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Original Article

Effects of total alkaloid of corydalis on gastric ulcer and immune regulation

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Abstract: Gastric ulcer is one of the common diseases in Department of Gastroenterology. Various drugs focus on gastric ulcer treatment, but the curative effect is still unsatisfactory. Corydalis alkaloid has multiple pharmacological effects, including analgesia, anti-inflammation, and anti-myocardial ischemia. There is still lack of report about its curative and immunoregulatory effect on gastric ulcer. A total of 50 male Wistar rats were randomly equally divided into 5 groups, including control, model group prepared by pylorus ligation method, and corydalis alkaloid high, middle, and low dose groups. PGE2 content, ulcer index (UI), gastric secretion, pepsin, and pH value were tested. SOD activity and MDA expression in gastric tissue. Supernatant IL-2, IL-6, IL-10, and TNF-α secretion were determined by ELISA. The gastric ulcer model group had increased UI, PGE2 reduction, pepsin secretion and gastric secretion enhancement, SOD activity declination, pH value decrease, MDA elevation, IL-2 and IL-10 secretion downregulation, and IL-6 and TNF-α secretion upregulation compared with control ($P < 0.05$). Corydalis alkaloid treatment significantly reduced UI, pepsin, gastric secretion, enhanced PGE2, pH value, and SOD activity, decreased MDA, IL-6, and TNF-α, and upregulated IL-2 and IL-10 compared with model group ($P < 0.05$). Corydalis alkaloid plays a curative effect on gastric ulcer by regulating inflammatory factors secretion and improving gastric mucosa attack/defense balance.

Keywords: Corydalis alkaloid, gastric ulcer, ulcer index, PGE2, TNF-α

Introduction

Gastric ulcer is a common disease in Department of Gastroenterology [1, 2]. Gastric ulcer is caused by different factors, which lead to gastric mucosa injury, repair system loss, and self-defense out of control, resulting in gastric mucosa progressive damage. Severe cases may combine bleeding, perforation, even cancer and death, which seriously threaten human health [3, 4]. Gastric ulcer induced pain has many characteristics, such as long-term, periodicity, and rhythmicity. The pathogenesis of gastric ulcer is influenced by various factors, such as psychology, spirit, physiology, pathology, and social factors [5, 6]. Abnormal gastric acid secretion, helicobacter pylori infection, improper drugs especially non-steroidal anti-inflammatory drugs application, stress, bad living habits, and hormone usage can cause gastric ulcer [7, 8]. However, its specific pathogenesis is still not fully elucidated. Following working environment changes, pace of life speed up, pressure from society, work, and family, psychological burden aggravating, lifestyle changes, and bad living habit, global gastric ulcers incidence increases year by year with younger trend [9]. Currently, there are various types of drugs for gastric ulcer, including mucosa protectant, antacids, and acid inhibitors, etc., whereas their curative effect is still unsatisfactory [10].

Corydalis alkaloid, also known as fumaric, is derived from papaveraceae and pouch dan subfamily [11]. An early study showed that corydalis alkaloid has the effect of relieve pain, activating blood, and promoting circulation [12]. More recent studies found that corydalis alkaloid has many pharmacological effects, including stop pain, anti-inflammation, sedative, and anti-ischemic diseases [13, 14]. However, its curative and immunoregulatory effect on gastric ulcer has not been reported. This

study aimed to test corydalis alkaloid curative effect on gastric ulcer and related mechanism through constructing gastric ulcer rat model.

Materials and methods

Materials and instruments

Corydalis alkaloid is provided by Xi'an Datown pharmaceutical co., LTD. The drug was extracted using ion exchange resin, organic solvents, ammonia, acid, and alkaline, and identified the purity as 65% by using spectral colorimetry and alkaloid precipitation reagent. SOD activity detection kit, pepsin activity kit, and MDA activity detection kit were purchased from Nanjing Jiancheng Bioengineering institute. IL-2, IL-6, IL-10, and TNF-α ELISA kits were from R&D. High-speed refrigerated centrifuge was from Beckman. Electronic ultrasonic homogenate was from Ultrasonic Technology Company. Stainless steel electronic digital caliper was from Shanghai instrument equipment factory. Electronic analytical balance was from Mettler-Toledo Company. Microplate reader was bought from BD.

Experimental animal selection

A total of 50 male Wistar rats in SPF grade were bought and fed in the Experimental Animal Center in Henan University of Science and Technology. The age and weight were 2 months old and 250 ± 20 g.

Male Wistar rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Yantai City Infectious Disease Hospital (Shandong, China).

Experimental methods

Grouping: The rats were randomly equally divided into five groups, including the normal control which fed in routine condition, model group which was constructed by pyloric ligation method, and corydalis alkaloid high, middle, and low dose groups with 2.0 mg/kg, 1.0 mg/kg, and 0.5 mg/kg treatment, respectively.

Gastric ulcer model construction: Pyloric ligation method was applied to construct gastric ulcer model. A midline incision was made from the sternum xiphoid to the medioventral line to expose stomach. The pylorus was ligated by sutures. Corydalis alkaloid was given through gastric gavage at 2.0 mg/kg, 1.0 mg/kg, and 0.5 mg/kg as high, middle, and low groups, respectively. The drug was given once a day for three consecutive days. The rat was killed after fasting for solids and liquids for 18 hours. Gastric juice was collected through gastric cardia ligation.

Sample collection: Gastric juice was collected through gastric cardia ligation. The gastric juice was calculated and centrifuged at 3000 rpm for 15 minutes to obtain the supernatant. Gastric acid pH value was measured by pH detector.

Ulcer index (UI) detection: The stomach was taken out and 5 ml formaldehyde solution at 1% was injected to the stomach cavity. The total stomach was soaked in 5% formaldehyde solution for 3 minutes, and rinsed by clean water. After open the stomach, the long diameter of ulceration was measured by electronic digital caliper. The number of ulcer was observed under microscope. UI was calculated on the basis of ulcer long diameter and degree. A total of 20 points was given for perforation or the length of diameter is greater than 10 mm. A total of 10 points was given for the length of diameter being 5-10 mm. Five points were given when the length of diameter was 2-5 mm. Two points were given when the length of diameter was less than 2 mm [15].

Pepsin activity detection: The gastric tissue was grinded on ice in normal saline to prepare gastric homogenate with concentration at 10%. Then the homogenate was centrifuged at 4000 rpm for 10 minutes. Pepsin activity was detected by the kit according to the manual.

Super oxide dismutase (SOD) activity detection: SOD activity was tested according to the specific instruction. Gastric tissue protein was extracted and water bathed at 95°C for 40 minutes. After centrifuged at 4000 rpm for 10 minutes, the tissue was added with ethanol-chloroform mixture (v/v, 5:3) to extract the ethanol phase from tissue homogenate for total SOD activity detection.

Malonaldehyde (MDA) content detection: The gastric tissue was grinded on ice in 4°C normal saline to prepare gastric homogenate with concentration at 10%. Then the homogenate was centrifuged at 4000 rpm for 10 minutes to obtain the supernatant. Protein content was

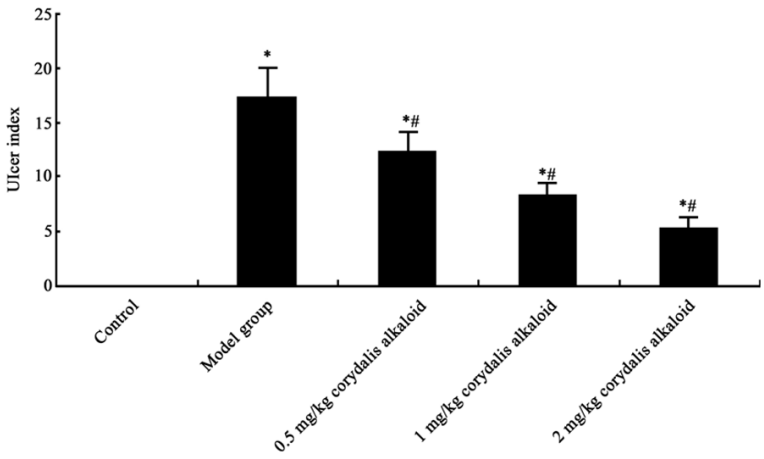

Figure 1. Corydalis alkaloid impact on UI in gastric ulcer model. After different treatments, the stomach was collected from rats followed by addition of 1% formaldehyde solution. Then the total stomach was soaked in 5% formaldehyde solution for 3 minutes and rinsed by clean water followed by measuring the long diameter of ulceration by electronic digital caliper under microscope. *P < 0.05, compared with the normal control. #P < 0.05, compared with the model group.

determined by Coomassie brilliant blue method. MDA content was detected by using BSA as standard substance.

Prostaglandin (PGE2) content detection: The gastric homogenate was centrifuged at 4000 rpm for 10 minutes to obtain the supernatant. After treated with 2 ml 0.5 mol/L KOH-methanol solution at 50°C for 20 minutes, the mixture was diluted by methanol and detected at 278 nm.

ELISA

The gastric homogenate was centrifuged to obtain the supernatant. IL-2, IL-6, IL-10, and TNF-α expression in supernatant was tested by ELISA according to the manual. In brief, a 96-well plate was incubated overnight at 4°C with 100 μl of coating antibody per well and then washed three times. After that, 200 μl of ELISA Diluent was added to each well and incubated at room temperature for 60 minutes. The wells were washed, and 100 μl of gastric homogenate supernatant or diluted standard was added to each well and incubated at room temperature for 2 hours. Subsequently, 100 μl of detection antibody was added per well and incubated at room temperature for 60 minutes. Finally, 100 μl of TMB reaction liquid was added per well followed by addition of a stop solution and then the plate was detected at a wavelength of 450 nm after incubation at room temperature for 15 minutes by a microplate reader.

Statistical analysis

All data analysis was performed on SPSS 16.0 software. Measurement data are presented as mean ± standard deviation ($\bar{x}$ ± SD) and compared by one-way ANOVA. P < 0.05 was considered as statistical significance.

Results

UI analysis

The impact of corydalis alkaloid on UI of gastric ulcer model was tested. The results confirmed that UI obviously increased in gastric ulcer model compared with normal control (P < 0.05). Corydalis alkaloid treatment significantly declined UI in gastric ulcer model with dose dependent (P < 0.05) (**Figure 1**). It suggested that corydalis alkaloid has protective effect on gastric ulcer model.

Corydalis alkaloid impact on gastric juice and pH value

The impact of corydalis alkaloid on pH value and gastric juice of gastric ulcer model was tested. The results revealed that gastric secretion enhanced, while pH value reduced in gastric ulcer model group compared with normal control (P < 0.05). Corydalis alkaloid treatment markedly reduced gastric secretion and elevated pH value compared with model group with dose dependence (P < 0.05) (**Table 1**).

Corydalis alkaloid impact on pepsin, SOD activity, and MDA expression

The impact of corydalis alkaloid on pepsin, SOD activity, and MDA expression in gastric ulcer model was analyzed. The results demonstrate that pepsin elevated, SOD activity decreased, and MDA overexpressed in gastric ulcer model group compared with normal control (P < 0.05). Corydalis alkaloid treatment significantly restored SOD activity, and reduced pepsin and MDA expression compared with model group with dose dependence (P < 0.05) (**Table 2**).

Table 1. Corydalis alkaloid impact on gastric juice and pH value

Index	Control	Model group	0.5 mg/kg corydalis alkaloid	1 mg/kg corydalis alkaloid	2 mg/kg corydalis alkaloid
Gastric secretion (ml)	3.21±0.45	14.37±2.21*	10.12±1.91*,#	8.43±2.26*,#	6.19±1.13*,#
pH	6.47±0.56	1.51±0.37*	3.53±0.48*,#	4.32±0.66*,#	5.29±0.71*,#

*$P < 0.05$, compared with the normal control. #$P < 0.05$, compared with the model group.

Table 2. Corydalis alkaloid impact on pepsin, SOD activity, and MDA expression

Index	Control	Model group	0.5 mg/kg corydalis alkaloid	1 mg/kg corydalis alkaloid	2 mg/kg corydalis alkaloid
Pepsin	16.21±1.52	34.61±2.31*	27.18±4.94*,#	22.11±3.29*,#	21.19±1.18*,#
SOD	156±53	61±16*	87±12*,#	117±21*,#	139±43*,#
MDA	40.21±6.55	57.35±4.51*	51.17±4.68*,#	47.67±3.9*,#	45.31±7.2*,#

*$P < 0.05$, compared with the normal control. #$P < 0.05$, compared with the model group.

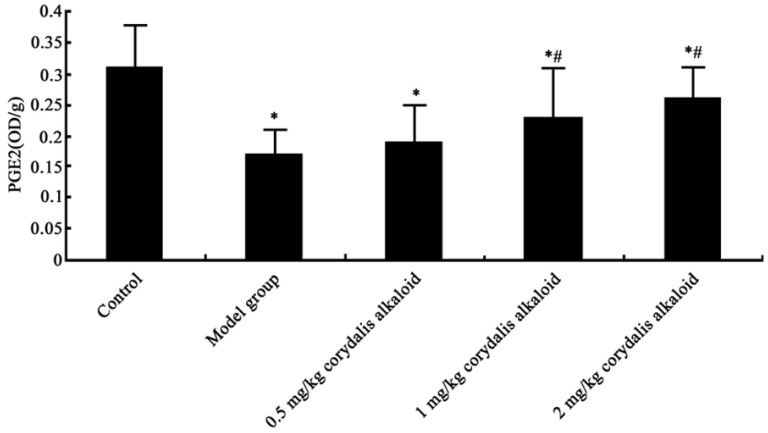

Figure 2. Corydalis alkaloid impact on PGE2. The gastric homogenate was collected and centrifuged to obtain the supernatant. After treatment with KOH-methanol solution, the mixture was diluted by methanol and the absorption value at 278 nm was detected by a microplate reader. *P < 0.05, compared with the normal control. #P < 0.05, compared with the model group.

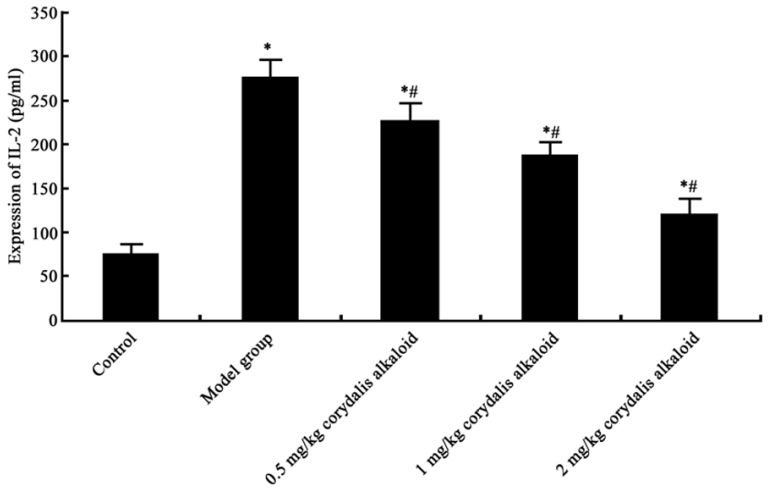

Figure 3. Corydalis alkaloid impact on IL-2 in gastric supernatant. The gastric homogenate was collected from rats of different groups and centrifuged to obtain the supernatant, which was used to measure the IL-2 level by ELISA. *$P < 0.05$, compared with the normal control. #$P < 0.05$, compared with the model group.

Corydalis alkaloid impact on PGE2

The impact of corydalis alkaloid on PGE2 in gastric ulcer model was analyzed. The results show that PGE2 declined in the supernatant from gastric ulcer model group compared with normal control ($P < 0.05$). Corydalis alkaloid treatment increased PGE2 secretion in supernatant compared with model group with dose dependence ($P < 0.05$) (**Figure 2**).

Corydalis alkaloid impact on IL-2 and TNF-α in gastric supernatant

ELISA was applied to test corydalis alkaloid influence on gastric supernatant IL-2 and TNF-α levels in gastric ulcer rat model. It was found that IL-2 and TNF-α levels upregulated in the supernatant from

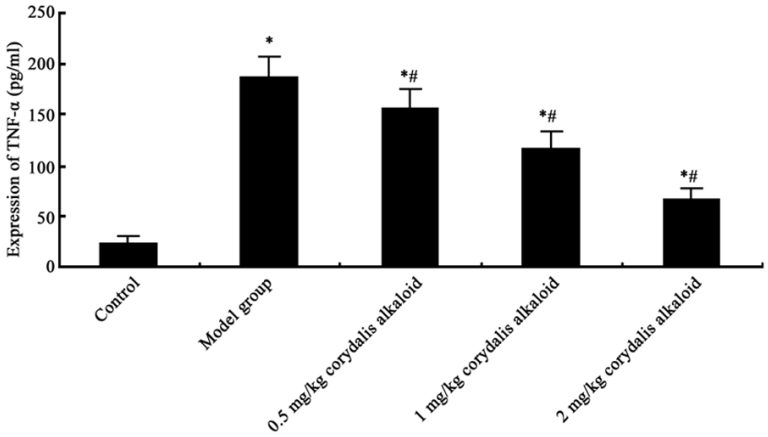

Figure 4. Corydalis alkaloid impact on TNF-α in gastric supernatant. The gastric homogenate was collected from rats of different groups and centrifuged to obtain the supernatant, which was used to measure the TNF-α level by ELISA. *P < 0.05, compared with the normal control. #P < 0.05, compared with the model group.

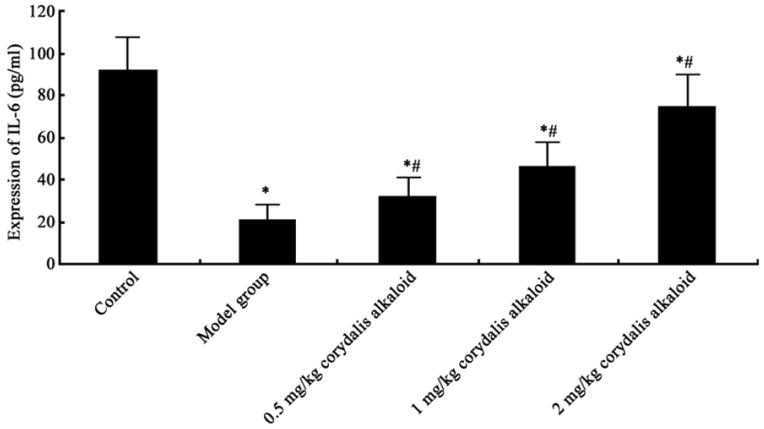

Figure 5. Corydalis alkaloid impact on IL-6 in gastric supernatant. The gastric homogenate was collected from rats of different groups and centrifuged to obtain the supernatant, which was used to measure the IL-6 level by ELISA. *P < 0.05, compared with the normal control. #P < 0.05, compared with the model group.

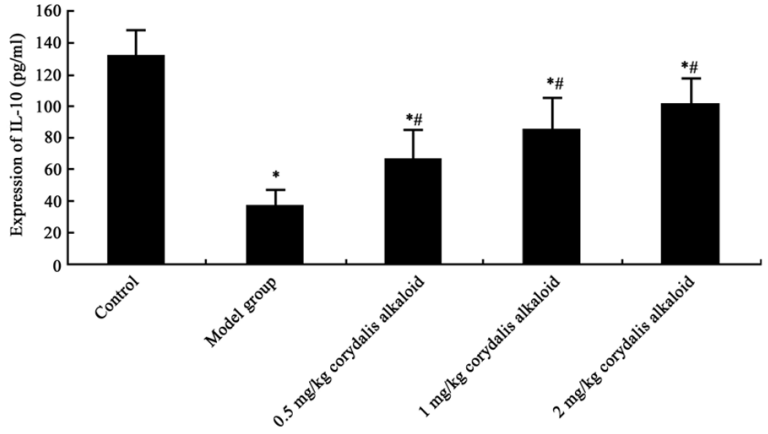

Figure 6. Corydalis alkaloid impact on IL-10 in gastric supernatant. The gastric homogenate was collected from rats of different groups and centrifuged to obtain the supernatant, which was used to measure the IL-10 level by ELISA. *P < 0.05, compared with the normal control. #P < 0.05, compared with the model group.

gastric ulcer model group compared with normal control (P < 0.05). Corydalis alkaloid treatment markedly restrained IL-2 and TNF-α secretion in supernatant compared with model group with dose dependence (P < 0.05) (**Figures 3** and **4**).

Corydalis alkaloid impact on IL-6 and IL-10 in gastric supernatant

ELISA was adopted to measure corydalis alkaloid impact on gastric supernatant IL-6 and IL-10 levels in gastric ulcer rat model. It was showed that IL-6 and IL-10 levels downregulated in the supernatant from gastric ulcer model group compared with normal control (P < 0.05). Corydalis alkaloid treatment significantly elevated IL-6 and IL-10 secretion in supernatant compared with model group with dose dependence (P < 0.05) (**Figures 5** and **6**).

Discussion

Gastric ulcer attack is featured as regularity and periodicity, which is related to different regions, psychological spirit, race, lifestyle, environment, and climate. Because of its complex risk factors, it is difficult for gastric ulcer treatment that brings huge pain and economic burden to patients [16]. It was found that

corydalis alkaloid can be used to the stop pain, thus can be used as the adjuvant drug to stop cancer pain. Its other pharmacological effects include regulating inflammation, anti-myocardial ischemia through regulating redox reaction [13, 14]. However, it is still unclear about whether it can be used for gastric ulcer treatment, and the related mechanism has not been reported. This study confirmed that corydalis alkaloid intra-gastric administration can significantly decreased UI in gastric ulcer rat model with dose dependence, suggesting that corydalis alkaloid has the protective effect on gastric ulcer rat model.

Recent studies considered that the pathogenesis of gastric ulcer is closely associated with helicobacter pylori infection, gastric acid, inflammatory factor expression, and attack and defense factor imbalance. In the study of the attack factors, it was thought that the main attack factors of gastric ulcer are gastric acid and pepsin. Their secretion elevation leads to gastrointestinal mucosa defense system has no protection, resulting in mucosal damage factors increase and the defense and damage imbalance to form ulcer [17, 18]. Gastric ulcer occurrence may cause oxygen free radical elevation, thus increases MDA content and reduces SOD activity. MDA has cytotoxicity, which can attack the stomach and duodenum mucosa epithelial cells, leading to cell damage, gastric mucous membrane phospholipids degradation, and ulceration aggravation [19]. Gastric mucosa epithelial cells synthetize and secrete PGE2, which can form mucus-bicarbonate barrier and cells-mucus secretion double protection to enhance the effect of mucosal immunity [20]. Inflammatory factor study believed that IL-2 and TNF-α overexpressed, while IL-6 and IL-10 secretion declined in the process of gastric ulcer formation, leading to inflammation progress and gastric injury aggravation [20, 21]. This study investigated the impact of corydalis alkaloid on improving attack factor and defensive factor imbalance. The results confirmed that corydalis alkaloid treatment can significantly suppress gastric secretion, elevated pH value, and reduced pepsin secretion in gastric ulcer rats model, reduce gastric acid, increase the gastric pH, and to reduce the secretion of pepsin, indicating that corydalis alkaloid can inhibit attack factor to regulate gastric mucosal defense and damage balance. Furthermore, corydalis alkaloid can promote SOD activity, decline MDA, and enhance PGE2 secretion to promote gastric mucosal protective barrier formation and reduce the epithelial cell damage. Additionally, it also suppress inflammation through regulating the balance of inflammatory cytokines and anti-inflammatory factors to reduce ulcer lesions.

In summary, corydalis alkaloid plays a curative role on gastric ulcer by regulating inflammatory cytokines secretion and improving gastric mucosa attack factor/defense element balance. This study offers a new drug selection and provide theoretical basis for gastric ulcer treatment.

Disclosure of conflict of interest

None.

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