Gastroprotective Effects of Ascaridole on Gastric Ulcer in Rats

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Abstract: **Objective** To evaluate the gastroprotective activity of ascaridole. **Methods** The gastroprotective effect of ascaridole was evaluated on ulcer healing in rats with acetic acid-induced chronic gastric ulcer, pylorus ligation- and Aspirin-induced gastric ulcer. Ascaridole was ig administered with the dosages of 10 and 20 mg/kg once daily for 7 d. **Results** Ascaridole showed the significant anti-ulcer effects. In acetic acid-induced gastric ulcer rats, the ulcer areas after 10 and 20 mg/kg of ascaridole treatment were (65.1 ± 20.0) and (50.6 ± 11.0) mm^2, respectively, which were significant lower ($P < 0.01$) than that of the control group [(116.7 ± 35.8) mm^2]. For pylorus ligation model, ascaridole showed a gastric ulcer healing effect in a dose-dependent manner. Ascaridole at the dose of 20 mg/kg showed 50% ulcer protection and had a significant ($P < 0.05$) gastroprotective activity since it decreased the total acidity and pepsin activity. Compared to the control group, the two dosages of ascaridole showed the significant reduction ($P < 0.05$) in the ulcer index on Aspirin-induced ulcer. **Conclusion** This study provides evidence that ascaridole shows potential efficacy on the healing of gastric ulcers induced by acetic acid, Aspirin, and pylorus ligation.

Key words: ascaridole; Aspirin; chronic gastric ulcer; gastroprotective effect; pylorus ligation
DOI: 10.3969/j.issn.1674-6384.2012.01.009

Introduction

Jinghua Weikang (JHWK) Capsule, composed of the essential oil from *Chenopodium ambrosioides* L. and *Adina pilulifera* (Lam.) Franch. ex Drake is one of Chinese patent medicines that was approved in 1997 by China State Food and Drug Administration (SFDA). In past decades, JHWK Capsule has been popularly used for the treatment of acute or chronic superficial gastritis, chronic erosive gastritis, duodenum ulcer, gastric ulcer, duodenitis, and chronic atrophic gastritis. Xie and Huang (2001) described that JHWK Capsule significantly inhibited stress-induced gastric ulcer, pyloric ligation-induced gastric ulcer, and Reserpine-induced gastric ulcer in rats, reduced gastric acid and the activity of pepsin, and increased the gastric mucus secretion. JHWK Capsule had been reported that it could inhibit *Helicobacter pylori* 26695 and antibiotic-resistant *H. pylori* strains *in vitro*, and it was synergistic with Metronidazole or Clarithromycin (Huang *et al*, 2010). Sheng *et al* (2007) also reported it had therapeutic efficacy for functional dyspepsia. So far, although many literatures showed the anti-*H. pylori* and gastroprotective effects of JHWK Capsule, the active components of JHWK Capsule have not been reported.

The genus *Chenopodium* L. includes varieties of weedy herbs (more than 200 species) native to much of Europe, Asia, and both North and South America (Smith, 2006). *C. ambrosioides* has been widely known as anti-helminthic, vermifuge, emmenagogue, and abortifacient popular medicine (Bhargava, Shukla, and Ohri, 2005; Rimada, Jeandupeux, and Cafferata Lazaro, 2007). It is used for the treatment of digestive, respiratory, urogenital, vascular, and nervous disorders, and for metabolic disturbances, such as diabetes and hypercholesterolemia, and as sedative,

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Received: December 28, 2011; Revised: January 18, 2012; Accepted: January 30, 2012
Fund: National Key Special Project of Science and Technology for Innovation Drugs of China (2008ZX09401-006)

antipyretic, and antirrheumatic (de Feo and Senatore, 1993). Noumi and Yomi (2001) reported that the plant was also used against stomach cramps, syphilis, measles, and intestinal diseases. Ascaridole was first reported by a German pharmacist in 1895 and it has been attributed with most of the vermifuge actions of *C. ambrosioides.* Ascaridole is a bicyclic monoterpene that has an unusual bridging peroxide functional group. Smillie and Pessoa (1924) firstly showed that the anthelmintic properties of chenopodium oil were due to the compound ascaridole, which constituted more than 50% of oil. Ascaridole has been documented with sedative and pain-relieving properties as well as antifungal effects (Pare *et al*, 1993). Ascaridole was found to be a potent inhibitor of *in vitro* development of *Plasmodium falciparum* (Pollack, Segal, and Golenser, 1990), *Trypanosoma cruzi* (Kiuchi *et al*, 2002), and *Leishmania amazonensis* (Monzote *et al*, 2006). MacDonald (2004) reported that ascaridole caused a reduction of carbachol-induced contractions in rat gastro-intestinal smooth muscle at certain concentrations.

In our previous study, the main chemical components of JHWK Capsule were analyzed by GC-MS. Twenty-two compounds were identified in MS, boiling point, retention index, and the relative content of each component was calculated by area normalization. Ascaridole is the major component of JHWK Capsule, which constituted more than 60% of the oil weight. Now the separation and purification method of ascaridole were established by our group. Although its many activities were studied, the gastroprotective effect on ulcer healing of ascaridole has not been reported. In the present study, we preliminary investigated the gastroprotective effect of ascaridole on ulcer healing of acetic acid- and pylorus ligation-induced gastric ulcer in rats. The study on gastroprotective effects of other components in JHWK Capsule is also underway in our laboratory.

Materials and methods

Drugs and chemicals

Ascaridole was separated and purified from JHWK Capsule (Tianjin Tasly Group, Tianjin, China).

Ouhuada (ranitidine hydrochloride) was purchased from Guangzhou Ouhua Pharmaceutica Co., Ltd. and Aspirin was purchased from Kaifeng Pharmaceutica. Glacial acetic acid and other solvents used were of analytical grade and were purchased from local chemical company (Tianjin, China). Pepsin test box (A080-1) was purchased from Nanjing Jiancheng Bioengineering Institute.

Determination of ascaridole purity

An HPLC method was used for the purity determination of ascaridole. It was performed with an Agilent series 1100 HPLC equipped with a quaternary gradient pump, autosampler, and diode array detector using an Agilent Zorbax SB-C_{18} analytical column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (A) and water (B) with a gradient elution of 30%—40% A in 0—30 min, 40%—58% A in 30—50 min, 58%—70% A in 50—75 min, 70%—100% A in 75—95 min and 100% A in 95—105 min. The flow rate was 1.0 mL/min and column temperature was maintained at 25 ℃. The chromatograms were monitored at 226 nm.

Reference solution of ascaridole was prepared by dissolving the accurately weighed reference compounds in mobile phase to give final concentration of 5 mg/mL. Ascaridole solution (5 μL) was applied to the chroma-tographic system for the determination of purity.

Experimental animals

Male SD rats weighing 200—250 g were used for this study. The animals were maintained under reference conditions in an animal house for one week prior to the study.

Acetic acid-induced chronic gastric ulcer

Fifty rats were divided into five groups and fasted for 24 h prior to the experiment. Under 10% chloral hydrate anaesthesia, ulcers were induced by injecting 20% glacial acetic acid (0.04 mL) under the anterior serosal surface of the stomach. Four groups of animals were treated, respectively with Ranitidine (50 mg/kg, ig), JHWK Capsule (10 mg/kg, ig), low or high doses (10, 20 mg/kg, ig) of ascaridole, once daily, for 7 d after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on day 7, and the stomachs were removed and cut open along the greater curvature (Xue *et al*, 2004).

Pylorus ligation-induced ulcers

Four groups, each comprised of eight rats, were included in the anti-ulcer studies. One group was treated with the vehicle, the other were treated with Ranitidine (50 mg/kg, ig), low or high doses (10, 20

mg/kg, ig) of ascaridole respectively, once daily, for one week. After 2 h of ascaridole or Ranitidine treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats in respective group under ether anaesthesia. The stomachs were isolated and the content collected and centrifuged. The volume of the gastric juice was measured and this was used for the estimation of free acidity, total acidity pepsin content, and total proteins. The ulcer index and gastric mucous content were determined (Khare *et al*, 2008). The percentage of ulcer protection was determined as follows: ulcer protection (%) = (ulcer index of control – ulcer index of test) / ulcer index of control × 100%

Aspirin-induced gastric ulcers

Animal grouping and treatment are the same as the method in pylorus ligation-induced ulcers. The animals had been fasted previously for 24 h to empty the stomach of food, and to increase the gastric acid level, thereby facilitating the induction of gastric ulcer upon ig administration of 10 mL/kg Aspirin at the concentration of 20 mmol/L. After 4 h, all the animals were killed and ulcer indexes were determined as mentioned by Khushtar *et al* (2009). In each rat, the macroscopic injury of each ulcer was scored by an independent observer according to a scale ranging from 0 to 4 as follows: (0) no macroscopic changes; (1) mucosal erythema only; (2) mild mucosal edema, slight bleeding or small erosions; (3) moderate edema, bleeding ulcers or erosions; and (4) severe ulceration, erosions, edema, and tissue necrosis. Ulcer index was then calculated by adding the total score of ulcers per stomach.

Statistical analysis

The values were expressed as $\bar{x} \pm$ s and $P < 0.05$ was considered significant. The statistical significance was assessed using One-way analysis of variance (ANOVA) process of the general linear models in SPSS11.0.

Results

Determination of ascaridole purity

The purity of ascaridole was more than 90%, which was calculated by area normalization method. Fig. 1 shows the purity of ascaridole by HPLC.

Acetic acid-induced chronic gastric ulcer

Here, the gastroprotective effects of ascaridole on ulcer healing of acetic acid-induced gastric ulcer in rats

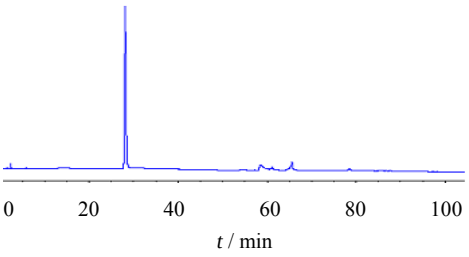

Fig. 1 Purity determination of ascaridole using HPLC

were preliminary investigated here. The two doses of ascaridole were evaluated in the experiment, in which 20 mg/kg is set up in reference of the clinical equivalent dose of JHWK Capsule since LD_{50} of ascaridole in mice is close to that of JHWK Capsule.

There was no difference in weight gained among the five groups of animals. As shown in Fig. 2, the rats' ulcer areas after 10 and 20 mg/kg of ascaridole treatment were (65.1 ± 20.0) and (50.6 ± 11.0) mm^2, respectively, which were significant lower ($P < 0.01$) than that of the control group [(116.7 ± 35.8) mm^2]. The high dose of ascaridole was significantly more effective than the low dose group in reducing the ulcer area. The difference between the JHWK Capsule and the ascaridole groups was no statistically significant. And the anti-ulcer activity of ascaridole at 20 mg/kg is almost equipotent as those of JHWK Capsule at 10 mg/kg and Ranitidine at 50 mg/kg.

Pylorus ligation-induced gastric ulcers

Table 1 lists the effect of ascaridole on total acidity, pepsin content, and ulcer index in pylorus ligated rats. The results showed that the two doses of ascaridole had a

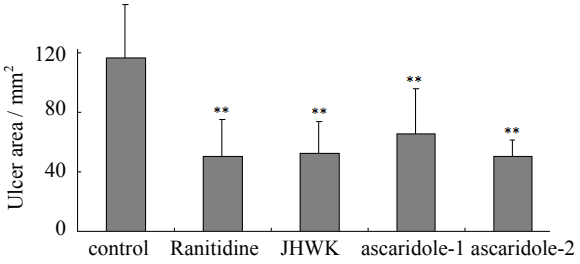

Fig. 2 Effect of ascaridole on acetic acid-induced chronic gastric ulcer in rats

Control group: ig administration of the vehicle; Ranitidine group: ig administration of Ranitidine at dose of 50 mg/kg; JHWK group: ig administration of JHWK Capsule at dose 10 mg/kg; Ascaridole-1 group: ig administration of ascaridole at dose of 10 mg/kg; Ascaridole-2 group: ig administration of ascaridole at dose of 20 mg/kg. Each column represents the $\bar{x} \pm$ s. ($n = 10$). $^{**}P < 0.01$ *vs* control group

Table 1 Effect on total acidity, pepsin content, and ulcer index in pylorus ligated rats ($\bar{X}$ ± s)

Groups	Dosage / (mg·kg^{-1})	Total acidity / pH	Pepsin content / U	Ulcer indexes	Protection / %
control	—	1.76 ± 0.34	60.90 ± 32.16	28.20 ± 14.49	—
Ranitidine	50	1.76 ± 0.28	37.42 ± 21.68	14.40 ± 6.74$^{\#}$	48.9
ascaridole-1	10	1.62 ± 0.07	43.33 ± 27.23	16.80 ± 9.15$^{\#}$	40.4
ascaridole-2	20	1.46 ± 0.10$^{\#}$	22.18 ± 27.31$^{\#}$	14.10 ± 9.92$^{\#}$	50.0

$^{\#}P < 0.05$ *vs* control group

protective effect against the gastic damage caused by pyloric ligation. It is also apparent that with increasing dosage of ascaridole, there is a proportional increase in gastric ulcer protective effect. Ascaridole at the dose of 20 mg/kg showed 50% ulcer protection, and had a significant gastroprotective activity ($P < 0.05$) since it decreased the total acidity and pepsin activity.

Aspirin-induced ulcers

Gastroprotective effect of ascaridole was observed on the Aspirin-induced gastric damage in rats. Fig. 3 showsthat the rats in groups with treatment of ascaridole had lower total ulcer scores, in comparison with the control group. The ANOVA results indicated that there were significant differences in ulcer index at dosage levels of ascaridole, compared with negative controls ($P < 0.05$). However, the two dosages of ascaridole did not show significant differences with Ranitidine (one of acid-suppressing drugs) ($P > 0.05$). This indicated that ascaridole as well as acid-suppressing drug, was capable of reducing the occurrence of gastric ulcer due to ig administration of Aspirin.

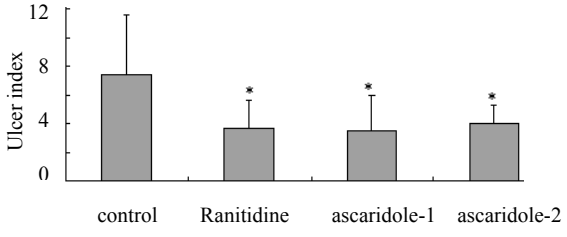

Fig. 3 Effect of ig administration of ascaridole on Aspirin-induced ulcers

Control group: ig administration of the vehicle; Ranitidine group: ig administration of Ranitidine at dose of 50 mg/kg; Ascaridole-1 group: ig administration of ascaridole at dose of 10 mg/kg; Ascaridole-2 group: ig administration of ascaridole at dose of 20 mg/kg. Each column represents the $\bar{X}$ ± s ($n = 8$). $^{*}P < 0.05$ *vs* control group

Discussion

The present study investigated the effect of ascaridole on the gastric ulcers in rats. Ascaridole shows potential efficacy on the healing of gastric ulcers induced by acetic acid, Aspirin, and pylorus ligation.

Application of glacial acetic acid (0.05 mL) under the serosal surface of the stomach produced deep penetrating gastric ulcer that resembles human peptic ulcer disease. Since the healing process of this ulcer closely resembles that of human peptic ulcers, this model is quite useful for studying the effect of drugs on the healing of peptic ulcers. From the above results, it could be inferred that the ascoridole showed marked anti-ulcer activity in acetic acid-induced gastric ulcer model in a dose-dependent manner. Ascaridole was effective in augmenting the gastric ulcer healing in this model as well as JHWK Capsule and Ranitidine. And the anti-ulcer activity of ascaridole at 20 mg/kg is almost equipotent effect as that of JHWK Capsule at 10 mg/kg and Ranitidine at 50 mg/kg. The results offer some evidences that ascaridole is one of the active components of JHWK Capsule.

The causes of gastric ulcer pyloric ligation are believed to be due to stress-induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to the exposure of unprotected lumen of the stomach to the accumulating acid (Raju *et al*, 2009). Pylorus ligation-induced ulcer is one of the most widely used methods for studying the effect of drugs on gastric secretion and mucus secretion. Agents that decrease gastric acid secretion and/or increase mucus secretion are effective in preventing the ulcers induced by this method. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach, leading to the development of ulcers in the stomach. Rat model involves fasting of the animals for 36 h followed by ligation of pyloric end of the stomach. The ulcer index is determined 18 h after pylorus ligation. Ascaridole (20 mg/kg) could significantly decrease the secretion of gastric aggressive factors, total acidity, and pepsin content.

Aspirin is an anti-inflammatory drug known for its gastric toxicity (Toruner, 2007), which produces erosions and ulcers in the stomach due to the inhibition of prostaglandin synthesis. Therefore, this drug is frequently used as a model in studies on *in vivo* cytoprotective activity of new substances or compounds (Wang *et al*, 2011). The result indicates that ascaridole is capable of reducing the occurrence of Aspirin-induced gastric ulcer similar to Ranitidine. From the lack of significant differences, it may be concluded that ascaridole at the dosages of 10 and 20 mg/kg, has a gastric ulcer protective power equivalent to Ouhuada (ranitidine hydrochloride) dosage of 50 mg/kg.

In conclusion, this study provides evidence that ascaridole possesses gastroprotective activity with healing of acetic acid-induced chronic gastric ulcers and preventing the development of gastric ulcers induced by pylorus ligation or Aspirin, demonstrating that ascaridole might be useful in the treatment of peptic ulcer. In future, this work would be extended by including more ulcer models to elucidate the mechanisms of ascaridole on increasing gastric ulcer healing for meaningful and tangible conclusion.

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