Pharmacology and Toxicology of Extract from *Arcangelisia gusanlung*

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Abstract: **Objective** To study the pharmacology and toxicology of the extracts from *Arcangelisia gusanlung* (EAG). **Methods** The anti-inflammatory activities were investigated using various inflammatory models including ear edema induced by xylene in mice, paw edema induced by carrageenan, and cotton pellet granuloma in rats. The analgesic effect was observed in hot-plate test and writhing test in mice and the antipyretic effect was observed in rat fever model induced by yeast. The antitussive action was tested in mice by sequential method and expectorant action was evaluated by tracheal excretion of phenol red. The antidiarrhea function was observed on normal intestinal propulsion of mouse model of diarrhea induced by decoction of *Sennae Folium*. The toxicity was measured by toxicological experiment. **Results** Each dose of EAG could significantly inhibit the paw edema, cotton pellet granuloma, and intestinal propulsion. EAG significantly reduced writhing times and amount of wet manure. Obvious antipyretic action to fevered rat was observed. EAG obviously increased the tracheal excretion of phenol red and prolonged the latency of cough. No toxic reaction was shown in the observed period, and the maximum tolerance dose of mice was equivalent to 1360 times of common-used dose in human. **Conclusion** The clinical dosage of EAG is safe, and its anti-inflammatory, analgesia, antipyresis, antitussive, expectorant, and antidiarrhea effects are significant.

Key words: analgesia; antidiarrhea; anti-inflammation; antipyresis; antitussive; *Arcangelisia gusanlung*; expectorant; toxicity
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Introduction

The rhizomes of *Arcangelisia gusanlung* H. S. Lo, are a kind of Li medicine (traditional medicine used by Li nationality) with a long history as an important part of Chinese materia medica (CMM). However, in the history, Li nationality does not have its own characters but the national language, its medicine information has been spread and preserved by oral teaching, which could lead to misinformation and unfounded rumor easily. To a certain extent, the inheritance and development of Li medicine are influenced. Based on this, it is necessary to develop rescuing and scientific research works of nationalistic medicine.

A. gusanlung was recorded to treat many diseases, such as enteritis, bacillary dysentery, malaria, bronchitis, pertussis, etc (State Administration of Traditional Chinese Medicine, 1998). Garcia *et al* (1970) isolated berberine, palmatine, and jatrorrhizine from the rhizomes of *A. gusanlung* for the first time in 1970. Afterward, five kinds of isoquinoline alkaloids, gusanlungs C and D, 8-oxyberberine, 8-oxythalifendine, and 8-oxyberberrubine, were yielded from the rhizomes of *A. gusanlung* (Zhang, Men-Olivie, and Massiot, 1995). All the studies known were focused on the structure and synthesis of the composition in *A. gusanlung*, but there is no report on the toxicity and efficacy, which brings difficulty to the clinical rational drug use and further development. This study puts emphasis on the research of pharmacology and toxicology in the 70% ethanol extract from *A. gusanlung*

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(EAG). The quality control of EAG was carried out by quantitative determination of alkaloids. The acute toxicity and anti-inflammatory, analgesia, antipyresis, antitussive, expectorant, and antidiarrhea effects were evaluated by the different animal models. This study provides a base for the further research.

Materials and methods

Preparation of EAG

Arcangelisia gusanlung H. S. Lo was collected in Wuzhishan city (Hainan, China) in October, 2011 and identified by Prof. CHEN Guo-biao, Hainan Provincial Institute for Drug Control. With alkaloid content as the index, ethonal volume, ethonal concentration, extraction times, and reflux time were screened by $L_9(3^4)$ orthogonal test (Zhang, Xu, and Sun, 2010). The results showed that the optimal conditions for the extraction of alkaloids were as follows: temperature of 80 ℃, alcohol volume fraction of 70%, ratio of solid to liquid of 1:10, reflux time of 1 h, and extraction for three times.

The powder of *A. gusanlung* (13.03 kg) was extracted under the optimum condition, then the hot solution filtered, the filtrates were collected. Alcohol in the filtrates was vaporized and collected. The residuals were concentrated into cream (2.62 kg). The yield of extract was 20.1%.

Determination of EAG alkaloids

The content of total alkaloids in EAG was determined 94.6 g/kg by the acidic dye colorimetry (Xu, Xiong, and Sun, 2010), which was performed with berberine hydrochloride as the reference substance, bromocresol green as the acidic dye, and $CHCl_3$ as the extractant under the condition of a buffer solution (pH 4). The berberine content was determined 78 g/kg by HPLC (Pharmacopoeia Committee of P. R. China, 2010) with Hypersil-C_{18} column (150 mm × 4.6 mm, 5 μm) and a mobile phase of acetonitrile-0.1% phosphoric acid (50:50, containing 0.1% sodium dodecyl sulfate solution).

Methodology validation showed that the two methods were simple and accurate, and had the good reprodubibility.

Drugs and reagents

EAG was dissolved in 5% CMC-Na solution to make up the suspension at various concentration, including the crude drug of 1.05, 2.1, and 4.2 g/mL, respectively. The Aspirin (Asp), berberine, and Dexamethasone (DEX) were prepared by CMC-Na solution with the concentration of 5 mg/mL. The suspension was prepared every time just prior to the administration.

Aspirin Tablets, Berberine Hydrochloride Tablets, Dexamethasone Acetate Tablets, and Pentoxyverine Citrate Tablets were all purchased from Guangantang Large Pharmacy (Haikou, China). Carrageenan, CMC-Na, glacial acetic acid, ammonia, and xylene were analytically pure and purchased from Guangzhou Chemical Reagent Factory (Guangdong, China).

Instruments

LC2010A/C High Performance Liquid Chromatograph, UV—2450 UV-Vis Spectrophotometer, AEL—200 Electronic Balance (Shimadzu, Japan); 402—A Ultrasonic Nebulizer (Jiangsu Yuyue Medical Instrument Co., Ltd., China); HWS—26 Thermostatic Water Bath (Shanghai Yiheng Technology Company, China); GJ—28402 Pain Threshold Detector (Zhejiang Ninghai Baishi Electronic Medical Instrument Company, China); WMY—01 Digital Thermometer (Shanghai Medical Panel Work, China); Foot Volume Recognizer (Hainan Provincial Institute, China).

Experimental animals

Male and female Kunming mice (SPF, 18—22 g) and SD rats (120—160 g) were provided by the Center of Experimental Animal in Guangdong Province, Animal Certificate of Conformity [SCXK(Yue) 2003-0002]. The animals were maintained under the condition of (22 ± 2) ℃ and (55 ± 15)% relative humidity.

Experimental animal grouping and administration

In each experiment, unless otherwise specified, a total of 50 mice or rats were randomly divided into five groups (n = 10 in each group), solvent control, positive control, low-, mid-, and high-dose EAG (EAG-L, EAG-M, and EAG-H) groups, with the equal male and female in each group. The animals in the control group were given CMC-Na solution. The animals in all groups were administered with CMC-Na solution once daily for consecutive 7 d with the dose of 20 mL/kg.

Toxicology and pharmacology

Maximum toleration test Thirty mice were used in the toxicity experiment. EAG was given orally at the concentration of 3.33 g/mL with a dose of 20

mL/kg each time, for three times daily, which was equivalent to daily administration dosage of human (50 kg) by 666 times (State Administration of Traditional Chinese Medicine, 1998). There was no death and abnormal reaction in observation period. The half lethal dose (LD_{50}) could not be obtained, and therefore, the maximum tolerated dosage (MTD) was obtained to evaluate the acute toxicity of EAG.

Twenty mice were grouped by gender and fasted for 12 h before administration. The EAG was given orally in maximum concentration (6.8 g/mL) with a dose of 20 mL/kg each time (Wo, Hong, and Gao, 2000). The weights, diet, and activity condition were observed for 9 d.

Ear edema induced by xylene in mice Male mice were used in this study. The mice in the positive group were administered with Asp. After 1 h of the 8th administration, xylene (0.05 mL) was applied to the surface of the right ear. The left ear was considered as control. After 30 min of xylene application, mice were killed and both ears were removed. Circular sectons were taken using cork borer with a diameter of 6 mm (Xu, Bian, and Chen, 2001), and weighed to calculate the swelling degrees and the inhibitory rate.

Paw edema induced by carrageenan in rats Male rats were used. The rats in the positive group were given DEX. After 1 h of the 8th administration, right paws of all the rats were sc injected with 0.1 mL of 1% carrageenan (Xu, Bian, and Chen, 2001), and then measured at 1, 2, and 4 h after inflammation, respectively to calculate the paws edema degree and the inhibitory rate.

Cotton pellet granuloma in rats Male rats were used. Two sterilized cottons, weighing 19—21 mg, were sc inoculated in both axilla of rats. The rats in the positive group were given DEX. After 1 h of the 8th administration, rats were weighed and killed. The cotton pellets covered by the granulomatous tissue were obtained and dried in hot air oven at 60 ℃ for 12 h (Xu, Bian, and Chen, 2001). Considering the effect of weights, the paw edema was evaluated according to the formula $W = A/B$ (A denotes the weight change of cotton pellets and B denotes per hundred kilogram body weight).

Acetic acid writhing test in mice The mice in the positive group were given Asp. After 1 h of the 8th administration, the mice were ip injected with 0.6% glacial acetic acid at a dose of 0.2 mL (Xu, Bian, and Chen, 2001). Times of writhing in 20 min after the injection were observed.

Hot-plate test in mice Male mice were used. The pain threshold of each mouse was measured before the official test. The pain response temperature was (55.0 ± 0.5) ℃; The latency of licking hindfoot was regarded as reactive marker of pain threshold. The mice with the threshold less than 5 s or more than 30 s were eliminated (Xu, Bian, and Chen, 2001). The mice in the positive group were given Asp. The pain threshold was measured at 30 min after the 8th administration to calculate the enhancement rate.

Fever induced by yeast in rats The rats (180—220 g), with the temperature between 36.6—38.0 ℃ and the temperature difference less than 0.3 ℃, were used. The rats in the positive group were given Asp. After successive administration for 10 d, the rats were sc injected with 10% yeast on the back at a dose of 10 mL/kg (Xu, Bian, and Chen, 2001). The temperature was measured before the 10th administration, and also measured at 2, 4, 6, and 8 h after the injection of yeast.

Cough induced by ammonia The mice in the positive group were given Pentoxyverine. After 30 min of the 8th administration, the mice were treated with 28% ammonia till schedule time. Times of representative cough in 1 min greater than or equal to 3 was observed by using sequential experimental method (Liu and Jiang, 2009). Stimulation time of ammonia was 20.0, 25.1, 31.6, 39.8, 50.1, 60.1, and 79.4 s. The standard stimulation time was selected as 39.8 s. Times of cough in half (EDT_{50}) was obtained and was used to calculate according to the formula $R = A/B$ (A denotes the EDT_{50} of EAG group and B denotes EDT_{50} of the control group).

Tracheal excretion of phenol red in mice Male mice were used. The mice in the positive group were given Asp. After 1 h of the 8th administration to 12-h fasting mice, 5% phenol red solution was ip injected at a dose of 10 mL/kg. After 30 min, all the mice were euthanized. Trachea from thyroid cartilage to tracheal branch was obtained and put in test tubes that contained 2 mL normal saline and 0.1 mL sodium hydroxide solution (1 mol/L). All the tubes were stirring for 30

min (Xu, Bian, and Chen, 2001). The liquid of each tube was determined by colorimetric analysis at the wavelength of 546 nm.

Diarrhea of mice due to *Sennae Folium* Sixty male mice weighing 22—26 g were used. The mice in the positive group were given berberine. After the 8th administration, the mice were treated with 8% *Sennae Folium* suspension at a dose of 0.25 mL/kg and 1 h later were put into boxes with filter paper (Xu, Bian, and Chen, 2001). Counts of damp excrement in 24 h were recorded.

Intestinal propulsion of mice The mice in the positive group were given berberine. After the 8th administration, the mice were treated with ink at a dose of 20 mL/kg and after 20 min were killed (Xu, Bian, and Chen, 2001). Small intestinal canal was obtained to calculate the ink propulsive rate.

Statistical analysis

The results were expressed as $\bar{x} \pm s$ and carried on the statistical analysis by the SPSS 17.0 statistics software, and the data of multiple groups were compared by Independent Sample *t* test. $P < 0.05$ was taken as significance, and $P < 0.01$ was taken as higher significance.

Results

Maximum toleration test

There was no death and toxic reaction. All observation indexes and organs were normal. The weights of the male mice changed from (20.4 ± 1.58) g to (24.3 ± 2.00) g, and those of the female mice changed from (20.2 ± 1.32) g to (23.2 ± 1.4) g. The increased food intake of mice was also shown in Fig. 1. The toxicological experiment showed that the maximum tolerance dose of EAG in mice was 408 g/kg and it was 1360 times higher than the expected clinical dosage. The test showed the drug did not have obvious toxicity.

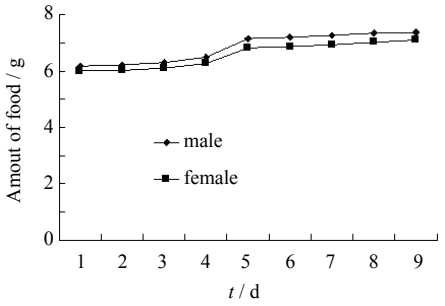

Fig. 1 Effect of EAG on food intake of mice

Ear edema induced by xylene in mice

Table 1 showed that the mice in the treatment groups had lower ear edema compared with those in the control group, but only the inhibitory rate in EAG-H group reached statistical significance ($P < 0.05$). Asp showed the significant anti-inflammatory action ($P < 0.01$).

Table 1 Effect of EAG on ear edema induced by xylene in mice ($\bar{x} \pm s$, $n = 10$)

Groups	Dose / ($g \cdot kg^{-1}$)	Ear edema / mg	Inhibitory rate / %
control	—	13.50 ± 3.72	—
Asp	0.1	6.70 ± 4.47**	50.37
EAG-L	21	11.70 ± 5.79	13.33
EAG-M	42	11.30 ± 3.37	16.30
EAG-H	84	8.70 ± 5.89*	35.56

*$P < 0.05$ **$P < 0.01$ *vs* control group, same as below

Paw edema induced by carrageenan in rats

Table 2 listed the effects of EAG on the paw edema at different time after the injection of carrageenan. Each dose of EAG could obviously inhibit paw edema at 1, 2, and 4 h after administration compared with the control group ($P < 0.01$, 0.05). The greater of the dosage was, the longer the effect lasted.

Cotton pellet granuloma in rats

Table 3 showed that the weight of granuloma of rats in the treatment groups was obviously lighter than that in the control group ($P < 0.01$).

Table 2 Effect of EAG on paw edema induced by carrageenan ($\bar{x} \pm s$, $n = 10$)

Groups	Dose / ($g \cdot kg^{-1}$)	Normal foot volume / mL	Paw edema / mL			Inhibitory rate / %		
			1 h	2 h	4 h	1 h	2 h	4 h
control	—	0.95 ± 0.05	0.48 ± 0.03	0.70 ± 0.07	0.85 ± 0.08	—	—	—
DEX	0.1	0.93 ± 0.04	0.36 ± 0.09**	0.40 ± 0.07**	0.34 ± 0.10**	25.0	42.9	60.0
EAG-L	21	0.98 ± 0.03	0.43 ± 0.05*	0.58 ± 0.09**	0.70 ± 0.03**	10.4	17.1	17.6
EAG-M	42	0.99 ± 0.07	0.39 ± 0.10*	0.55 ± 0.09**	0.60 ± 0.22**	18.8	21.4	29.4
EAG-H	84	0.95 ± 0.07	0.36 ± 0.04**	0.52 ± 0.07**	0.51 ± 0.11**	25.0	25.7	40.0

Table 3 Effect of EAG on cotton pellet granuloma in rats ($\bar{x} \pm s$, $n = 10$)

Groups	Dose / ($g \cdot kg^{-1}$)	Weight of cotton pellet granuloma / mg	Inhibitory rate / %
control	—	130.05 ± 20.32	—
DEX	0.1	44.55 ± 4.58**	65.72
EAG-L	21	106.67 ± 12.22**	17.99
EAG-M	42	100.20 ± 12.76**	22.98
EAG-H	84	86.28 ± 19.17**	33.66

Acetic acid writhing test in mice

As shown in Table 4, each dose of EAG could significantly reduce the writhing times of acid-induced mice in 20 min after injection ($P < 0.01$), and it inhibited the time in a dose-dependent manner with the inhibitory rates of 18.89%, 31.00%, and 51.83%, respectively.

Table 4 Effect of EAG on writhing test in mice ($\bar{x} \pm s$, $n = 10$)

Group	Dose / ($g \cdot kg^{-1}$)	Writhing times	Inhibitory rate / %
control	—	27.90 ± 5.59	—
Asp	0.1	10.56 ± 4.67**	62.15
EAG-L	21	22.63 ± 4.47**	18.89
EAG-M	42	19.25 ± 4.68**	31.00
EAG-H	84	13.44 ± 7.96**	51.83

Hot-plate test in mice

Table 5 showed that each dose of EAG elevated pain threshold by 55.75%, 90.32%, and 91.62% respectively at 30 min after administration ($P < 0.05$).

The enhancement of pain threshold of mice treated with EAG-M and EAG-H was almost the same, and it was greater than that in Asp group.

Fever induced by yeast

As shown in Table 6, the action time of EAG-H group was longer than those of EAG-L and EAG-M groups. EAG-H remarkably decreased the value rising temperature of fever rats at 4, 6, and 8 h after injection of yeast ($P < 0.01$). EAG-M remarkably decreased the value at 6 and 8 h after the injection ($P < 0.01$). EAG-L remarkably decreased the value at 4 h after the injection ($P < 0.01$). The change value of rectal temperature in EAG-L and EAG-M groups was not significantly decreased compared with that in the control group at 8 h ($P > 0.05$).

Cough induced by ammonia through sequential experimental method

Table 7 showed that the latency of coughing in half mice was significantly prolonged in all the treatment groups ($R > 130\%$). The effect of EAG was suitable to the Pentoxyverine group ($P > 0.05$).

Tracheal excretion of phenol red in mice

The excretion of phenol red in the five groups was 1.25 ± 0.33, 1.87 ± 0.32, 1.67 ± 0.29, 1.71 ± 0.26, and 1.84 ± 0.11. Each dose of EAG promoted the secretion of phenol red by trachea obviously ($P < 0.01$), as shown in Fig. 2. The effect of EAG-H group equaled to that of Pentoxyverine group ($P > 0.05$).

Diarrhea of mice due to *Sennae Folium*

Fig. 3 showed that each dose of EAG inhibited diarrhea induced by *Sennae Folium* obviously with the amount of wet manure 12.8 ± 4.8, 12.5 ± 1.8, and 11.8 ± 2.3, compared with the control group, in which the

Table 5 Effect of EAG on hot-plate test in mice ($\bar{x} \pm s$, $n = 10$)

Groups	Dose / ($g \cdot kg^{-1}$)	Normal pain threshold / s	Pain threshold after administration / s	Enhancement rate / %
control	—	21.00 ± 8.18	22.50 ± 9.42	7.14
Asp	0.1	13.80 ± 7.12	24.80 ± 14.88*	79.71
EAG-L	21	17.40 ± 7.41	27.10 ± 11.27*	55.75
EAG-M	42	12.40 ± 4.97	23.60 ± 10.45*	90.32
EAG-H	84	16.70 ± 5.27	32.00 ± 18.07*	91.62

Table 6 Effect of EAG on fever rats induced by yeast ($\bar{x} \pm s$, $n = 10$)

Groups	Dose / ($g \cdot kg^{-1}$)	Basal temperature / ℃	Value rising temperature / ℃			
			2 h	4 h	6 h	8 h
control	—	37.84 ± 0.25	0.23 ± 0.19	0.81 ±0.13	1.37 ± 0.23	1.84 ± 0.23
Asp	0.1	37.83 ± 0.29	0.16 ± 0.70	0.51 ± 0.11**	0.93 ± 0.15**	1.44 ± 0.17**
EAG-L	21	37.73 ± 0.34	0.20 ± 0.10	0.67 ± 0.15**	1.22 ± 0.18	1.69 ± 0.20
EAG-M	42	37.70 ± 0.40	0.18 ± 0.11	0.58 ± 0.16**	1.11 ± 0.25**	1.67 ± 0.26
EAG-H	84	37.71 ± 0.38	0.12 ± 0.06	0.55 ± 0.16**	1.10 ± 0.21**	1.60 ± 0.22**

Table 7 Effect of EAG on latency of coughing in half of mice ($\bar{x} \pm s$, $n = 10$)

Groups	Dose / (g·kg^{-1})	EDT_{50} / s	R / %
control	—	32.4	—
Pentoxyverine	0.1	44.7	140$^{\#}$
EAG-L	21	44.7	140$^{\#}$
EAG-M	42	44.7	140$^{\#}$
EAG-H	84	46.8	144$^{\#}$

$^{\#}R > 130\%$

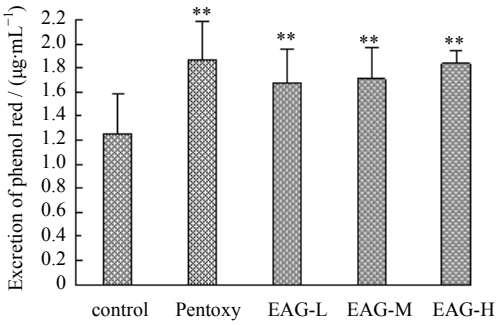

Fig. 2 Effect of EAG on tracheal excretion of phenol red ($\bar{x} \pm s$, $n = 10$)

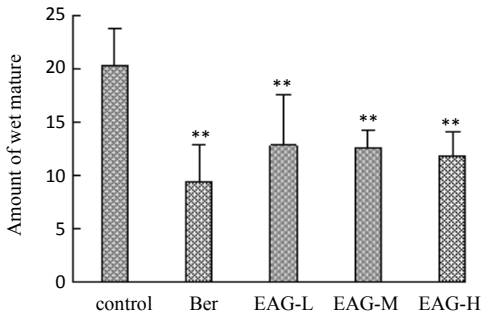

Fig. 3 Effect of EAG on diarrhea of mice due to *Sennae Folium* ($\bar{x} \pm s$, $n = 10$)

amount was 20.33 ± 3.5 ($P < 0.01$). The effect of EAG-H was suitable to berberine ($P > 0.05$). The effects of EAG-L and EAG-M were equivalent.

Intestinal propulsion of mice

The pushing rates of the five groups were 72.4 ± 3.6, 51.2 ± 17.7, 59.9 ± 12.3, 58.8 ± 4.7, and 50.9 ± 7.7 (Fig. 4). Each dose of EAG could obviously inhibit the intestinal propulsion of mice ($P < 0.01$), and the effect of each group was equivalent to that of berberine. There was little difference between the effects of EAG-L and EAG-M ($P > 0.05$).

Discussion

The roots of *A. gusanlung* have been used for cleaning away heat, moistening aridity, purging fire,

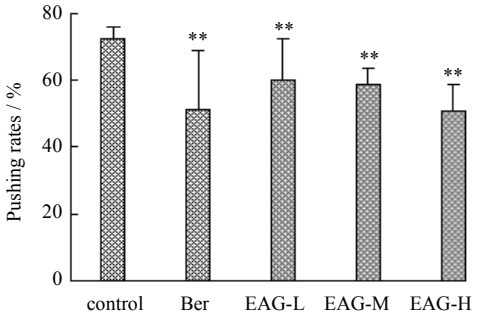

Fig. 4 Effect of EAG on normal intestinal propulsion of mice ($\bar{x} \pm s$, $n = 10$)

and detoxifying toxicosis in accordance with the theory of CMM. Classical inflammatory models were used to test its anti-inflammatory effects. The results indicated that EAG had the effect on the acute and chronic inflammation. By means of hot-plate test and peripheral analgesic model of the acetic acid injection in mice, it was found that EAG had analgesia effect on the central nervous system, but its peripheral analgesic effect was greater. Its antipyresis effect was obvious, the greater of the dosage was, the longer the effect lasted. The toxicologic study showed that EAG was safe with ig administration at normal clinical dosage.

Fourteen known compounds containing isoquinoline alkaloids and glycosides in EAG have been found. Gusanlungionosides A—D had the effect on inhibiting melanogenesis (Yu *et al*, 2011). The modern researches show that the isoquinoline alkaloids, such as berberine, palmatine, and jatrorrhizine, are of great importance to humanity because of their medicinal values and different structures (Bruneton, 1995). Jatrorrhizine is one of the main antimicrobial ingredients of *Coptis chinensis* L. and the activity is worse than that of berberine (Yang, Ye, and Li, 2007). Berberine and palmatine are important protoberberine alkaloids. They have the same tetracyclic structures but with different subsitituents on the benzo ring. They have common pharmacological and biological activities including anti-inflammatory, antimicrobial, and anti-diarrhoeal effects (Giri, Hossain, and Kumar, 2006; Da-Cunha *et al*, 2005). However, the antidiarrhoeal mechanism is different. As is known, an increased Cl^- secretion is the major pathogenesis of secretory diarrhoea. Increased intracellular cAMP and cGMP levels could activate the cystic fibrosis transmembrane conductance regulator (CFTCR) Cl^- channel in the

apical membrane of enterocytes. Additionally, the cAMP-activated (voltage-dependent delayed activated K^+) channel and Ca^{2+}-activated (small-conductance Ca^{2+}-activated K^+) channel were two types basolateral K^+ channels in the apicalmembrane of enterocytes, which maintains a negative electrical driving force for luminal Cl^- secretion when they were open. Inhibition of both the CFTCR Cl^- channel and the basolateral cAMP-activated K^+ channel has been recognized as major pharmacological targets for the treatment of secretory diarrhoea (Oprins, Meijer, and Groot, 2000; Albano *et al*, 2005; McNamara *et al*, 1999). Berberine blocked basolateral K^+ channels, while palmatine inhibited both Ca^{2+}- and cAMP-activated pathways (Taylor *et al*, 1999; Wu *et al*, 2008). This study indicated that the inhibitory effect of EAG on the intestinal function was remarkably significant, but the substance which is responsible for the inhibition is unknown. Further research is needed to confirm whether berberine and palmatine is related to the inhibitory action on the intestinal function and what the mechanism is.

Bronchitis is chronic nonspecific inflammation of trachea and bronchial mucosal tissues with the symptom of cough, cough with sputum, and asthma. This study showed that EAG could remarkably prolong the cough incubation period and increase the phlegm liquid secreting capacity of mice, and there was no statistical difference as compared with the Pentoxyverine group. Whether EAG is effective in bronchitis needs more research.

Chinese herb has the characteristics of multi-components, multi-targets, and multi-pathway regulation, and the effect of which was not due to one component but effective fractions (Chen and Zhao, 2010). The studies on single compounds and multicomponents are both important. The index component of berberine and the content of total alkaloids were determined in our former studies, but the effective fractions of EAG remain to be screened so as to enhance the quality control.

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