Effect and mechanism of ethanol extracts of muxiang (*Radix Aucklandiae*) on gastric ulcers in rats

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**Supported by** the Science and Technique Plan of the Education Department of Yunnan Province: the Effect of the Ethanol Extract of Muxiang (*Radix Aucklandiae*) on Gastric Ulcer in Rats (No. 2018JS285), Yunnan Innovation Team of Application Research on Traditional Chinese Medicine Theory of Disease Prevention at Yunnan University of TCM (No. 2017HC011), and Yunnan Provincial University Key Laboratory of Aromatic Chinese Herb Research
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**Accepted:** January 1, 2019

**Abstract**

**OBJECTIVE:** To investigate the effect of ethanol extracts of Muxiang (*Radix Aucklandiae*) (RA) on gastric ulcers in rats and explore the potential mechanisms.

**METHODS:** A model was established by ethanol (0.75 mL/kg). According to body weight, rats were pretreated with RA extracts (2.5 or 5 g/kg). The rats were administered 95% ethanol orally after 1 h. The effects of ethanol were evaluated by measuring the gastric secretion volume, pH, pepsin activity, and ulcer area. Histological analysis and immunohistochemistry were also conducted. Furthermore, the effect of the ethanol extract of RA on transiting activity of the gastrointestinal tract was observed in mice.

**RESULTS:** Intragastric administration of RA extracts protected the gastric mucosa from ethanol-induced gastric ulcers, while reducing submucosal edema and preventing hemorrhagic damage. Moreover, the extracts increased the production of gastric mucus, upregulated Bcl-2, and downregulated Bax expression. Importantly, pretreated rats exhibited no significant change in the gastric secretion volume, gastric juice acidity, or pepsin. Furthermore, pretreatment prominently (*P* < 0.05) enhanced propulsive movement of the gastrointestinal tract in normal mice and mice with gastrointestinal motility disorders.

**CONCLUSION:** Ethanol extracts of RA ameliorated gastric lesions in the gastric ulcer rat model. The mechanisms of action were related to improvement of gastrointestinal dynamics, maintenance of mucus integrity, and inhibition of apoptosis by downregulating proapoptotic Bax protein and upregulating anti-apoptotic Bcl-2 protein.
INTRODUCTION

Gastric ulcer (GU) affect many people worldwide and are one of the most common digestive system diseases. Development of a GU is caused by many factors such as stress, malnutrition, alcohol consumption, smoking, excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), and infection by Helicobacter pylori. Although many drugs are currently being used to treat gastric ulcers, almost all of these drugs have significant side effects and lead to high rates of recurrence after discontinuation. Therefore, it is very important to discover antiulcer agents that are more effective. Traditional Chinese Medicine (TCM) has been used for several centuries in China and is a rich source of medicine. For example, there are many herbal medicines used for the treatment of digestive system diseases. In particular, some medicinal plants have been shown to possess anti-ulcer properties. Muxiang (Radix Aucklandiae) (RA) is the dry root of Aucklandia lappa decne, a Compositae plant. It originated from India and was later introduced to China. According to its different habitats, Muxiang (Radix Aucklandiae) is further classified as Aucklandiae Radix and Vladimiriace Radix. RA has been studied intensively and found to have anti-inflammatory, antioxidant, anticancer, antibacterial, and antiallergic effects. In TCM, RA is locally called “one important medicine for promoting Qi circulation and relieving pain”. The plant is widely used to treat stomach ache, abdominal distension, belching, and appetite loss. It is believed that RA enhances the effect of prescriptions for Qi stagnation, abdominal pain, and gastric ulcer. Indeed, a study has demonstrated that RA ameliorates gastric lesions in various experimental gastric ulcer models. However, there have been no studies on its mechanism in gastric ulcers. In the present study, we investigated the possible protective effects of ethanol extracts of RA on an acute ethanol-induced gastric ulcer rat model. Moreover, we examined the underlying molecular mechanisms.

To investigate the pathogenesis and pathophysiology of human gastric ulcers, a large number of gastric ulcer animal models have been established. The most common type of animal model is established by ethanol that penetrates quickly into the gastric mucosa, inducing damage to gastric tissues. Ethanol also damages the integrity of the gastric mucosa, resulting in submucosal edema, inflammatory cell infiltration, and ulcer formation. Although RA has been used as an antiulcer medicinal plant in TCM and demonstrated good clinical effects, the gastroprotective mechanism of RA has not been studied yet. Hence, this study was designed to define the anti-ulcer effects of RA and elucidate the possible mechanism(s).

MATERIALS AND METHODS

Ethics statement

This study was performed strictly according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experiments were approved by the Institutional Animal Care and Use Committee of Yunnan University of Chinese Medicine.

Ethanol extracts

The plant materials were purchased from the Branch Company of Herbal Pieces of Yunnan Baiyao Group, Yunnan, China. Identification of the plant was performed by an expert from the Identification Teaching Research Office of Yunnan University of Chinese Medicine. The ethanol extracts of RA were prepared by macerating 50 g dried and fragmented plant material in 250 mL of 95% alcohol. The extract was dried in a rotary evaporator.

Animals

Sprague-Dawley male rats (200-220 g) and their feed pellets were purchased from Dossy Experimental Animals Co., Ltd. (Chengdu, China). All animals were housed at (20 ± 2) °C with 43% humidity in 12 h light/dark cycles and provided with distilled water in the animal facility at the Experimental Animal Center, Yunnan University of Chinese Medicine. Rats were divided into several groups with eight rats in each group. The animals were acclimatized for 1 week.

Acute gastric injuries model

Rats were fasted for 24 h prior to the experiment. The animals were divided randomly into five groups (n = 8). Group 1 was the normal control, group 2 was the GU model control, group 3 was administered with omeprazole (20 mg/kg), and two GU groups were pretreated with either 2.5 or 5.0 g/kg ethanol extracts of RA. The extracts were administered to the two experimental groups [10 mL/kg bodyweight (bw)]. Groups 1 and 2 were orally administered 5% Tween 80. Absolute ethanol (7.5 mL/kg) was administered to all animals after 1 h. After monitoring continuously for 1 h, all rats were sacrificed and their stomachs were removed immediately, unfolded along the greater curvature, and rinsed with cold saline. The ulcer area on the gastric mucosa was measured with ImageJ. Inhibition percentage (%) was calculated by the following formula: 1%

\[ \text{Inhibition percentage} = \frac{[\text{UI model group} - \text{UI treated group}]}{\text{UI model group}} \times 100\% \]

Each gastric tissue was cut in half and prepared for histological analyses.

HE staining and PAS staining

Stomach samples were fixed with 10% formalin and dehydrated in alcohol and xylene. The tissues were then
embedded in paraffin. Tissues were sectioned by a Leica rotation microtome at a thickness of 5 μm and then stained with hematoxylin and eosin (HE). Histopathological changes in gastric tissues, such as epithelial cell loss, edema, and hemorrhage, were examined under a light microscope. The results were scored as follows: epithelial cell loss (score: 0–4), edema in the submucosa (score: 0–4), and hemorrhagic damage (score: 0–4). The evaluation was conducted by a pathologist without awareness of the treatment. Gastric tissues were also stained with Periodic acid–Schiff (PAS) to assess mucus production.

**Immunohistochemistry (IHC) assay**
For further analysis, the tissue sections underwent immunohistochemical staining with an anti-Bcl-2 antibody [E17] (ab32124, 1:200, abcam, USA) to observe localization of Bcl-2 protein. An anti-Bax antibody [E63] (ab32503, 1:200, abcam) was used to define the localization of Bax protein. Immunohistochemical staining was analyzed by ImageJ and divided into four grades: high positive (4), positive (3), low positive (2), negative (1).

**Antisecretory analysis**
According to a published method, we analyzed the effects of RA on gastric acid secretion. Sprague-Dawley rats were allocated into five groups. Sham-operated and model control groups were orally administered 5% Tween 80. The positive control group was administered cimetidine (42 mg/kg). Low and high dose groups were administered 2.5 and 5 g/kg RA, respectively. Medicines were administered once per day for 4 d. On day 5, all rats were anaesthetized by chloral hydrate (10%, 3 mL/kg). The sham-operated animals had their abdomen opened without ligation of the pylorus. For the other groups, immediately after opening their abdomen, exposing the stomachs, and ligating the pylorus, each animal received one intraduodenal treatment (10 mL/kg bw). The abdomen was then sutured. After 4 h of observation, we opened the abdomen, ligated the cardia, and removed the whole stomach immediately. Then, gastric contents were collected into tubes and centrifuged at 3000 × g for 10 min with a fixed gastric secretion volume (mL). The pH values of gastric juice were monitored by a digital pH meter. Pepsin activity was detected by a pepsin activity kit.

**Charcoal meal GI transit test for mice with gastrointestinal motility disorders**
Rats were divided into four groups (n = 10). Group 1 was a normal control group and group 2 was model animals. The other two groups were pretreated with either 3.4 or 6.8 g/kg RA orally once a day for 6 d. On day 7, at 30 min after receiving the treatment, all groups except for the normal control group received atropine (10 mg/kg, 10 mL/kg, i.m) at 30 min prior to the charcoal meal. Each animal was treated with 0.6 mL charcoal meal. After 15 min, animals were sacrificed and the whole gastrointestinal tract was removed. The methods of emptying the stomach and transporting through small intestine are described above.

**Statistical analysis**
Data are presented as the mean ± standard deviation (x ± δ). Statistical differences were assessed using one-way analysis of variance, followed by Dunnnett’s multiple comparison tests. Statistical analyses were conducted with SPSS 24 (SPSS Corp., Armonk, NY, USA). P < 0.05 indicated statistical significance.

**RESULTS**

**Effects of RA on gastric lesions in rats**
In comparison with the normal control group (Figure 1A), acute ethanol-induced gastric mucosal damage was presented by a slender hemorrhagic band (Figure 1B). Animals pretreated with omeprazole (Figure 1C) or RA (Figure 1D, E) showed considerably less gastric lesions. Quantitative analysis showed that omeprazole at 20 mg/kg bw and RA at doses of 2.5 and 5 g/kg bw significantly (P < 0.05) protected against ethanol-induced ulcers by 63.54%, 70.21%, and 77.58%, respectively (Figure 1 and Table 1).

**RA protect mucosal from ethanol-induced injury**
We investigated the protective effects of RA against ethanol-induced injury and apoptosis by staining with HE, PAS and IHC. Histopathological changes in gas-

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**Figure 1 Gross observations**
A: normal control group; B: ulcer model control group; C: positive control group pretreated with omeprazole; D: RA low dose (RAL) at 2.5 g/kg; E: RA high dose (RAH) at 5 g/kg. The normal control group had an intact lesion-free stomach. The ulcer model group exhibiting extensive lesions showed an elongated band of hemorrhaging. Omeprazole at 20 mg/kg resulted in moderate injuries to the gastric mucosa. However, compared with group B, rats pretreated with different doses of RA (RAL, 2.5 g/kg; RAH, 5 g/kg) had less mucosal injuries. RAL: *Radix Aucklandiae* low dose; RAH: *Radix Aucklandiae* high dose.
The normal group was administered with 5% Tween 80; the ulcer model control group was administered with 5% Tween 80 and treated with ethyl alcohol at 7.5 mL/kg; omeprazole: positive control (20 mg/kg); RAL and RAH groups were administered with an ethanol extract of RA at 2.5 and 5.0 g/kg, respectively, and treated with ethyl alcohol at 7.5 mL/kg. Samples were orally administered via a stomach tube. RAL: Radix Aucklandiae low dose; RAH: Radix Aucklandiae high dose.

Table 1 Effect of RA on gastric lesions ( ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ulcer area (mm²)</th>
<th>Ulceration-inhibiti on rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>132±9³</td>
<td>-</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>8</td>
<td>48±14³</td>
<td>63.54</td>
</tr>
<tr>
<td>RAL</td>
<td>8</td>
<td>39±20³</td>
<td>70.21</td>
</tr>
<tr>
<td>RAH</td>
<td>8</td>
<td>29±13³</td>
<td>77.58</td>
</tr>
</tbody>
</table>

Notes: the normal control group was administered with 5% Tween 80; the ulcer model control group was administered with 5% Tween 80 and treated with ethyl alcohol at 7.5 mL/kg; omeprazole: positive control (20 mg/kg); RAL and RAH groups were administered with an ethanol extract of RA at 2.5 and 5.0 g/kg, respectively, and treated with ethyl alcohol at 7.5 mL/kg. Samples were orally administered via a stomach tube. RAL: Radix Aucklandiae low dose; RAH: Radix Aucklandiae high dose.

Figure 2 Histopathological change of gastric mucosa in rats by HE staining (× 400)

A: normal control group; B: ulcer model control group; C: positive control group pretreated with omeprazole; D: RAL at 2.5 g/kg; E: RAH at 5 g/kg. A-E: Low amplification (× 400) of HE staining in representative gastric mucosa sections from the five groups. A: normal group showed normal arrangement of the gastric epithelium and gland. B: the ulcer model group displayed disruption to the surface epithelium and submucosal layer edema, and hemorrhaging. Moreover, rats pretreated with omeprazole or different doses of RA, the levels of Bax were downregulated compared with the ethanol ulcer model control group (Figure 4C-E versus 4B). For Bcl-2, ethanol induced a marked decrease in Bcl-2 staining in comparison with the normal group (Figure 4A versus 4B). In rats pretreated with omeprazole or different doses of RA, the levels of Bax were downregulated compared with the ethanol ulcer model control group (Figure 4C-E versus 4B).

PAS staining

PAS staining was used to examine the gastric mucus layer. In the normal group, the mucus layer covering the entire surface of the gastric mucosa showed an expansion of continuous PAS positivity (Figure 3A). Ethanol resulted in loss of the mucus layer (Figure 3B). In rats pretreated with omeprazole (Figure 3C) or different doses of RA (Figure 3D, E), the mucus layer showed significantly less damage induced by ethanol. These results showed that RA has a protective effect against ethanol-induced glycoprotein reduction.

Table 2 Lesion scores of ethanol-induced gastric injuries in rats ( ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Epithelial cell loss (score 0-4)</th>
<th>Edema (score 0-4)</th>
<th>Hemorrhagic damage (score 0-4)</th>
<th>Total (score 0-12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>4 (3-4)³</td>
<td>4 (4-4)³</td>
<td>4 (4-4)³</td>
<td>12³</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>8</td>
<td>3 (2-3)</td>
<td>3 (2-4)</td>
<td>0²</td>
<td>6²</td>
</tr>
<tr>
<td>RAL</td>
<td>8</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>1 (0-1)³</td>
<td>7³</td>
</tr>
<tr>
<td>RAH</td>
<td>8</td>
<td>1 (0-1)¹</td>
<td>1 (0-1)¹</td>
<td>0²</td>
<td>2²</td>
</tr>
</tbody>
</table>

Notes: the normal control group was administered with 5% Tween 80; the ulcer model control group was administered with 5% Tween 80 and treated with ethyl alcohol at 7.5 mL/kg; omeprazole: positive control (20 mg/kg); RAL and RAH groups were administered with ethanol extracts of RA at 2.5 and 5.0 g/kg, respectively, and treated with ethyl alcohol at 7.5 mL/kg. Samples were orally administered via a stomach tube. RAL: Radix Aucklandiae low dose; RAH: Radix Aucklandiae high dose. *P < 0.01 vs normal; †P < 0.05; ‡P < 0.01 vs ulcer model group.
tochemical scores of Bax and Bcl-2 in each group were determined by ImageJ and are presented in Table 3.

**Effect of RA on gastric acid secretion, pH, and pepsin activity**

To examine the effects of RA, it was administrated to the duodenum at doses of 2.5 or 5.0 g/kg bw after pylorus ligature. Cimetidine (42 mg/kg) was used for comparison. Cimetidine significantly (*P* < 0.05) reduced acid output of the gastric secretion and inhibited the activity of pepsin compared with the ulcer model control group during a 4-h period. Compared with animals in the ulcer model group, rats pretreated with RA showed no difference in gastric acid secretion or pepsin activity (Table 4).

**Effect of RA on charcoal meal intestinal transit in normal mice**

The results showed that pretreating animals with RA (6.8 g/kg) reduced the gastric residual volume and considerably prolonged the propulsive movement of charcoal meal compared with the normal control group (Table 5).

**Effect of RA on charcoal meal intestinal transit in mice with gastrointestinal motility disorders**

The experimental mice were injected intraperitoneally with atropine and gastrointestinal excitation was reduced greatly. However, RA at 6.8 g/kg largely reduced gastric residue and increased the charcoal meal transit in mice with gastrointestinal motility disorders (Table 6).

**DISCUSSION**

In this study, we showed that ethanol extracts of RA exerted a protective effect against ethanol-induced ulcers in rats. Intragastric administration of RA at 2.5 and 5 g/kg effectively protected the gastric mucosa from ethanol damage in a dose-dependent manner.
consistent with this notion, we propose that plant extracts may provide protective action. They speculated that accelerating gastric emptying reduces the occurrence of experimentally induced gastric ulcers. Consistent with this notion, we observed the effect of RA on normal mice and gastrointestinal inhibition model mice. The results indicated that the extracts at high doses considerably promoted the transit of charcoal meal in both normal mice and mice with gastrointestinal motility disorders. These findings support that the anti-ulcer effect of RA might be mediated via promotion of gastrointestinal peristalsis.

Gastric ulcers are a common disease triggered by an unbalance between mucosal protective factors and aggressive factors. Aggressive factors, which include gastric acid, pepsin, Helicobacter pylori infection, alcohol consumption, and Non-Steroid Anti-Inflammatory Drugs (NSAIDs), can induce ulcers. While the integrity of the mucosal barrier, the regenerative ability of mucosal epithelial cells, and mucosal blood flow have protective functions against ulcers. Currently, the therapeutic strategies for gastric ulcers focus on inhibiting aggressive factors or enhancing gastroprotective factors.

We investigated the effect of RA on the pylorus ligation model in rats. The pylorus ligation model is considered as a good model to assess alterations of gastric acid. We found that the extracts did not increase the output of gastric acid secretion or enhance the activity of pepsin induced by pylorus ligation. Thus, the anti-ulcer effect of the plants might not inhibit gastric secretion.

Some studies have reported that neostigmine protects the stomach from ethanol-induced ulcers. They speculated that accelerating gastric emptying reduces the occurrence of experimentally induced gastric ulcers. Consistent with this notion, we observed that plant extracts with anti-ulcer functions promote gastrointestinal motility and decrease the time that ethanol persists in the stomach. We observed the effect of RA on normal mice and gastrointestinal inhibition model mice. The results indicated that the extracts at high doses considerably promoted the transit of charcoal meal in both normal mice and mice with gastrointestinal motility disorders. These findings support that the anti-ulcer effect of RA might be mediated via promotion of gastrointestinal peristalsis.

The mucus layer of the stomach acts as the first line of defense and physical barrier against the aggressive effect of endogenous and exogenous substances. In our study, the extracts of RA significantly inhibited ulcer formation and had positive effects against gastric damage induced by ethanol that is destructive to stomach tissue and the gastric mucosal barrier. PAS staining showed that ethanol damaged the gastric mucus layer of rats, but the herb increased glycoproteins in the stomach mucus. Ethanol increased the production of ROS and upregulated Bax, a proapoptotic protein, while reducing the level of Bcl-2, an anti-apoptotic protein. Indeed, RA countered the effects of ethanol on these two proteins and may possess a powerful anti-apoptotic effect that may contribute to the gastroprotective activity.

In summary, our study showed that ethanol extracts of RA alleviate ethanol-induced gastric lesions in gastric ulcer model rats. The beneficial effects of the plant may be related to its ability to increase propulsive movement of the stomach and intestines, and maintain the integrity of the gastric mucosal surface, which may lead to increased production of gastric mucus and downregulation of proapoptotic protein expression. Our study provides further evidence that RA also has an antisecretory effect that may augment the gastroprotective action.

### ACKNOWLEDGMENTS

We thank Zhao Yi, Yunnan Provincial University Key Laboratory of Aromatic Chinese Herb Research, Yunnan University of Chinese Medicine, for providing...
### Table 5 Effect of RA on charcoal meal intestinal transit in normal mice (% normal vs Ra)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gastric residual rate</th>
<th>Intestine propulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>39±11</td>
<td>45±11</td>
</tr>
<tr>
<td>RAL</td>
<td>10</td>
<td>32±13</td>
<td>48±7</td>
</tr>
<tr>
<td>RAH</td>
<td>10</td>
<td>26±11</td>
<td>61±12</td>
</tr>
</tbody>
</table>

Notes: the normal group was administered with 5% Tween 80 for 6 d and treated with charcoal meal; RAL and RAH groups were administered with ethanol extracts of RA at 3.4 and 6.8 g/kg, respectively, and treated with charcoal meal. RAL: Radix Aucklandiae low dose (3.4 g/kg); RAH: Radix Aucklandiae high dose (6.8 g/kg). *P < 0.05; †P < 0.01 vs normal group.

### Table 6 Effect of RA on charcoal meal intestinal transit in mice with gastrointestinal motility disorders (% normal vs Ra)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gastric residual rate</th>
<th>Intestine propulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>41±17</td>
<td>58±9</td>
</tr>
<tr>
<td>Atropine</td>
<td>10</td>
<td>81±21†</td>
<td>29±7†</td>
</tr>
<tr>
<td>RAL</td>
<td>10</td>
<td>74±15</td>
<td>32±5</td>
</tr>
<tr>
<td>RAH</td>
<td>10</td>
<td>59±10†</td>
<td>38±7†</td>
</tr>
</tbody>
</table>

Notes: the normal group was administered with 5% Tween 80 for 6 d and treated with charcoal meal. Atropine: model control group was administered with 5% Tween 80 and received atropine prior to the charcoal meal; RAL and RAH groups were administered with RA at 3.4 and 6.8 g/kg, respectively, and received atropine prior to the charcoal meal. RAL: Radix Aucklandiae low dose (3.4 g/kg); RAH: Radix Aucklandiae high dose (6.8 g/kg). *P < 0.01 vs normal group; †P < 0.05 vs atropine group.

technical support and financial assistance. We also thank Qian Zhonyi, Department of Pathology, Kunming Medical University, for assisting with immunohistochemistry.

## REFERENCES


