Research on the protection and mechanism of coptis alkaloids on acetic acid type gastric ulcer

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Abstract. This paper studied the therapeutic effect of Coptis alkaloids on acetic acid type gastric ulcer and its possible mechanism. Acetic acid type gastric ulcer model is used and administered for 7 days, and the rats are killed, and then IPP software is used to calculate the area of ulcer of gastric tissue and the inhibition rate of ulcer. ELISA kit is also used to measure the expression level of epidermal growth factor (EGF) in serum of rats, 5-hydroxytryptamine (5-HT) in brain and noradrenaline (NE) in adrenal tissue. At the same time, HE staining is used to observe the healing state of gastric tissue. The experiment result shows that the ulcer area of Coptis total alkaloids group 25 mg/kg/day is significantly lower than that of the model group \((P < 0.01)\), and the inhibition rate of ulcer is more than 50%; at the same time, it increases the content of EGF in serum \((P < 0.05)\), promotes the healing of injured gastric tissue; and it also significantly increases 5-HT content in brain tissue and NE content in adrenal tissue \((P < 0.01, P < 0.01)\), and to some extent, it regulates the mood of rats and prevents the recurrence of ulcer; the HE staining results shows that the Coptis total alkaloids promotes the recovery of gastric injury. Therefore, the Coptis total alkaloids can promote the healing of acetic acid type gastric ulcer, and its mechanism may be that it improves the healing quality of gastric ulcer by regulating the emotion of rats, which results in the effect of treating gastric ulcer.

1. Introduction

Gastric ulcer is a common, frequently-occurring and recurrent digestive system disease. It is a kind of ulcer-like lesion formed by the gastric mucosa self digestion caused by gastric juice [1]. According to relevant data, about 10% of people have suffered from gastric ulcer in their lifetime, and the recurrence rate within 5 years reaches 100% [2]. Domestic data in recent years show that the incidence rate of gastric ulcer is increasing, which has been rated as one of the diseases with great harm to human health, and gastric ulcer has seriously affected people’s normal life [3]. The incidence factors include gastric acid, Helicobacter pylori, inflammation, pressure and local ischemia [4]. In recent years, due to the use of western medicine, gastric ulcer can be cured in a short period of time, but the recurrence rate after drug withdrawal is high. Modern research shows that traditional Chinese medicine can treat gastric ulcer well and reduce its recurrence rate at the same time. China has a long history of treating diseases with herbal medicine, and there are many kinds of medicinal plants, so the prospect of developing new anti ulcer drugs from these plants is broad.
**Coptidis Rhizoma** is the dry rhizome of Coptis Chinese Franch, Coptis deltoidea or Coptis teeta wall. It is one of the commonly used drugs of traditional Chinese medicine, and it were recorded in all the important medical books of each dynasty. It is bitter in taste and cold in nature, and it has the functions of clearing away heat, drying dampness, purging heat and detoxifying. Clinically, it is used to treat vomiting, diarrhea, jaundice, high fever, coma, vexation and sleeplessness, toothache, heart-fire hyperactivity, abscess furuncle and other diseases [6]. It has been reported that the chemical components of Coptis are mainly alkaloids, which show good pharmacological effects of antibiosis [7-8], anti-inflammation [9], anticancer [10], hypoglycemic [11], hypolipidemia [12] and anti-ulcer [13], but there is no report about its mechanism of treating gastric ulcer from the aspect of neurohumoral regulation. In this study, we used the common acetic acid type gastric ulcer model to study the effect of Coptis total alkaloids on the expression of epidermal growth factor (EGF) in serum of rats, 5-hydroxytryptamine (5-HT) in brain and noradrenaline (NE) in adrenal tissue of rats, and elucidate the therapeutic effect Coptis total alkaloids on gastric ulcer and related mechanism, so as to provide a certain research basis for clinical screening of anti ulcer drugs.

2. **Material**

2.1. **Instrument**
SARTORIUS-BT25S electronic balance (Sartorius Scientific Instrument (Beijing) Co., Ltd.); HH-6 digital display thermostat water bath (Guohua Electric Instrument Co., Ltd.); KQ-250E ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); rotary evaporator RE-5203 (Shanghai Yarong Biochemical Instrument Factory); BHX type electric constant temperature blast drying oven (Nanjing Keer Equipment PTY., Ltd.); DZF-6020 type vacuum drying oven (Shanghai Yiheng Technology Co., Ltd.); low temperature and high speed centrifuge (Nanjing Keer Equipment PTY., Ltd.).

2.2. **Drugs and reagents**
Berberine hydrochloride, jatrorrhizine hydrochloride and palmatine hydrochloride standards were all purchased from National Institutes for Food and Drug Control; omeprazole (Cisen Pharmaceutical Co., Ltd., China Food and Drug Administration Approval No.: H20083815, batch No.: 160203511, 20mg/tablet×7 tablets/plate×2 plates/box); enzyme linked immunosorbent assay, ELISA kit was purchased from Nanjing Dizhao Biotechnology Co., Ltd.

2.3. **Medicinal materials**
Coptis materials were purchased from Bozhou Traditional Chinese Medicine Company in Anhui Province, and its origin is Chongqing, China. It was identified as dry rhizome of Coptis Chinese Franch by Li Yong, associate professor of Jiangsu Animal Husbandry & Veterinary College, the total alkaloid content is 90%.

2.4. **Animals**
SD rats, with a weight of 180-220g, are provided by the animal experiment service center of Jiangsu University, and the animal quality certificate number is SCXK (Su) 2016-0001.

3. **Method**

3.1. **Experimental groups**
Take 48 healthy male SD rats with a body weight of 180-220g and feed them regularly. After 7 days of adaptation to the environment, randomly divide them into 6 groups with 8 rats in each group. They were: sham-operated group (S0), model group (S1), positive control group (S2), Coptis alkaloids low dose group (S3), Coptis alkaloids middle dose group (S4), Coptis alkaloids high dose group (S5). For the positive control group, omeprazole was selected as the control (4.2 mg·kg⁻¹·d⁻¹,i.g.), and the doses of
Coptis alkaloids filled into the stomach in the low, medium and high dose groups were respectively 12.5, 25 and 50 mg·kg⁻¹·d⁻¹.

3.2. Modeling and administration
Forbid the rats to eat for 24 hours, and then anesthetize it with 10% chloral hydrate, let the rats lie on their backs, bound its limbs firmly, fix them on the board, disinfect the lower part of xiphoid process, cut a 2cm opening along the midline of abdomen, take out its stomach, and make the model by sticking acetic acid filter paper. The specific operation is as follows: first, use dry cotton ball to wipe off the body fluid on the gastric serosa, and then apply 100% acetic acid filter paper on it, stimulate it twice, and 30s for each time. After the stimulation, use cotton swab stained with normal saline to scrub, and then send the stomach into the abdominal cavity, and use the greater omentum to cover the sticking part of the acetic acid paper to avoid the adhesion of the peritoneum. Sew up the inner and outer wounds respectively, and apply iodophor solution and penicillin respectively for disinfection. For the sham-operated group, replace acetic acid filter paper with saline filter paper, and the operation method is the same. After modeling, water and rat food are given according to normal standards. For sham-operated group and model group, give 0.5% CMC-Na solution, for positive control group, give 4.2 mg·kg⁻¹·d⁻¹ omeprazole, for test drug group, give 12.5, 25 and 50 mg·kg⁻¹·d⁻¹ Coptis alkaloids respectively. 24 hours after the completion of modeling, administer the drug once a day for a week. Observed and record the body weight, behavioral activity and mental state of rats.

3.3. Sampling and staining observation
During the experiment, feed all the animals in the same environment, 12 hours, day and night cycle, keep the room temperature to be 225±1℃, let animals eat and drink freely. After the last administration, forbid the rats to eat for 24 hours, but let them drink freely. Then take blood from their eyes, and then take off their cervical vertebra to kill the rats, cut off their heads and take brain, stomach, adrenal gland and other tissues. In each group, select the half part of the stomach modeling position of 3 rats to carry out fixing, and the rest are used for index determination. Blood samples are centrifuged at 3000 rpm for 15 min within 2h, and then we take the supernatant and store it at -80℃ for standby. All tissues and organs were stored at -80℃ for future use.

Use 10% formalin to fix the gastric tissue of the ulcer site for 24 hours. Use paraffin embedded section method to cut sections, and the thickness of the sections is about 4~5μm, then carry out HE staining. Let pathology professional researchers to observe the sections and observe the histological changes of gastric mucosa under the light microscope. Histological observation indexes: whether the four layers of gastric wall is clear, whether the surface of gastric mucosa is smooth, whether the epithelium is complete, and whether the glands are arranged orderly.

3.4. Calculation of ulcer inhibition rate
After removing the residue in the stomach, cut it along the great curvature of the stomach. Use digital camera to take photos to obtain the image of the gastric wall of rats. Then use IPP software to calculate the ulcer area and ulcer inhibition rate of rats.

Ulcer inhibition rate= (average ulcer area of model group - average ulcer area of experimental group)/average ulcer area of model group×100%

3.5. Use ELISA method to detect EGF in serum, 5-HT in brain tissue and NE in adrenal gland
Before the determination, the samples of serum and tissues and organs were thawed at 4℃. Take 9 times normal saline homogenate of each tissue sample, and centrifugate it at 3000rpm for 10min, and take supernatant for test. Use ELISA kit to determine EGF in serum, 5-HT in brain tissue and NE in adrenal gland. The specific operation method should be carried out according to the instructions of the kit.

3.6. Statistical treatment
For all the statistical treatment, we use SPSS 19.0, and for the chart, we use GraphPad Prism 6.0, and use the average value±SD to express the results. For data comparison, we use one-way ANOVA (Fisher's protected LSD multiple comparison test or Tamhane's T2 test). P< 0.05 means that there is a significant difference. Use Image-Pro Plus (IPP) 6.0 to analyze the data about ulcer area.

4. Results

4.1. Macroscopic picture of the wall of the stomach with gastric ulcer
Observed the surface of the rat’s stomach macroscopically (Fig. 1). In the sham-operated group (S0), there is no ulcer formation on the rat's stomach. It can be seen that the selected modeling method in the experiment is reliable, and there is no mechanical damage in the modeling process. The gastric ulcer in the model group is the most serious and the area of the ulcer is the largest, which indicates that the modeling is successful. Compared with the model group (S1), the ulcers of each administration group are somewhat relieved, and among them, the ulcer areas in omeprazole group and total alkaloid middle dose group (25 mg·kg⁻¹·d⁻¹) are small, and the stomach shows red, which is similar to the macroscopic results in the sham-operated group (S0).

Figure 1. Macroscopic picture of gastric ulcer of rats.

4.2. Gastric ulcer area and ulcer inhibition rate
IPP software can accurately obtain the area size of areas with different light intensity in the image, and calculate and analyze out the ulcer area. Compared with the model group, the positive control group and Coptis alkaloids group have a certain inhibitory effect. In the total alkaloids middle dose group (25 mg·kg⁻¹·d⁻¹), the ulcer area of rats is significantly reduced, and the ulcer inhibition rate is as high as 50%, and the results are significantly different (P < 0.05), and the effect is similar to omeprazole. (Table 1)

Table 1. Statistical table of the results of ulcer area and ulcer inhibition rate (X±S, n=8).

| Groups          | Ulcer area(mm²) | Ulcer inhibition rate (%) |
|-----------------|-----------------|--------------------------|
| S0              |                 |                          |
| S1              |                 |                          |
| S2              |                 |                          |
| S4              |                 |                          |

Table 1.
|   | S0       | S1       |
|---|---------|---------|
|   | 15.8±0.5| 1.1±0.1 |
| S2| 8.2±0.9*| 46.7±0.7**|
| S3| 14.6±0.7| 3.8±0.9 |
| S4| 5.9±0.8**| 50.6±0.4**|
| S5| 11.8±0.7| 26.7±0.8 |

Note: compared to the model group (S1), **means $P<0.01$; *means $P<0.05$

4.3. Pathological tissue analysis

From the pathological section of gastric tissue, that is, figure 2, we can see that in the sham-operated group, glandular epithelium of gastric mucosa doesn’t not proliferate or atrophy, there is no inflammatory cell infiltration in the inherent layer, there is no pathological change in the muscularis mucosa, submucosa, muscularis and serosa, there is no erosion or ulcer in the surface layer of mucosa, and everything is normal. In the model group, in the local part of the gastric wall, there are incomplete epithelium and epithelial cell abscission; there are many epithelial cell fragments that fell off in the gastric pit, and focal ruptures can be found in the gastric gland, some of the wall cells are swollen, and the cytoplasm staining is light. A lot of inflammatory cell infiltration can be seen in the lamina propria and submucosa. In omeprazole group, epithelial cell denaturation is found in part of the gastric wall, local rupture is found in the gastric gland, part of the wall cells are swollen, and the cytoplasm staining is light. In the total alkaloids middle dose group, a small amount of inflammatory cell infiltration can be seen in the lamina propria and submucosa of the stomach, and there are dilation and hyperemia phenomena in capillaries. Compared with the model group, the area and severity of ulcer in the administration group are significantly reduced, and the tissue repair effects in omeprazole group and middle dose group are good, which indicates that Coptis alkaloids can promote the healing of ulcer.

4.4. Expression levels of EGF in serum, 5-HT in brain and NE in adrenal gland

EGF is a kind of epidermal repair factor, and the increase of EGF content can promote the repair of damaged parts [14]. Compared with the model group, the Coptis total alkaloids can improve the expression level of EGF in the serum of rats, and there is a significant difference between the middle
dose group (25 mg·kg\(^{-1}\)·d\(^{-1}\)) and the high dose group (50 mg·kg\(^{-1}\)·d\(^{-1}\)) (P < 0.05), so it can be seen that the Coptis total alkaloids can promote the proliferation and differentiation of gastric wall cells and accelerate the healing of gastric wall and gastric mucosa, while the omeprazole has no promotion effect on the expression of EGF level.

5-HT is a kind of monoamine neurotransmitter closely related to nerves, which plays an important role in the regulation of hypothalamus – pituitary – adrenocortical axis (HPA)\(^{[15]}\). Compared with the model group, the middle dose group (25 mg·kg\(^{-1}\)·d\(^{-1}\)) of Coptis total alkaloids significantly increases the level of 5-HT in the brain tissue of rats, and there is a significant difference between the two groups (P < 0.01). Therefore, it can be seen that Coptis total alkaloids can activate the HPA axis to a certain extent, regulate the nervous system of rats, make the mood of rats to be in a happy state and promote the recovery of diseases.

NE is also a kind of monoamine neurotransmitter closely related to neurohumoral regulation, which plays an important role in the regulation of hypothalamus – pituitary – adrenocortical axis (HPA) and endocrine system \(^{[15]}\). Compared with the model group, the middle dose group (25 mg·kg\(^{-1}\)·d\(^{-1}\)) of Coptis total alkaloids significantly increases the level of NE in the adrenal tissue of rats, and there is a significant difference between the two groups (P < 0.01). Therefore, it can be seen that Coptis total alkaloids can increase the NE content of rats and to a certain extent, the increase of NE content can regulate HPA axis, alleviate rats’ mood, release pressure and promote the recovery of disease.

### Table 2. Statistical table of the results of expression level of EGF in serum of rats (X±S, n=8).

| Groups | EGF(pg/mL) | NE(nM) | 5-HT(nM) |
|--------|------------|--------|----------|
| S0     | 207.1±0.5  | 61.3±0.2 | 89.6±0.5 |
| S1     | 225.3±0.6  | 62.6±0.3 | 92.5±0.1 |
| S2     | 223.7±0.9  | 57.8±0.2 | 87.7±0.7 |
| S3     | 246.8±0.7  | 65.1±0.3 | 97.3±0.6 |
| S4     | 250.9±0.8**| 73.3±0.3**| 102.3±0.6**|
| S5     | 241.8±0.7* | 66.7±0.2 | 98.1±0.8 |

Note: compared to the model group (S1), **means P< 0.01; *means P< 0.05

5. **Discussion**

In today’s competitive society, people bear more and more psychological pressure. Anxiety, depression and fidgety have become the main reasons that threaten people’s health. According to traditional Chinese medicine, poor mood can lead to liver dysfunction and liver-qi stagnation. Stagnation of liver results in bad mood, which can lead to liver qi stagnation and qi stagnation and fidgety have become the main reasons that threaten people’s health. According to traditional Chinese medicine, poor mood can lead to liver dysfunction and liver-qi stagnation. Stagnation of liver results in bad mood, which can lead to liver qi stagnation. Acetic acid type gastric ulcer model is a classic model, and it is the pharmacological model whose pathogenesis is most similar to the pathogenesis of human chronic gastric ulcer, so it is very common in the study of the pathogenesis and treatment mechanism of gastric ulcer\(^{[3]}\). Studies show that the occurrence of gastric ulcer is often due to the dysfunction of cerebral cortex and the nutritional disorders in the stomach. After getting gastric ulcer, the receptors in the stomach will send disease impulses to the cerebral cortex, deepening the dysfunction of the cortex \(^{[18]}\). At the same time, the autonomic nerve center will also be affected, resulting in various disorders of autonomic functions. There are many monoamine neurotransmitters in the central nervous system, and the Coptis total alkaloids (including berberine, jatrorrhizine, palmatine, etc.) are mostly inhibitors of monoamine oxidase \(^{[19-20]}\). Studies \(^{[21]}\) show that when depression attacks depressed patients, the expression of central monoamine neurotransmitters, 5-HT and NE, in the is relatively low. The experiment results show that the Coptis total alkaloids, on the one hand, increase the content of EGF in serum, promote the proliferation and differentiation of gastric parietal cells and accelerate the healing of gastric parietal and gastric mucosa, which shows a good therapeutic effect; and on the other hand, it increases the level of 5-HT in the brain tissue of rats, increase the level of NE in the adrenal tissue of rats within
a certain range and prevents the recurrence of ulcer. It can be seen that the Coptis total alkaloids can increase the 5-HT and NE content of rats who suffer from gastric ulcer by regulating the HPA axis, and can relieve their mood to a certain extent, reduce their anxiety, depression and other negative emotions after getting the disease, and can promote the recovery of the disease after releasing the pressure. At the same time, because NE plays a key role in noradrenergic pain regulation, the increase of NE content may help to alleviate the pain caused by gastric ulcer.

6. Conclusions
Coptis alkaloids have a good effect on promoting the healing of acetic acid type gastric ulcer, and its mechanism may be that it increases the content of EGF in serum and 5-HT and NE in brain tissue of rats, which promotes the healing of gastric mucosa and also relieves the anxiety and pain of rats when they are ill, so as to improve the healing quality of rats’ ulcer and achieve the effect of treating gastric ulcer.

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