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Gastroprotective effect of a traditional Chinese herbal drug "Baishouwu" on experimental gastric lesions in rats

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Abstract

"Baishouwu" is an appellative name of dried root tubers from three Asclepiadaceae plants: *Cynanchum auriculatum* Royle ex Wight, *Cynanchum bungei* Decne and *Cynoctonum wilfordii* Maxim. In order to establish the pharmacological basis for the ethnomedicinal use of Baishouwu in gastric disorders, this study examined the effects of ethanol extracts and fractions from root tubers of *Cynanchum auriculatum*, *Cynanchum bungei* and *Cynoctonum wilfordii* on ethanol-, indomethacin-induced gastric lesions and histamine-induced gastric acid secretion in rats. Plant materials were collected from various areas of China. Oral administration of ethanol extract and chloroform fraction of *Cynoctonum wilfordii* collected from *Changbai* Cordillera at doses of 150 and 68 mg/kg, respectively, significantly inhibited the development of ethanol- and indomethacin-induced gastric lesions and also caused significant decrease of gastric acid secretion after histamine-induced gastric lesion. Oral administrations of ethanol extract and chloroform fraction of *Cynanchum auriculatum* collected from Binhai at the doses of 300 and 69 mg/kg, respectively, significantly inhibited ethanol- and indomethacin-induced gastric lesions. This study demonstrates the gastroprotective property of Baishouwu for the first time.

Keywords: Gastroprotective activity; Baishouwu; *Cynanchum auriculatum*; *Cynanchum bungei*; *Cynoctonum wilfordii*

1. Introduction

Baishouwu is an appellative name of root tubers from *Cynanchum auriculatum* Royle ex Wight, *Cynanchum bungei* Decne and *Cynoctonum wilfordii* Maxim, all of which belong to Asclepiadaceae. It is a famous tonic herbal drug in traditional Chinese medicine (TCM), and has been used for over 1000 years from *Tang* Dynasty of China. Baishouwu is known for its functions in enriching vital essence and enhancing immunity. Modern pharmacological studies have shown that the extract and fractions of Baishouwu have varieties of pharmacological actions, including anti-tumor (Shan et al., 2005), clearing away free radicals (Song and Ding, 1997), enhancing immunity (Gu et al., 1987) and reducing high serum cholesterols (Niu et al., 1988). A recent ethnobotanical survey conducted by our research group revealed that Baishouwu is an important herbal drug for the treatment of gastric disorders (e.g. indigestion and stomachache) in the ethnomedicine of the Tujia and Hmong/Miao.

The misuse of different herbal drugs with the same appellative name may produce unexpected or even harmful effects in the patients. This kind of misuse probably exists in the ethnomedical practice of Baishouwu because the authentic original plant material of it remains indefinite. Now the most commonly accepted but not necessarily the correct opinion is that root tubers from *Cynanchum auriculatum*, *Cynanchum bungei* and *Cynoctonum wilfordii* can all be used as Baishouwu. The three plant species of Baishouwu distribute in different areas: *Cynoctonum wilfordii* mainly in *Changbai* Cordillera in northeastern China, *Cynanchum bungei* only in *Tai* Mountain of Shandong Province and *Cynanchum auriculatum* in most areas of southern China, which distributes most widely of the three species

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and provides the greatest amount of Baishouwu for the market. There are, however, no scientific reports until now in support of Baishouwu's ethnomedicinal claims for gastric infections. Comparisons of efficacy among Baishouwu of different species and from various growing areas have not been reported, either. In this study, three species of Baishouwu from five provinces, including both crude ethanol extracts and different fractions of crude extract, were tested against experimental-induced gastric lesions in rats. And by the comparison and analysis of experimental results, we try to give a possible resolution for the confusion of Baishouwu's authentic original plant material for gastric infections treatment.

2. Materials and methods

2.1. Animal

Male Sprague–Dawley rats (180–200 g) were used for the experiment. The animals were maintained in propylene cages at 26 ± 2 °C in a 12-h light:12-h dark cycle and had free access to standard pellet chow and water. Groups of eight animals were used for experimentation.

2.2. Plant materials

The plant materials of Baishouwu were collected in Jiangsu, Shandong, Liaoning and Guizhou Provinces from 2002 to 2004. After identification and authentication by Professor Hanmin Zhang of the Department of Medical Botany, Second Military Medical University of Shanghai, China, voucher specimens (Nos. 3167–3172) were deposited at the Herbarium of this University.

2.3. Preparation of plant extracts and fractions

The root tubers were washed clean, sliced and dried in the sun. After that, dried root tubers were macerated with ethanol (50%) at room temperature for a period of 10 days. The ethanol extract was separated by filtration and evaporated in vacuo. The residue was suspended in water, and then partitioned with petroleum ether (PE), chloroform ($CHCl_3$), acetic acid ethyl ester (EtOAc), *n*-butyl alcohol (*n*-BuOH) and aqueous (H_2O), affording the fractions. The five fractions were evaporated in vacuo. The yields of the dried ethanol extract and fractions tested in this study are shown in Fig. 1, as a percentage weight of the dried root tuber. All

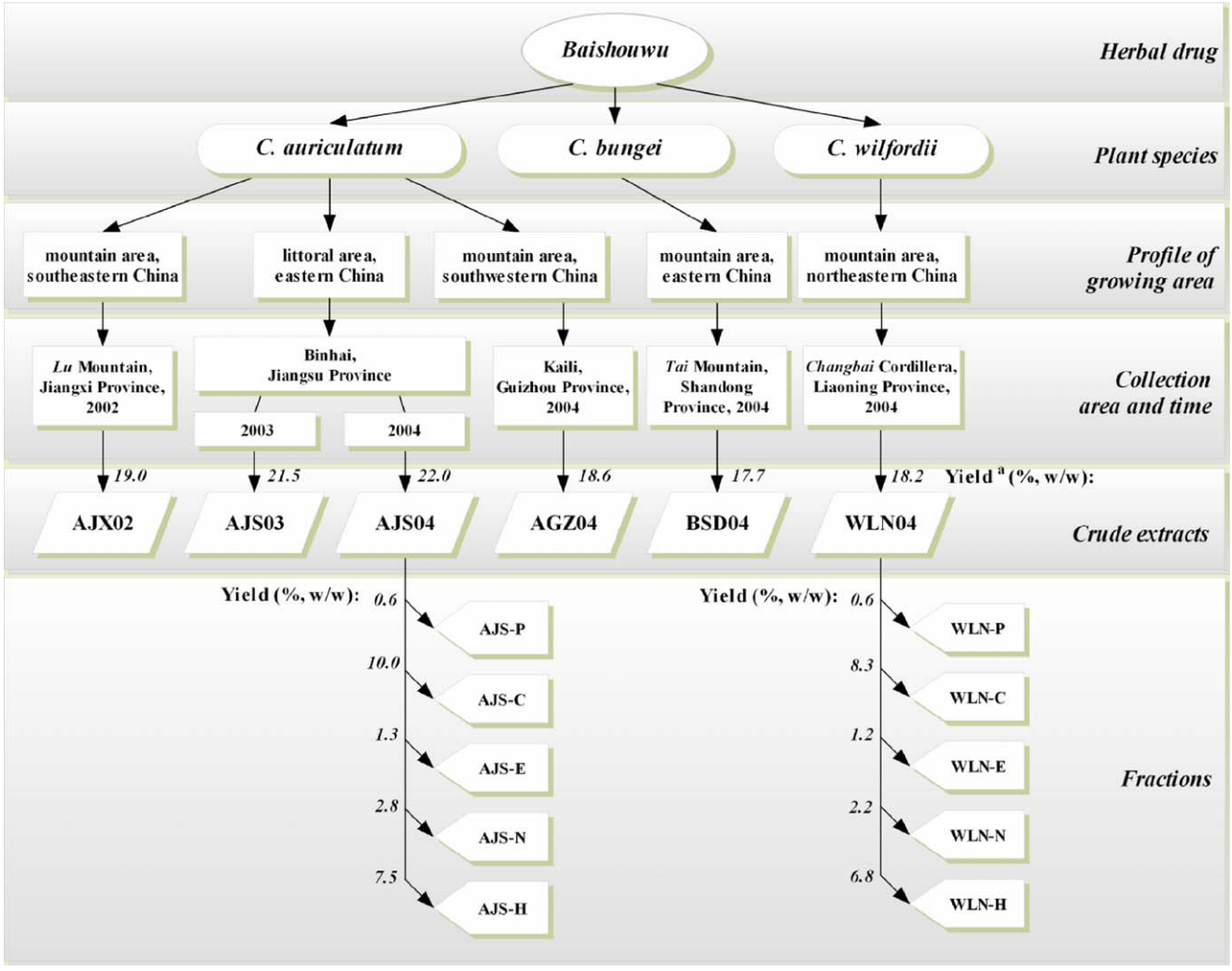

Fig. 1. Information of plant materials and isolation flowing of the tested samples. (a) Yield was calculated in terms of starting crude material.

Table 1
Effects of Baishouwu on ethanol-, indomethacin-induced gastric lesions and histamine-induced gastric acid secretion[a]

Code of tested samples	Dose (mg/kg)	Number of rats	Ethanol		Indomethacin		Histamine	
			Lesion index (mm)	Percentage inhibition	Lesion index (mm)	Percentage inhibition	pH	H^+ (μmol/4 h)
Experiment 1								
Control	–	8	94.9 ± 6.6	–	31.0 ± 3.0	–	2.5 ± 0.2	91.5 ± 13.9
AJX02	150.0	8	79.4 ± 10.5	16.3	21.4 ± 5.9	31.0	2.4 ± 0.1	78.6 ± 11.3
AJS03	150.0	8	82.4 ± 8.2	13.3	9.6 ± 2.7**	69.0	2.2 ± 0.1	101.7 ± 17.5
AJS04	150.0	8	76.8 ± 5.3	19.0	7.4 ± 1.9**	76.1	2.9 ± 0.5	67.4 ± 14.1
AGZ04	150.0	8	42.7 ± 9.8**	55.0	40.4 ± 9.3	–	2.5 ± 0.2	89.6 ± 12.9
WLN04	150.0	8	40.5 ± 12.1**	57.3	14.7 ± 6.9*	52.6	3.7 ± 0.4*	44.9 ± 8.8*
BSD04	150.0	8	74.4 ± 10.3	21.6	11.1 ± 3.5**	64.2	2.7 ± 0.2	81.7 ± 8.9
Experiment 2								
Control	–	8	102.5 ± 7.8	–	31.7 ± 3.3	–	2.5 ± 0.3	93.5 ± 15.2
AJS04(H)	300	8	50.2 ± 12.5*	51.0	5.9 ± 0.9**	81.4	2.3 ± 0.2	65.9 ± 13.7
Experiment 3								
Control	–	8	107.0 ± 22.1	–	32.0 ± 5.0	–	2.42 ± 0.1	96.6 ± 13.9
WLN-P	5.0	8	101.0 ± 15.5	6.1	35.9 ± 10.4	–	2.05 ± 0.3	90.5 ± 17.6
WLN-C	62.0	8	46.5 ± 12.7**	56.5	15.7 ± 4.1*	50.9	3.88 ± 0.5*	48.4 ± 6.8*
WLN-E	9.0	8	102.6 ± 10.2	4.1	42.0 ± 5.7	–	2.82 ± 0.4	90.6 ± 12.1
WLN-N	17.0	8	107.7 ± 13.8	–	29.9 ± 4.6	6.6	2.24 ± 0.1	105.4 ± 18.4
WLN-H	51.0	8	92.9 ± 14.5	13.2	50.4 ± 8.7	–	2.21 ± 0.5	93.7 ± 16.1
AJS-P	5.0	8	116.0 ± 18.2	–	47.9 ± 11.6	–	2.28 ± 0.1	104.0 ± 18.4
AJS-C	75.0	8	43.7 ± 4.3**	59.2	12.4 ± 1.3**	61.3	2.27 ± 0.1	93.7 ± 14.3
AJS-E	10.0	8	105.6 ± 14.1	1.3	49.0 ± 7.7	–	2.32 ± 0.2	103.6 ± 16.3
AJS-N	21.0	8	91.0 ± 12.6	15.0	21.1 ± 2.9*	44.1	2.63 ± 0.4	107.3 ± 25.3
AJS-H	56.0	8	93.9 ± 11.4	12.2	52.4 ± 8.8	–	2.39 ± 0.1	93.7 ± 16.1

[a] Values are expressed as mean ± S.E. ($n = 8$).
* Level of significance relative to the control value: $P < 0.05$.
** Level of significance relative to the control value: $P < 0.01$.

the extracts and fractions were prepared in 0.5% carboxymethyl cellulose (CMC) and given to the rats in a volume of 5 ml/kg.

2.4. Induction of gastric lesions

2.4.1. Ethanol-induced gastric lesions

Rats fasted for 24 h were treated with vehicle (CMC), or extracts/fractions (doses are shown in Table 1). One hour later, 1.0 ml absolute ethanol was administered intragastrically to control and drug-treated animals. Each animal was sacrificed by ether overdose 1 h after administration of ethanol. The stomachs were removed, washed gently with saline (0.9%) and inflated with 1% formalin solution (10 ml) and immersed in the same solution to fix the outer layer of stomach. After 10 min, each stomach was then opened along the greater curvature and examined under dissecting microscope to assess the formation of ulcers (Robert et al., 1979).

2.4.2. Indomethacin-induced gastric lesion

Rats fasted for 24 h were treated with vehicle (CMC), or extracts/fractions (doses are shown in Table 1). One hour later, the ulcerogenic agent indomethacin (Sigma, USA) prepared in 2% sodium bicarbonate solution was given to rats at an oral dose of 200 mg/kg. The animals were sacrificed after 4 h, the stomachs were removed, washed gently with saline (0.9%) and injected with 1% formalin solution (10 ml) to fix the outer layer of stomach (West, 1982). After 10 min, each stomach was then opened along the greater curvature and examined under dissecting microscope to assess the formation of ulcers.

2.4.3. Histamine-induced gastric acid secretion enhancement in rats

Rats were fasted for 24 h but permitted for water freely. Gastric lesion was induced by intragastrical administration of histamine acid phosphate (Sigma) (300 mg/kg). The animals were sacrificed after 4 h following histamine administration and the stomach was dissected out after pylorus and cardia ligation (Parmar and Desai, 1993). The gastric contents was collected and evacuated into a centrifuge tube for centrifugation (3000 rpm, 15 min).

2.5. Measurement of gastric lesions and acid secretions

2.5.1. Gastric lesion index (LI)

In ethanol- and indomethacin-induced gastric lesion models, stomachs were removed after sacrifice, opened along the greater curvature. The sum of length (mm) of all lesions for each stomach was calculated and used as the lesion index, and the inhibition percentage was calculated by the following formula: [(LI control − LI treated)/LI control)]/100.

2.5.2. Gastric acid secretion

In histamine-induced gastric acid secretion model, the gastric contents of each animal were individually assayed for the total

acidity. Total acidities in the supernatants were determined after centrifugation by titration with 0.01 M sodium hydroxide and the results were expressed in μmol/4 h and pH.

2.6. Statistical analysis

The values are expressed as mean ± standard deviations. The results were analyzed statistically by analysis of variance. Values of P less than 5% ($P < 0.05$) were considered to be statistically significant.

3. Results

Results of gastroprotective effect of crude extracts (7 samples in all) and fractions (10 samples in all) of Baishouwu against three models are shown in Table 1.

3.1. Effects of Baishouwu on ethanol-induced gastric lesion

In the 17 tested samples including crude extracts and fractions, 5 samples showed statistically significant gastric protective effect against lesion induced by ethanol. They are AJS04(H), AGZ04, WLN04, AJS-C and WLN-C. The other samples showed no significant gastric protection activity in this model.

List of abbreviations

Sample code	Definition
AJX02	Crude extract of *Cynanchum auriculatum* collected in *Lu* Mountain, Jiangxi Province, in 2002
AJS03	Crude extract of *Cynanchum auriculatum* collected in Binhai, Jiangsu Province, in 2003
AJS04	Crude extract of *Cynanchum auriculatum* collected in Binhai, Jiangsu Province, in 2004
AJS04(H)	Crude extract of *Cynanchum auriculatum* collected in Binhai, Jiangsu Province, in 2004 (high dose)
AGZ04	Crude extract of *Cynanchum auriculatum* collected in Kaili, Guizhou Province, in 2004
BSD04	Crude extract of *Cynanchum bungei* collected in *Tai* Mountain, Shandong Province, in 2004
WLN04	Crude extract of *Cynoctonum wilfordii* collected in *Changbai* Cordillera, Liaoning Province, in 2004
AJS-P	Petroleum ether (PE) fraction of AJS04
AJS-C	Chloroform ($CHCl_3$) fraction of AJS04
AJS-E	Acetic acid ethyl ester (EtOAc) fraction of AJS04
AJS-N	*n*-Butyl alcohol (*n*-BuOH) fraction of AJS04
AJS-H	Aqueous (H_2O) fraction of AJS04
WLN-P	Petroleum ether (PE) fraction of WLN04
WLN-C	Chloroform ($CHCl_3$) fraction of WLN04
WLN-E	Acetic acid ethyl ester (EtOAc) fraction of WLN04
WLN-N	*n*-Butyl alcohol (*n*-BuOH) fraction of WLN04
WLN-H	Aqueous (H_2O) fraction of WLN04

3.2. Effects of Baishouwu on indomethacin-induced gastric lesion

In the 17 tested samples, 8 samples showed statistically significant gastric protective effect against lesion caused by indomethacin. They are AJS03, AJS04, WLN04, AJS04(H), BSD04, AJS-C, AJS-N and WLN-C. The other samples showed no significant gastric protection activity in this model.

3.3. Effects of Baishouwu on histamine-induced gastric acid secretion

In the 17 tested samples, only WLN04 and WLN-C showed statistically significant gastric acid secretion inhibition effect after histamine-induced gastric lesion. The pH value of treated group (WLN04 and WLN-C) increased nearly to the normal level. The other samples showed no significant gastric protection activity in this model.

4. Discussion

4.1. Determination of extraction method and dosage of oral administration in rats

In ethnomedical practice of Baishouwu, cleaned and dried root tubers of plants are macerated in bottles of alcohol for about 2 weeks before use. The medicated wine is then drunk for treatment of gastric complaints when needed. So maceration with ethanol (50%) for 10 days at room temperature was adopted as the extraction method in the study.

Dose of crude plant material of Baishouwu for a person is about 30 g/day according to folk remedies. The average weight of Chinese adult is about 60 kg, and rats used in this experiment about 0.25 kg. The average yield of crude extracts is about 20%. Taking the specific surface area difference between human and rat into consideration, the moderate dosage of crude extract for the rats can be calculated by the following formula:

$$\left[\left(\frac{30\,\mathrm{g}}{60\,\mathrm{kg}}\right) \times 0.25\,\mathrm{kg} \times 6\right] \times 20\% \times 1000 = 150\,\mathrm{mg/kg}$$

So a dose of 150 mg/kg crude extracts was set for the experimentation. This dose is thought to be rational because it represents the ethnomedically therapeutic dose of Baishouwu for gastric disorders. Dosage of fractions was calculated by dose of crude extract and the yield of the fractions. Thus, the crude extracts and fractions were ensured to be equal in dose converting into crude plant materials.

4.2. Diversity of gastroprotective effect of Baishouwu among various original plants and growing areas

By analysis of experimental data of crude extracts, a notable diversity in gastroprotection effect can be found between different samples of Baishouwu prepared from various original plant materials. Moreover, the diversity can also be found between plants of the same species collected from different original growing areas.

In all the three plant species of Baishouwu, *Cynoctonum wilfordii* was proved by our experiments to be the most effective species on the experimentally induced gastric lesions and gastric acid secretion. At the dose of 150 mg/kg crude extract, *Cynoctonum wilfordii* showed statistically significant anti-lesion

and anti-acid secretion effects in rats compared with the control group. However, at the same dose, *Cynanchum auriculatum* and *Cynanchum bungei* were only effective on either the indomethacin-induced gastric lesion or the ethanol-induced gastric lesion. Neither *Cynanchum auriculatum* nor *Cynanchum bungei* had efficacy on histamine-induced gastric acid secretion. Even at the doubled dose of 300 mg/kg crude extract, *Cynanchum auriculatum* (sample AJS04(H) in the test) did not show statistically significant effect on the acid secretion compared with the control group. Therefore, it is conceivable that *Cynoctonum wilfordii* is the most effective species of Baishouwu. It is suggested, from our results, to use *Cynoctonum wilfordii* from *Changbai* Cordillera in the prescription of ethnomedical practice and the manufacture of gastroprotective drugs derived from Baishouwu, instead of *Cynanchum auriculatum* and *Cynanchum bungei*.

The same original plants of Baishouwu collected from different areas were also found to have activity diversity. For example, the tested four samples of *Cynanchum auriculatum* collected from the eastern littoral area (AJS03 and AJS04), southeastern mountain area (AJX02) and southwestern mountain area (AGZ04) were found to have different gastroprotective activities. At the dose of 150 mg/kg crude extract, AJS03 and AJS04 were only effective on the indomethacin-induced lesion; AGZ04 was only effective on the ethanol-induced lesion, while AJX02 was not effective in all the three test models. The homological relationship of *Cynanchum auriculatum* from different growing areas may only lie in the effect on histamine-induced acid secretion: all the samples of *Cynanchum auriculatum* showed no significant activity in this model.

Cynanchum auriculatum of Binhai is cultivated by the local inhabitants while all the other species are wild plants, so growing periods and environments of them are different: *Cynanchum auriculatum* of Binhai grows about 7 months (July–November) in plantation, and the wild species grows about 3–5 years in woodland. Taking this into consideration, it is reasonable that the content of secondary metabolism products (especially the active constituents) in *Cynanchum auriculatum* from Binhai is low than that of the wild species. It was presumed to be the reason for the inefficacy on the ethanol-induced gastric lesion and histamine-induced gastric acid secretion enhancement and a higher dose of *Cynanchum auriculatum* from Binhai may show a better effect on experimental gastric lesion models. In order to validate this assumption, we promoted further experiment by doubled dosage of crude extract (AJS04(H), 300 mg/kg). The assumption was partly proved: 300 mg/kg crude extract of *Cynanchum auriculatum* from Binhai showed statistically significant gastroprotective effect on both ethanol- and indomethacin-induced gastric lesions, but it was still ineffective on experimental acid secretion enhancement. Therefore, it is conceivable that the diversity of gastroprotective effect between different samples of Baishouwu may be due to the type of active constituents as well as the content of them.

It should be emphasized that the evaluation of different original materials of Baishouwu is limited to the treatment of gastric infections. And a comprehensive conclusion of the activity of Baishouwu is awaiting further studies.

4.3. Determination of effective fractions of Baishouwu

As was designed in the research program, 30 fractions (6 crude extracts × 5) would be tested in the experiment. However, in the preliminary study of crude extracts, AJX02, AGZ04 and BSD04 were found to have a weak or even no gastroprotective effect. It seemed that, for AJX02, AGZ04 and BSD04, it was senseless to further investigate the effective fractions and fractions of these samples should be rejected from the test group. *Cynanchum auriculatum* from Binhai (AJS03 and AJS04) did not show satisfying gastroprotective effect in the preliminary assay of crude extracts. The fractions (AJS-P, AJS-C, AJS-E, AJS-N and AJS-H) of AJS04, however, were put forward into the investigation for effective fractions along with those of WLN04 because *Cynanchum auriculatum* from Binhai plays an essential role in the ethnomedicinal use of Baishouwu in China. The yield of *Cynanchum auriculatum* of Binhai is over 5,000,000 kg each year, accounting for more than 85% of the total yield of Baishouwu in China. The results showed that the chloroform fraction was more effective than the crude extract and the other fractions: it was the effective fraction.

Ethanol extract of *Cynoctonum wilfordii* (*Changbai* Cordillera) is effective in all the three models, which provides an ideal research material for the determination of effective fraction. Given the same dose (converted to crude plant material), chloroform fraction (WLN-C) showed similar activity profile to that of the crude extract (WLN04) in all the three models. The activities of other fractions (WLN-P, WLN-E, WLN-N and WLN-H) were distinct from that of the crude extract. So the chloroform fraction is suggested to be the effective fraction for gastroprotective activity of Baishouwu. This deduction is in accordance with the result of *Cynanchum auriculatum* (Binhai). The result indicates the active constituents for gastric protection were concentrated into the chloroform fraction. It can be predicted that a potential gastroprotective drug may be developed from the chloroform fraction of *Cynoctonum wilfordii* (*Changbai* Cordillera).

5. Conclusions

1. Baishouwu is demonstrated by this study to possess gastroprotective property as evidenced by its significant inhibition in the formation of gastric lesions induced by ethanol, indomethacin and the gastric acid secretion induced by histamine.
2. *Cynoctonum wilfordii* from *Changbai* Cordillera is the best original plant material of Baishouwu for the treatment of gastric infections, better than *Cynanchum auriculatum* from Binhai which is now much more normally used in China.
3. The chloroform fraction derived from the ethanol extract is the effective fraction of crude plant material and may lead to a novel gastroprotective drug.

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