway of antitussive properties (Chao and Hu, 1993), mechanism and screening methods are widely discussed by domestic and foreign scholars (Pi and Wu, 2004). The chemical composition of *Fritillaria* plant is alkaloids and non-alkaloid section, in which alkaloids section is the main part of the activity. *F. unibracteata* is used in single herb or compound traditional medicine for the cough treatment (Zhou *et al*., 2006; Chen *et al*., 2007), because *F. unibracteata* is one of the highest *Fritillaria* species on acquisition volume, especially, and the expectorant, antitussive and other physiological activity are slightly stronger than others (Li, 1995). Concerning antitussive effect study on *F. unibracteata*, it is found that there has been no research on *F. unibracteata* cultivated in the Qinghai-Tibet Plateau (Gao *et al*., 2000). Therefore, this article takes the *F. unibracteata* cultivated in the ecological environment of Qinghai-Tibet Plateau as an experimental material, adopting the Ammonia Citation Cough Method in mice (Zhang *et al*., 2007) to determine the antitussive effect of its ethanol extract.

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Materials and Methods

Plant material

Fritillaria unibracteata is supplied by Prof. Chen Zhi of China-Korea Plateau Wild Flower Institute. Male and female mice were purchased from Qinghai Biology Research Institute at 18~22 g weighing.

Extraction

Firstly, crush the dry *F. unibracteata* for 500 g. Then extract with 75%, 50%, 30% ethanol solvent (2,000 mL). There are 4 times of extraction totally, each lasts 4 hours with extraction temperature of 60℃, and the crude concentrated extract is evaporated to obtain at 45℃~50℃ by Rotary evaporator. Average yield is 5.28%, and then the crude extracts are prepared to *F. unibracteata* suspension with different concentrations. Concentrations are 10, 20, 40 mg/kg respectively.

The control group

Codeine phosphate with effective antitussive effect is selected as the positive control group, a dose of 25 mg/kg. 0.9% sodium chloride injection as the blank control group.

Table 1. Medicine of controlled conditions.

Division	Control medicine
Positive control	Codeine Phosphate (25 mg/Kg)
Blank space control	Sodium chloride injection 0.9% (25 mg/kg)

The positive control group and blank control group of *F. unibracteata* extract are shown in Table 1.

Ammonia coughs method in mice

Ammonia coughs method is executed by Zhang *et al*. (2007). Mice are randomly divided into groups of 10 each (male and female in half). Investigational medicine is set into high, medium and low dose group, respectively 10, 20, 40 mg/kg, and suspended with solution of 0.9% sodium chloride. Positive control group receives same solvent (25 mg/kg); the above groups receive the medicine of irrigation stomach for 5 days, once a day. Mice are placed in an inverted beaker under which 0.15ml concentrated ammonia (25.0~ 28.0%) in the Heating bath is evaporated; when ammonia steam stimulation to mice within the scheduled time, the mice are quickly transferred to a large beaker, then the cough times of mice in 3 minutes are recorded. Occurring 3 times cough and more within 1 minute is typical known as “cough”, less than 3 times as “no cough”. The cough is subject to abdominal severe contraction and mouth sound (with stethoscope) on mice.

Determination on antitussive effect of *F. unibracteata*

Mice are randomly divided into groups of 10 each (male and female in half). Ammonia cough method in mice (Zhang *et al*., 2007) is used in experiments, the above-mentioned groups receive the irrigation stomach for 3 different doses (10, 20, 40 mg/kg) of ethanol extracts of *F. unibracteata* with 75, 50, 30; the control group receives the medicine (codeine phosphate, sodium chloride injection 0.9%) dose of 25 mg/kg. The groups are given medicine of irrigation stomach once a day, for 5 days, and the experiment is on the sixth day.

Results and Discussion

The antitussive experiment in 75% ethanol extract of *F. unibracteata*, 10, 20, 40 mg/kg dose and the positive control group (25 mg/kg, codeine phosphate) on arousing cough inhibition in mice are 16.55%, 30.05%, 31.84%, 18.04% respectively; the antitussive experiment in 50% ethanol extract of *F. unibracteata*, 10, 20, 40 mg/kg dose on arousing cough inhibition in mice are 17.03%, 29.89%, 32.95 % with antitussive effects; the antitussive experiment in 30% ethanol extract of *F. unibracteata*, 10, 20, 40 mg/kg dose on arousing cough inhibition in mice are 14.56%, 24.72%, 26.55% with good antitussive effect. The antitussive effect of *F. unibracteata* ethanol extract is shown in Table 2, 3, 4 and Figure 1, 2, 3.

Table 2. Effect of *F. unibracteata* 75% ethanol extracts on incubation period of cough by ammonia liquor in mice (n=10).

Solvent	Dose (mg/kg)	Incubation period of cough/s
Ethanol extracts[1] 《Ⅰ》	10 mg	18.3 ± 3.4
Ethanol extracts[1] 《Ⅱ》	20 mg	25.3 ± 5.7
Ethanol extracts[1] 《Ⅲ》	40 mg	26.6 ± 5.2
Codeine Phosphate[2]	25 mg	20.6 ± 2.4
Sodium chloride injection[3]	25 mg	14.3 ± 3.6

[1]Ethanol extracts, [2]Positive control, [3]Blank space control

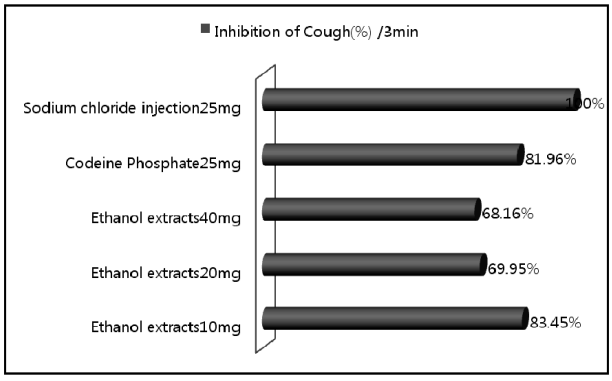

Fig. 1. *F. unibracteata* 75% ethanol extracts on cough induced by ammonia liquor in mice controlled conditions.

F. unibracteata ethanol extract of 10, 20, 40 mg/kg dose has a significant antitussive effect to mice in ammonia liquor, especially high, middle dose group with obvious antitussive effects. Middle and high doses of *F. unibracteata* ethanol extract is better than the positive control group (codeine phosphate) on antitussive effect. The experimental results show that the ethanol extracts of *F. unibracteata* has the antitussive effect.

Table 3. Effect of *F. unibracteata* 50% ethanol extracts on incubation period of cough by ammonia liquor in mice (n = 10).

Solvent	Dose (mg/kg)	Incubation period of cough/s
Ethanol extracts[1] 《Ⅰ》	10 mg	17.7 ± 4.2
Ethanol extracts[1] 《Ⅱ》	20 mg	24.7 ± 7.6
Ethanol extracts[1] 《Ⅲ》	40 mg	27.1 ± 5.4
Codeine Phosphate[2]	25 mg	20.6 ± 2.4
Sodium chloride injection[3]	25 mg	14.3 ± 3.6

[1]Ethanol extracts, [2]Positive control, [3]Blank space control

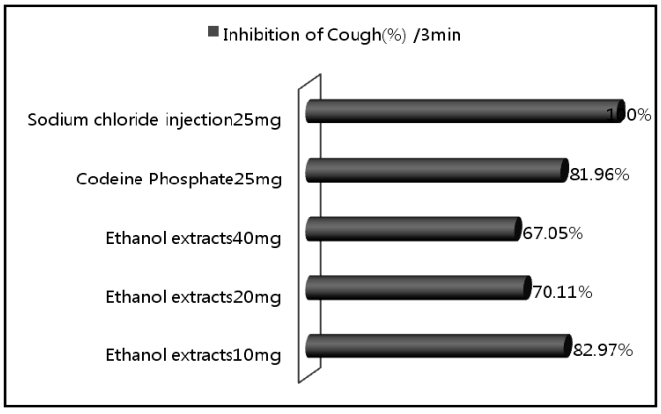

Fig. 2. *F. unibracteata* 50% ethanol extracts on cough induced by ammonia liquor in mice controlled conditions.

Table 4. Effect of *F. unibracteata* 30% ethanol extracts on incubation period of cough by ammonia liquor in mice (n = 10).

Solvent	Dose (mg/kg)	Incubation period of cough/s
Ethanol extracts[1] 《Ⅰ》	10 mg	16.2 ± 3.4
Ethanol extracts[1] 《Ⅱ》	20 mg	20.8 ± 5.7
Ethanol extracts[1] 《Ⅲ》	40 mg	22.1 ± 5.2
Codeine Phosphate[2]	25 mg	20.6 ± 2.4
Sodium chloride injection[3]	25 mg	14.3 ± 3.6

[1]Ethanol extracts, [2]Positive control, [3]Blank space control

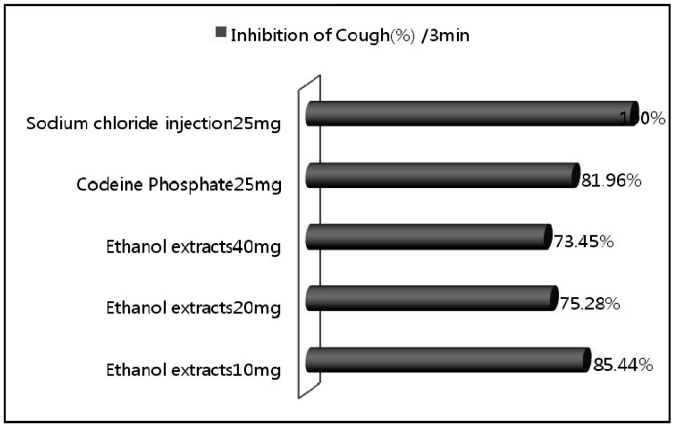

Fig. 3. *F. unibracteata* 30% ethanol extracts on cough induced by ammonia liquor in mice controlled conditions.

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