

Activities of Antitussive of even Alkaloids from Bulbus *Fritillariae cirrhosae*

Dongdong Wang*, Shu Wang and Wenhua Chen

Department of Pharmacognosy, West China College of Pharmacy, Sichuan University, No.17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China

Abstract

The objective of this study was to isolate active alkaloids from the Bulbus *Fritillariae Cirrhosae* (BFC), to evaluate their anti-tussive to find the highly active substance from the BFC for further new drug development. Materials and methods: The BFC was extracted with chloroform: methanol and alkaloids were isolated by phytochemical methods. The anti-tussive activity was investigated using ammonia induced mice coughing. Results: The seven alkaloids (1)~(7) were isolated from the BFC and the structure were determined. The seven alkaloids significantly inhibited mice's frequency of cough induced by ammonia, increased mice's latent period of cough and show activity dose-dependently. The inhibitions of cough number of these alkaloids are 65.33% (1), 60.28% (2), 35.06% (3), 37.26% (4), 56.37% (5), 57.80% (6), 59.07% (7). The results of the present study provide a convincing proof to put BFC as one of the most popular antitussive Traditional Chinese Medicine (TCM) in China.

Keywords: Antitussive; Alkaloids; Bulbus *Fritillariae Cirrhosae***Introduction**

Coughing is a symptom of respiratory illness which causes chest and thorax pain [1]. Presently available therapies to treat cough are limited for lack of effective and safe medications. Moreover, most existed antitussive drugs could bring inevitable or intolerable side effects [2]. In TCM, many plants are recorded to treat respiratory complaints such as cough, bronchial inflammation, pneumonia and expectoration [3], which have been used for hundreds of years. So it is a noticeable path to search for effective medicines to treat cough in the rich traditional Chinese medicine in the world [4].

BFC, is a well known antitussive TCM used for a long time. It has been used in clinic for more than 2000 years in China due to the positive potent therapeutic effects, low toxicity and minimal side effects. The pharmacological studies indicate that the crude alkaloids extract of BFC have good antitussive activities [5,6]. Alkaloids were established to be the major biologically active components in the genus of *Fritillaria*. BFC which has been considered as the most valuable medicine in the genus of *Fritillaria* lacks further phytochemical study and pharmacological research of monomers of alkaloids *in vivo*. So we focused our studies on the phytochemical and pharmacological research on BFC. The isolation of alkaloids, evaluation of the anti-tussive activities of different alkaloids are the subject of this paper.

Materials and Methods**Plant materials**

The Bulbs of *Fritillariae Cirrhosae* was collected in April 2010 in Chengdu International Trade City Hehuachi Chinese Medicinal Herbal Market, Chengdu city, Sichuan province, China. The BFC was authenticated by Dr. Wang Shu, Professor in Department of Pharmacognosy, West China College of Pharmacy, Sichuan University. The voucher specimen was deposited in the herbarium of West China College of Pharmacy, Sichuan University.

Extraction and isolation

Dried ground bulbs (15 kg) were powdered and treated in ammonia (1200 ml) for 15 hours, then extracted with chloroform to obtain crude extract (38 g). TAF was obtained by using previously described methods [7,8]. The concentrated extract was dissolved in 3% HCl and partitioned with chloroform. The pH of the aqueous solution was readjusted with NH_4OH to 10.0 and extracted with CHCl_3 . The

CHCl_3 extract (crude alkaloid, 9.5 g) and was chromatographed over Si gel using petroleum ether-acetone-diethylamine (6: 1: 2~1: 1: 2) of increasing polarity as eluent to obtain seven fractions [9].

Animals and administration

Kunming mice (18–22 g) were purchased from Experimental Animal Center, West China College of Pharmacy, Sichuan University (Chengdu, China). All animals were housed at room temperature (22–24°C) and constant humidity (40–70%) under a 12 h light–dark cycle and provided with food and water ad libitum.

After 3–5 days of adaptation, the eligible animals were randomly assigned to nine groups and orally administered, including group 1 (Control) (0.5% carboxyl methylcellulose solution, 0.2 ml/20 g), group 2 (the positive control) (codeine phosphate/30 mg/kg), group 3–9 (alkaloid (1)~(7), respectively). The doses of the group 3–9 in the antitussive test were 3.0 mg/kg.

Anti-tussive activity

Kunming mice were randomly divided into 9 groups of 6 each and administered according to Section 2.3. Anti-tussive activity was investigated on a classical mouse cough model induced by ammonia liquor [10,11]. Briefly, each mouse was placed in a 1000 ml special glass chamber and exposed to, 0.3 ml 25% NH_4OH for 45 seconds. Then the frequency of the cough for 5 min and latent period of cough were observed. The number of coughs produced 60min after the administration of antitussive agents (C_1) was compared with the number of control coughs (C_0). The antitussive effect was expressed as the percentage of inhibition of the number of control coughs $[(C_0 - C_1) / C_0 \times 100\%]$.

Statistical analysis

The experimental results are expressed as mean \pm standard error

*Corresponding author: Dongdong Wang, Department of Pharmacognosy, West China College of Pharmacy, Sichuan University, No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China, Tel: +86-028-85503950; Fax: +86-028-85503950; E-mail: wddong1988@hotmail.com

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Group	Dose (mg/kg)	No. of animals	Latent period of cough(s)	No. of coughs	Inhibition (%)
0.5% CMC-Na	-	6	15.73 ± 2.74	63.77 ± 9.16	-
Codeine Phosphate	30	6	26.62 ± 3.16*	19.18 ± 3.98***	69.92
(1)	3	6	34.19 ± 6.55*	22.11 ± 6.58**	65.33
(2)	3	6	59.23 ± 8.31**	25.33 ± 5.81**	60.28
(3)	3	6	39.24 ± 5.40*	41.41 ± 8.21*	35.06
(4)	3	6	43.25 ± 6.71*	40.01 ± 9.38*	37.26
(5)	3	6	30.22 ± 5.65*	27.82 ± 7.37**	56.37
(6)	3	6	58.47 ± 10.31**	26.91 ± 6.80**	57.80
(7)	3	6	46.79 ± 8.49**	26.10 ± 6.00***	59.07

Values expressed as mean ± S.E.M. (n=10)

* P<0.05 for comparison of treated groups with control

** P<0.01 for comparison of treated groups with control

*** P<0.001 for comparison of treated groups with control

Table 1: Effects of alkaloids of medium dose on the ammonia liquor induced cough in mice.

of mean (S.E.M.). One-way analyses of variance (ANOVA) test was performed for multiple group comparison and Student's t-test was carried out for direct comparison of two groups. Values of p<0.05 imply significance of the pharmacological effects in the experiments.

Results

Alkaloids from BFC

Alkaloids (1)~(7) were isolated from BFC and identified on the basis of spectral data (1H-, 13C-NMR, IR, and mass spectra etc.).

Effect of alkaloids on the ammonia liquor induced cough in mice

The effects of the alkaloids on the ammonia liquor induced cough in mice are shown in Table 1. All alkaloids showed potent anti-tussive effects. Positive and tested groups could enhance latent period of cough and all the increase presented significantly, compared with the period of Control.

Discussion and Conclusion

The BFC is one of the most popular antitussive and expectorant TCM in Chinese communities. Potent antitussive, expectorant and antiasthmatic activities of the crude alkaloids extract from BFC were previously reported in the pharmacological studies [5,6,12]. In one word, the traditional knowledge and available clinical efficiency led us to isolate and characterize the active constituents present in BFC. In the present study, seven alkaloids were isolated from BFC.

In conclusion, the results of the present study provide a convincing proof to put BFC as one of the most popular antitussive and expectorant TCM in Chinese communities. Through the pharmacological evaluation on antitussive, expectorant and anti-inflammatory activities of all alkaloids respectively, (1), (2), (5), (6), (7) were highly active constituents of crude alkaloids extract from BFC. The mechanism of action explained for the observed activities have not been established, and thus further investigation needs conducted.

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