EVALUATION OF ANTITUSSIVE AND EXPECTORANT ACTIVITIES OF "CAM CUM BA GIANG" HARD CAPSULES IN THE EXPERIMENT

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The study aimed to evaluate the antitussive and expectorant activities of "Cam cum Ba giang" hard capsules. The antitussive effect of "Cam cum Ba giang" hard capsules was assessed using a murine model of ammonia-induced cough. The expectorant effect of "Cam cum Ba giang" hard capsules was evaluated by measuring tracheal phenol red output in mice. The results showed that "Cam cum Ba giang" hard capsules at 355.2 mg dried extract/kg/day and 710.4 mg dried extract/kg/day significantly inhibited cough and cough frequency and increased the latent period of cough. Moreover, "Cam cum Ba giang" hard capsules at both doses tended to pose the expectorant effect by slightly increasing the mice's tracheal phenol red output compared to the control group. In conclusion, "Cam cum Ba giang" hard capsules, derived from herbal medicine, expressed antitussive and expectorant effects in experimental mice. Therefore, "Cam cum Ba giang" hard capsules may be a potential product for supporting the treatment of symptoms related to respiratory diseases in clinical settings.

Keywords: "Cam cum Ba giang" hard capsules, antitussive, expectorant, experiment.

I. INTRODUCTION

Cough is a common symptom that requires patients to go to the primary and specialized healthcare units for diagnosis and treatment. Although cough is an important protective reflex of the respiratory system, patients with a prolonged cough should be given a comprehensive examination and early treatment.[1] Cough with copious phlegm is a common symptom of respiratory diseases. Increased sputum may irritate the respiratory mucosa, which leads to coughs. In some cases, excessive phlegm can cause difficulty breathing and congestion.[2] To date, the prevalence of respiratory diseases is increasing dramatically. Worldwide, by 2017, approximately 545 million people (7.4%) suffered from chronic respiratory diseases, of which 3.9 million died from these conditions.[3]

In addition to synthetic cough suppressants and expectorants that are commonly used in clinical practice, natural drugs are also widely used according to folk experience and have shown high effectiveness. However, they have not been systematically studied to prove their efficacy. Therefore, finding and researching cough suppressants and expectorants from medicinal sources with high efficiency, low toxicity, and low cost is an urgent issue with scientific and practical value.[4]

"Cam cum Ba giang" hard capsules are

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prepared from natural materials including *Radix Angelicae dahuricae, Rhizoma Chuanxiong, Rhizoma Cyperi, Rhizoma Zingiberis, Cortex Cinnamomi,* and *Radix et Rhizoma Glycyrrhizae*. The expectorant and antitussive effect of each component was proven in various research;[5,6] however, there have been no report available on the impact of the combination of these ingredients in Vietnam and worldwide. Thus, we aimed to investigate the expectorant and antitussive effect of "Cam cum Ba giang" hard capsules in experimental mice.

II. MATERIALS AND METHODS

1. Subjects

The preparation of "Cam cum Ba giang" hard capsules

"Cam cum Ba giang" hard capsules were produced by Ba Giang Traditional Medicine Factory, Bagiaco Pharmaceutical Company, in Ha Nam, Vietnam. The product achieved basic standards. The product was formulated in the form of hard capsules, and each capsule contained 400mg dried extract, including:

Radix Angelicae dahuricae: 1200mg
Rhizoma Chuanxiong: 1000mg
Rhizoma Cyperi: 900mg
Rhizoma Zingiberis: 600mg
Cortex Cinnamomi: 200mg
Radix et Rhizoma Glycyrrhizae: 100mg

The capsules were dissolved with distilled water before being given orally to rats.

Drugs and chemicals

Codeine phosphate in powder form was supplied by Mediplantex National Pharmaceutical Joint Stock Company. Ammonia solution 25% was the product of Xilong company, China, CAS 1336-21-6. Ambroxol in capsule form, brand name Ambroxol Boston, content 30mg, was produced by Boston Vietnam Pharmaceutical Joint Stock Company. Red phenol in powder form was made by Xilong Scientific Co., Ltd, China, registration number 143-74-8.

Laboratory equipment

Electronic scale model 321LX type 2200C, Precisa, Switzerland, serial number 327-9454-002. Specialized glass chamber with a capacity of 1.5 L. SmartSpecTM plus Spectrophotometer from BIO-RAD (U.S.), serial number 273 BR05679. Hettich EBA20 Centrifuge with a maximum centrifugal speed of 6000 rpm, manufactured by Andreas Hettich GmbH & Co. KG, 78532 Tuttlingen, Germany. Chronometer, feeding needle for mice, graduated cups, 1 ml syringe.

2. Methods

Experimental animals

Swiss mice, both sexes, weighing 20 ± 2g, were supplied from the National Institute of Hygiene and Epidemiology. The animals were housed in cages (groups of ten mice/cage) in a room with access to a standard certified rodent diet and water ad libitum. They were acclimated to housing for 1 week before the investigation at the Department of Pharmacology, Hanoi Medical University.

Research units

The study was conducted in the laboratory at the Department of Pharmacology, Hanoi Medical University. The absorbance of the trachea output was measured at the Department of Pathophysiology and Immunology, Hanoi Medical University.

Evaluation of antitussive activity

Mice were divided into four groups, 10 mice per group:

- Group 1 (control): Mice were administered orally with distilled water at a volume of 0.2 ml/10g b.w.

- Group 2 (codeine phosphate): Mice were administered codeine phosphate orally at 30

mg/kg/day at a volume of 0.2 ml/10g b.w.

- Group 3 ("Cam cum Ba giang" hard capsules at the low dose): Mice were administered orally with "Cam cum Ba giang" hard capsules at 355.2mg dried extract/kg/day (equivalent to the human recommended dose as four tablets/day, conversion ratio 12), at a volume of 0.2 ml/10g b.w.

- Group 4 ("Cam cum Ba giang" hard capsules at the high dose): Mice were administered orally with "Cam cum Ba giang" hard capsules at 710.4 mg dried extract/kg/day (2 times as high as the low dose), at a volume of 0.2 ml/10g b.w.

Mice were given distilled water, codeine phosphate, or "Cam cum Ba giang" hard capsules orallly for three continuous days. On the 3rd day of the study, after 1 hour of the last administration, all mice were induced to cough through an inhalation of 0.5mL ammonia solution in a specialized glass chamber. Each mouse was put into a glass chamber; the cough frequency was measured for 5 minutes. The cough can be identified by mouth opening, the accompanying sound of coughing, and contraction of the thoracic and abdominal muscles.

Research indexes include:

- The latent period was when mice were put into the chamber until they first appeared to cough.

- The frequency of cough in 5 min.

- The percentage of inhibition of cough times was calculated as follows:

% Inhibition= $(C_0 - C_t)/C_0 \times 100\%$

where C_0: the cough time of control group; C_t: the cough time of the treatment group

Evaluation of expectorant activity

Expectorant activity was assessed following the method of Yu P. et al.[2]

Mice were divided into four groups, 10 mice per group:

- Group 1 (control): Mice were administered orally distilled water at a volume of 0.2 mL/10g b.w.

- Group 2 (ambroxol): Mice were administered orally with ambroxol at 250 mg/kg/day in at a volume of 0.2 mL/10g b.w.

- Group 3 ("Cam cum Ba giang" hard capsules at the low dose): Mice were administered orally with "Cam cum Ba giang" hard capsules at 355.2mg dried extract/kg/day (equivalent to the human recommended dose as four tablets/day, conversion ratio 12) at a volume of 0.2 mL/10g b.w.

- Group 4 ("Cam cum Ba giang" hard capsules at the high dose): Mice were administered orally with "Cam cum Ba giang" hard capsules at 710.4mg dried extract/kg/day (2 times as high as the low dose) with the volume of 0.2 mL/10g b.w.

Mice were given oral administration with distilled water, ambroxol, or "Cam cum Ba giang" hard capsules for three continuous days. On the 3rd day of the study, after 1 hour of the last administration, all mice were injected i.p with red phenol 5%, at a volume of 0.1 mL/10 g.

Thirty minutes after the injection of red phenol, mice were sacrificed, the trachea was exposed with a blunt needle, washed with 0.8ml of 5% $NaHCO_3$, and centrifuged at 2500 rpm for 5 minutes. The absorbance of the centrifuged supernatant was measured at a wavelength of 546 nm using a microplate reader.

The higher the absorbance of the centrifuged supernatant corresponding to the amount of red phenol secreted from the trachea, the better the expectorant effect. The average absorbance and phenol red concentration among groups were compared.

Statistical analysis

Data were collected and analyzed using

SPSS ver 26.0. The significance levels among the experimental groups were determined using Student's t-test. Data were shown as mean ± standard deviation. All data were considered significant at $p < 0.05$.

III. RESULTS

1. Antitussive effect of "Cam cum Ba giang" hard capsules

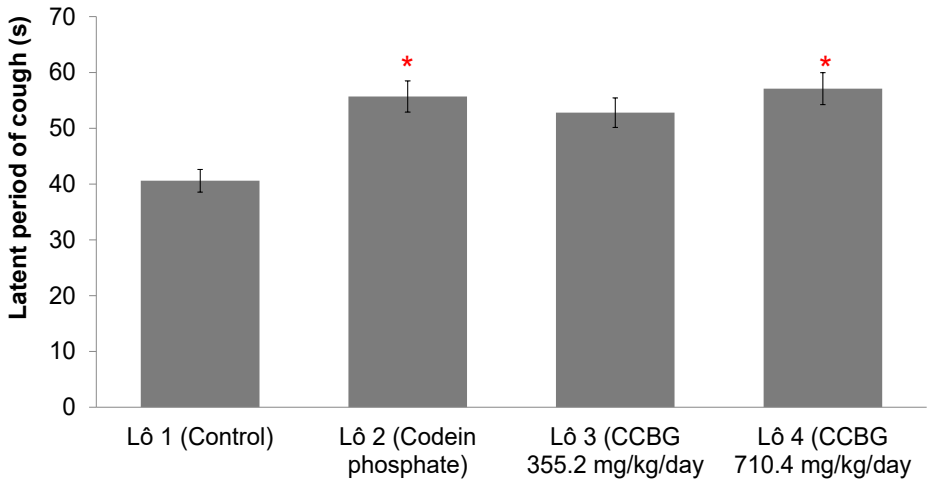

Chart 1. Effect of "Cam cum Ba giang" hard capsules on the latent period of the cough induced by ammonia in mice

**compared with group 1 (control) with p < 0.05, CCBG: "Cam cum Ba giang" hard capsules*

The results from Figure 1 showed that codeine phosphate significantly increased the latent period of cough compared to the control group ($p < 0.05$). "Cam cum Ba giang" hard capsules at 710.4 mg dried extract/kg/day improved significantly the latent period of cough compared to the control group ($p < 0.05$). There was an upward trend in the latent period of cough in the group treated with Cam cum Ba giang" hard capsules at 355.2 mg dried extract/kg/day. No significant difference was observed among groups treated with codeine phosphate and "Cam cum Ba giang" hard capsules ($p > 0,05$).

Table 1. Effect of "Cam cum Ba giang" hard capsules on the frequency of cough in 5 min in mice induced cough by ammonia

Groups	n	Frequency of cough in 5 min	% inhibition
Group 1 (Control)	10	23.40 ± 8.69	-
Group 2 (Codeine phosphate 30 mg/kg/day)	10	12.80 ± 5.16**	45.30
Group 3 "Cam cum Ba giang" hard capsules 355.2mg dried extract/kg/day)	10	14.80 ± 4.37*	36.75
Group 4 "Cam cum Ba giang" hard capsules 710.4mg dried extract/kg/day)	10	12.90 ± 4.43**	44.87

**, ** compared with group 1 (control) with p < 0.05; p < 0.01*

Results from Table 1 showed that codeine phosphate dramatically decreased the cough frequency compared to the control group ($p < 0.001$). There was a significant reduction in the frequency of cough at groups treated with "Cam cum Ba giang" hard capsules at 355.2mg dried extract/kg/day and 710.4mg dried extract/kg/day as compared with the control group ($p < 0.05$ and $p < 0.01$, respectively). No significant difference in the frequency of cough among groups treated with codeine phosphate and "Cam cum Ba giang" hard capsules ($p > 0.05$).

Codeine phosphate had a percentage of inhibition of cough times of 45.30%. The percentage of inhibition of cough times of "Cam cum Ba giang" hard capsules at 355.2mg dried extract/kg/day and 710.4mg dried extract/kg/day was 36.75% and 44.87%, respectively.

2. Expectorant effect of "Cam cum Ba giang" hard capsules

Table 2. Effect of "Cam cum Ba giang" hard capsules on the tracheal phenol red output

Groups	n	Tracheal red phenol output (µg/mL)
Group 1 (Control)	10	0.919 ± 0.270
Group 2 (Ambroxol 250 mg/kg/day)	10	1.691 ± 0.926*
Group 3 ("Cam cum Ba giang" hard capsules 355.2mg dried extract/kg/day)	10	1.061 ± 0.327
Group 4 ("Cam cum Ba giang" hard capsules 710.4mg dried extract/kg/day)	10	1.136 ± 0.402

** compared with group 1 (control) with $p < 0.05$*

Results from Table 2 demonstrated that ambroxol at 250 mg/kg substantially increased the tracheal phenol red output secreted from the trachea compared to the control group ($p < 0.01$). There was an upward trend in the tracheal phenol red output in groups treated with "Cam cum Ba giang" hard capsules at 355.2mg dried extract/kg/day and 710.4 mg dried extract/kg/day ($p > 0.05$). No significant change in the tracheal phenol red output were observed among groups treated with ambroxol and "Cam cum Ba giang" hard capsules ($p > 0.05$).

IV. DISCUSSION

Experimental animals selected in antitussive and expectorant effects studies can be mice, rats, guinea pigs, dogs, and cats. The choice of experimental animals depends on the research method, route of administration, and laboratory conditions.[7] In this study, mice were chosen to assess the effect of the research product.

Cough inducers used in experimental studies can be mechanical or chemical. Chemicals (ammonia, citric acid, etc.) are the most commonly used agents to induce cough in mice.[8] In this study, the cough inducer chosen is ammonia because of ease of application, low cost, and ability to cause a variety of cough responses in the experiment.

Codeine and ambroxol were selected to be positive drugs in the study of antitussive and expectorant effects of "Cam cum Ba giang" hard capsules. These drugs were commonly used in clinical as well as experimental studies.[9]

The results demonstrated that "Cam cum Ba giang" hard capsules at both doses of 355.2mg dried extract/kg/day and 710.4 mg dried extract/kg/day prolonged the latent period of cough, decreased substantially the frequency

of cough in 5 min, and significantly inhibited cough (36.75% and 44.87% respectively) compared to the control group. However, the low dose of 355.2mg dried extract/kg/day only improved the positive effect on the mouse cough model induced by ammonia. There was a slight difference in the percentage of inhibition of cough among groups treated with "Cam cum Ba giang" hard capsules at both doses of 355.2mg dried extract/kg/day and 710.4mg dried extract/kg/day and group treated with codeine phosphate (8.55% and 0.43% respectively). Thus, "Cam cum Ba giang" hard capsules at both doses showed the antitussive effect on the ammonia-induced cough model; the more apparent effect was observed in the group treated with the higher dose of 710.4 mg dried extract/kg/day.

"Cam cum Ba giang" hard capsules at both doses of 355.2 mg dried extract/kg/day and 710.4 mg dried extract/kg/day tended to increase the mice's tracheal phenol red output compared to the control group. No significant difference was observed in tracheal phenol red output between groups treated with "Cam cum Ba giang" hard capsules and the control group. Thus, "Cam cum Ba giang" hard capsules tended to express the expectorant effect in the experimental model.

The effect of the components in "Cam cum Ba giang" hard capsules can be explained by the antitussive and expectorant effects of the components. The study of Kamei J (2003) suggested that *Glycyrrhizae radix* contains a potent antitussive compound, liquilitin apioside, whose antitussive effect may depend on both peripheral and central mechanisms.[5] Chinese describe ginger (Shengjiang) as pungent and warm; and as a cold-discutient, mucolytic, and antitussive.[6]

V. CONCLUSIONS

In terms of the antitussive effect, "Cam cum Ba giang" hard capsules at both doses of 355.2 mg dried extract/kg/day (equivalent to the human recommended dose as 4 tablets/day) and 710.4 mg dried extract/kg (equivalent to the human recommended dose as 8 tablets/day) significantly posed the antitussive effect on the mouse cough model induced by ammonia through increasing the latent period of cough, decreasing the frequency of cough, and expressing clearly the inhibition potential of cough.

In terms of the expectorant effect, "Cam cum Ba giang" hard capsules at both doses of 355.2 mg dried extract/kg and 710.4 mg dried extract/kg tended to have the expectorant effect by slightly increasing the mice's tracheal phenol red output.

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