

Saussurea Lappa Modulates Gastrointestinal Motility, Motilin and Cholecystokinin Expression in Ulcer Rats

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ABSTRACT

AIM: To investigate effects of *Saussurea lappa* (*S. lappa*) on rat chronic duodenal ulcer induced by ethylic acid and the underlying mechanisms.

METHODS: Wistar rat chronic duodenal ulcer was patterned according to Okabe model while two pairs of bipolar stainless steel electrodes were implanted onto the serosal layer of gastrointestinal tract. Then, the rats were treated with water, Omeprazole, diverse-dose *S. lappa* for 10 days. Gastrointestinal motility, intestine propulsion and duodenal ulcer crater were detected. Expressions of cholecystokinin and motilin in duodenum were detected by immunohistochemistry (IHC). Motilin serum level was measured with enzyme-linked immunosorbent assay (ELISA).

RESULT: Duodenal ulcer group rats had obvious disorders of gastrointestinal motility and brain-gut peptide secretion, including gastrointestinal myoelectric activity inhibition, migrating myoelectric complex (MMC) interruption, intestinal propulsion slowdown,

cholecystokinin and motilin secretion decrease. Similarity to Omeprazole, *S. lappa* accelerated the coalescence of ethylic acid-induced duodenal ulcer significantly ($p < 0.01$, vs model group), but no obvious dose-dependent effect was found. *S. lappa* also improved gastrointestinal myoelectric activity and accelerated intestinal propulsion. Furthermore, *S. lappa* could increase the expression levels of cholecystokinin and motilin in duodenum and increase motilin level in serum.

CONCLUSION: *S. lappa* can accelerate the coalescence of chronic duodenal ulcer, and increase gastrointestinal myoelectric activity and the expression of cholecystokinin and motilin in duodenum.

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Key Words: *Saussurea lappa*; Duodenal ulcer; Gastrointestinal motility; Brain-gut Peptides

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INTRODUCTION

Peptic ulcer (PU), as a common disease, has been decreasing according to the recent reports with the treatment of *Helicobacter pylori* (*H. pylori*) eradication^[1]. Nevertheless, this decreasing prevalence has "not resulted fewer hospital admissions for peptic ulcer disease-related complication"^[2]. Duodenal ulcer (DU), as the most common form of this disease, is still prevalent with a high incidence and frequently in China^[3,4]. The decline of the protective screen and the excessive secretion of gastric acid were known as one of important pathogenesis. Thus, it would be valuable to study human chronic DU by eroding intestinal wall with ethylic acid^[5].

Saussurea lappa (*S. lappa*, Chinese name is MuXiang) is compositae mostly cultivated in YunNan of China^[6]. As a traditional oriental medicine, it possesses extensive applications on resisting

bacteria^[7,8], protecting liver^[9,10], diminishing inflammation^[11,12] and so on. Recent studies indicate that *S. lappa* can decrease blood pressure, inhibit vasoconstrictile responses^[13], cancer^[14], and affect cytokine^[15]. Furthermore, it widely applies to alleviate pain in abdominal distention and tenesmus, indigestion with anorexia, dysentery, nausea and vomiting^[16], and has been a major composition of some oral drugs like Aplotaxis carminative pill, Pill of Aucklandia and Areca Seed, Simotang and so on nowadays.

Organic lesions usually complicate with function disorder, so gastrointestinal motility interruption is the sign of duodenal ulcer and becomes one of probable mechanisms in some studies^[17,18,19]. Besides, hormone secretions also influence gastrointestinal motility^[20-25].

Despite the increasing attention to the effects of digestive disorders including GU^[26], little is known about the mechanisms of these effects on DU, especially in chronic DU. The present study was designed to observe the effects of *S. lappa* on ethylic acid –induced chronic duodenal ulcer and to investigate the underlying mechanism on gastrointestinal motility and hormone levels.

MATERIALS AND METHOD

Animal Preparation

The present work was conducted according to the guidelines for the Care and Use of Laboratory Animals from the Experimental Animal Center of Lanzhou University. All rats were acclimatized to laboratory conditions (23 °C, 12 h/ 12 h light/dark, 50% humidity, ad libitum access to food and water) for a week prior to experimentation. Adult male Wistar rats (221.1 ± 28.7 g, provided by the experimental animal center of Lanzhou University) were utilized in this study and anesthetized with sodium pentobarbitone (30 mg/kg, ip) after fasting but drank for 24 h. Then the skins of the abdomen and the back of neck were prepared. Next, the operation was done under strict sterile conditions. The abdominal muscles and peritoneum were opened gradually by linea alba. Two pairs of bipolar stainless steel electrodes, 3mm apart, were implanted onto the serosal layer of gastrointestinal tract. One pair was on the antrum at 0.5 cm from pylorus and the other was on the duodenum at 1cm distal to pylorus respectively. The free ends of the electrodes were brought subcutaneously to the back of the neck where the skin and muscle were opened for 0.5 cm^[27]. Some rats were randomly made models that duodenal bulb was contacted by glass tube covered with 50% ethylic acid and others were contacted instead of normal saline for 30 s before electrodes were implanted^[2]. After the operation, they were individually housed with free access to water after a day and food after 2 days.

Experimental Procedure

At the fifth day after surgery, each survived rat was recorded the gastrointestinal myoelectric activity including slow wave and fast wave of antrum and duodenum for 1h. Then rats whose duodenum treated with acetic acid were coded and divided into 5 groups randomly. Randomized 6 groups of rats were given either water, omeprazole (9 mg/(kg. d), Hunan Dinuo Pharmaceutical Co., Ltd) or *S. lappa* (native to Yun Nan, China) by intragastric (ig) (gavage straight gavage needles: 150-300 g body weight: 16 gauge, 3 inch length, 3 mm ball diameter) 4 mL every time and 2 times a day for 10 days. To compare effects of concentration gradient on ethylic acid–induced duodenal ulcer in rats, the different concentration (12, 24 or 48 g/(kg.d)) of *S. lappa* was used in the present experiment. After 10 days' treatment, all rats executed by dislocating their cervical spine under barbiturate overdose (150 mg/kg pentobarbital

sodium, i.p.), and blood was collected from the femoral artery and duodenum was immediately removed, edulcorated and fixed in 4% paraformaldehyde.

Motility Recording

Recordings were conducted in conscious rats at 5th, 10th, 15th days after operation. And all rats were fasted with free access to water for 16 to 18 h before each test. The gastrointestinal myoelectric activity was recorded with the BL-420S experimental system of biologic function (TaiMeng Technology, Chengdu, China) by microcomputer, and each item was logged for 1h while rats were placed in an individual fixing frame.

Hematoxylin and eosin (H&E) staining and ulcer size calculation

The fixed well tissue was stained with hematoxylin and eosin (H&E) by Institute of Pathology of Lanzhou University. The length and the width of the duodenal ulcer crater were measured with vernier calipers in millimeters. And the ulcer areas were calculated with the ellipsoid formula.

Detection of Intestine Propulsion

Ten days after drug or water administration, rats fasted for 20 h while given drug or water as usual. Each rat was provided semi-solid paste (0.3 mL/100g, ig), then was stunned by striking their head after 30min and their cardia and pylorus were both ligatured before stomach and small intestine were took out at once. The intestine propulsion rate in 30 min was calculated according to the following formula^[28]: intestinal propulsion rate (%) = advanced length of black semi-solid paste/total length of the small intestine × 100%.

Immunohistochemistry

The expression of proteins including cholecystokinin (CCK) and motilin (MTL) were detected by SP method of immunohistochemistry (IHC) according to the protocol of SP KIT. In brief, paraformaldehyde-fixed tissue sample were embedded in paraffin, sectioned, and dried in oven at 60 °C overnight. Then the sections were dewaxed completely with dimethylbenzene, ethanol of concentration gradient, PBS and distilled water, and irradiated at microwave oven for 6min to boiling and repaired by cutting electricity for 5min, buffering pH 6.0 citrate solution two times, incubated at 37°C for 10 min with 3% hydrogen. After irrigation, they were immunostained with normal goat serum and anti-CCK-I (1:300) and anti-MTL (1:200) (All antibody produced by Bioss Technology), followed by biotinylated secondary antibodies after they were at 4°C overnight, and a peroxidase-labeled streptavidin-biotin staining technique. Finally, hematoxylin was used to counterstain. Results were observed under the light microscope and judged by two reviewers according to the criterion of immunohistochemical semi-quantitative integration.

Measurement of Serum MTL Level

Serum was collected from the femoral artery and preserved at 4,-20,-40, and -80°C gradually, and MTL level was measured with ELISA in according with its manufacturer's protocol (R&D,USA).

Statistical Analysis

All values are expressed as mean ± SD, and “n” means the number of Wistar rats utilized in the study. *P* values less than 0.05 were considered significant, and statistical analysis was performed by one-way ANOVA among groups and independent sample *t*-test between two groups with IBM SPSS Statistic 20.

RESULTS

Effects of *S. lappa* decoction on coalescence of duodenal ulcer

Hematoxylin and eosin (H & E) staining revealed defective glands in damaged mucosa after ethylic acid's administration and proliferative granulation tissue after *S. lappa*'s treatment, which indicated that ethylic acid could destroy duodenum while *S. lappa* healed chronic DU in Wistar rats (Figure 1A). Similarity to Omeprazole, *S. lappa* decreased ulcer size ($p < 0.01$, vs model). Furthermore, high-dose *S. lappa* (48 g/kg, d) dropped the sizes of ulcers from 5.15 ± 0.16 to $1.99 \pm 0.64 \text{ mm}^2$ ($p < 0.01$, vs model) (Figure 1B).

Effects of *S. lappa* decoction on gastrointestinal myoelectric motility

According to motility recording after 5 days, the administration of ethylic acid led a disorder of gastrointestinal myoelectric motility, representing the decrease of the amplitude of slow wave ($p < 0.01$) and the percentage of slow wave containing spike bursts ($p < 0.001$), but didn't affect the myoelectric motility of gastric antrum while slow wave frequencies of neither gastric antrum nor intestine was affected (Table 1). It appears regular spontaneous gastrointestinal myoelectric activity in normal rats, including adequate slow wave and migrating myoelectric complex (MMC) consisting of four definite phases: quiescence (phase I), irregular contractions (phase II), regular contractions with a duration of at least 2 to 3 minutes and migrating downwards in the gastrointestinal tract (phase III) and transitional period (phase IV) (Normal of Figure 2A). However, DU induced with ethylic acid interrupted MMC in duodenum and obvious phases were not found (Model of Figure 2A). Contrary to model, *S. lappa* improved MMC activity after 15 days, which showed four definite phases (*S. lappa* of Figure 2A). Besides, the result of intestinal propulsion trail indicated *S. lappa*, especially the lowest group (12 g/kg, d) *S. lappa*, enhanced intestinal peristalsis of ethylic-induced duodenum (Figure 2B).

Effects of *S. lappa* decoction on protein expression

CCK and MTL localized mostly in the cytoplasm of submucosa and mucosa layer in duodenum. Compared with model, Omeprazole and *S. lappa* both could upregulate CCK and MTL expression in duodenum (Figure 3).

Serum level of motilin among the groups

As shown in Figure 4, ethylic-induced DU significantly decreased the level of serum MTL, and *S. lappa* and Omeprazole could improve it. Especially medium-dose *S. lappa* enhanced the level of serum MTL to approach that of normal level.

DISCUSSION

It's widely known that the break in the normal balance between aggressive and defensive factors of gastroduodenal mucosa contributes to PU. The aggressive factors include gastric acid, pepsin, bile reflux, alcohol, drugs like non-steroidal anti-inflammatory drugs (NSAIDs) and *H. pylori*, while the defensive factors include mucosal

blood flow, surface epithelial cells, prostaglandin, gastric motility, and others^[29,30]. Acetic acid-induced DU model is similar to human's chronic DU by eroding intestinal wall (defensive factor), and it is beneficial to observe the repairing effect of *S. lappa* on ulcer. Data from our study indicates the inhibitory effect of *S. lappa* on ulcer, which is consistent with the reported findings^[26], and provides further evidence for anti-DU of *S. lappa*.

To observe the gastrointestinal motility, we made motility recording with BL-420S and intestine propulsion. Gastrointestinal myoelectric activity that is a sensitive index of gastrointestinal motility, comprises slow wave and spike activity (fast wave). Slow wave could influence the mechanical contraction by pacing and directing gastrointestinal contraction^[17], and the spike activity would improve gastrointestinal motility^[18]. Besides, MMC observed in gastrointestinal tract during fasting is a vivid pattern recording electromechanical activity and it consists of four phases as follows: quiescence, irregular contractions,

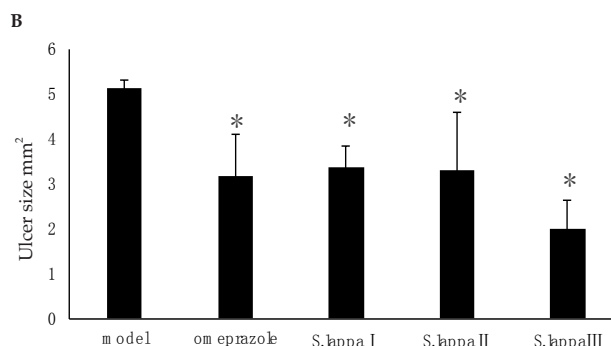
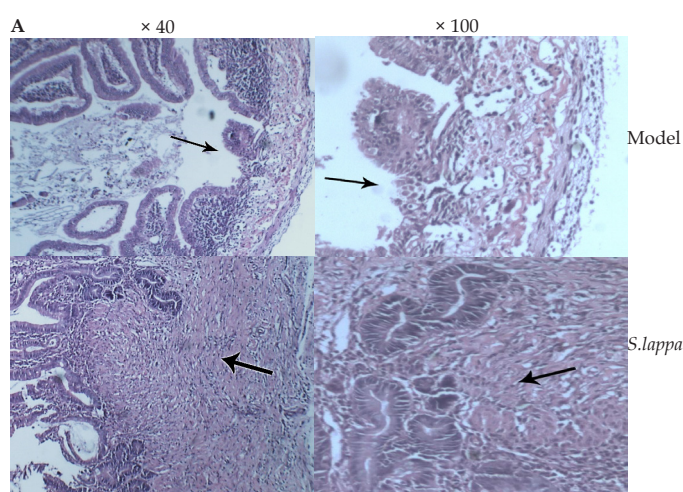


Figure 1 Effects of Saussurea lappa (*S. lappa*) decoction on coalescence of ethylic acid –induced duodenal ulcer. A Appearance of duodenal ulcers and coalescence after water and *S. lappa* treatment. Light microscopy of duodenal ulcer with Hematoxylin and eosin (H & E) staining, x40, x100. (The arrows represent damaged mucosa in model and proliferative granulation tissue in *S. lappa*). B Ulcer size after corresponding treatment. Major and minor diameters of duodenal ulcer in rats were measured with vernier calipers and areas of duodenal ulcer were calculated using the ellipsoid formula. Omeprazole: 9 mg/(kg, d); *S. lappa* I: 12g/(kg, d); *S. lappa* II: 24g/(kg, d); *S. lappa* III: 48g/(kg, d). Size of duodenal ulcers are expressed as mean \pm SD ($n=4$), * $P < 0.01$, vs model.

Table 1 Changes of antral and duodenal myoelectric activities after ethylic acid induction ($n = 9-16$, mean \pm SD).

Groups	Antrum			Duodenum		
	SF (num/min)	SA (μ V)	Spike burst (%)	SF (num/min)	SA (μ V)	Spike burst (%)
Model	4.73 \pm 0.26	282.08 \pm 68.99	46.14 \pm 6.41	38.28 \pm 10.14	101.16 \pm 22.64*	33.44 \pm 1.02**
Normal	4.71 \pm 0.29	289.07 \pm 36.07	51.30 \pm 7.29	36.29 \pm 6.10	198.84 \pm 80.90	54.67 \pm 7.12

SF: Slow wave frequency; SA: Slow wave amplitude; Spike burst (%): the percentage of slow wave containing spike bursts. * $p < 0.01$, ** $p < 0.001$, vs normal.

regular contractions with a duration of at least 2 to 3 minutes and migrating downwards in the gastrointestinal tract and transitional period^[19]. It is considered as a housekeeping role that sweeps undigested residue through the digestive tract. In the present study, our finding that the amplitude of slow wave and the percentage of

slow wave containing spike bursts was lower in chronic DU than in normal revealed that organic lesions were complicated with motility disorder. Meanwhile, the comparison among groups on MMC and intestine propulsion suggests that the probable mechanism involves sweeping undigested residue and improving intestinal motility.

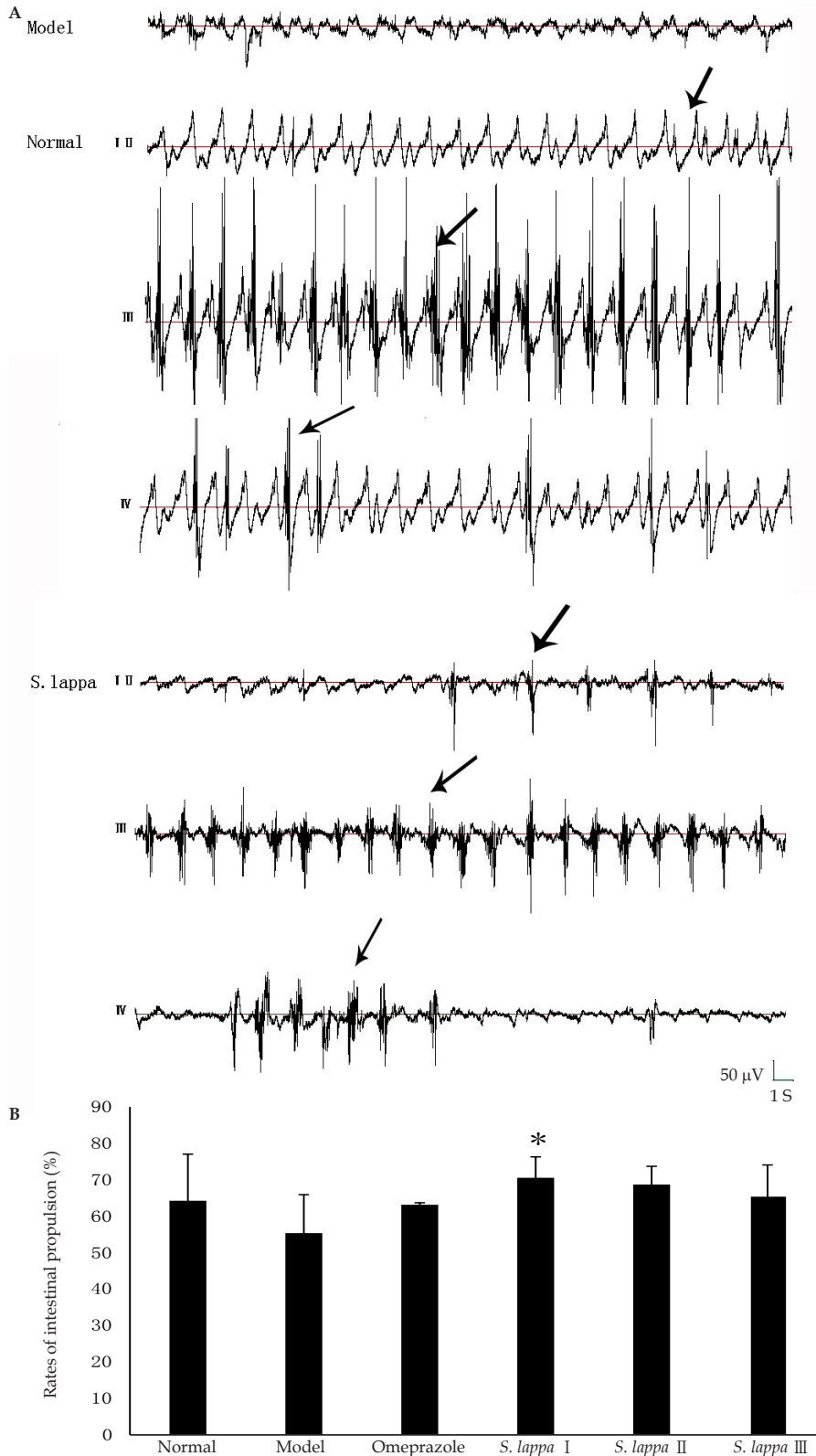


Figure 2 Effects of Saussurea lappa decoction on antral and duodenal activities in rats. A Effects of Saussurea lappa decoction on duodenal MMC activity (The arrows represent spike bursts). B Effects of Saussurea lappa decoction on rates of intestinal propulsion in rats. * $p < 0.05$, vs model; $n = 3-5$.

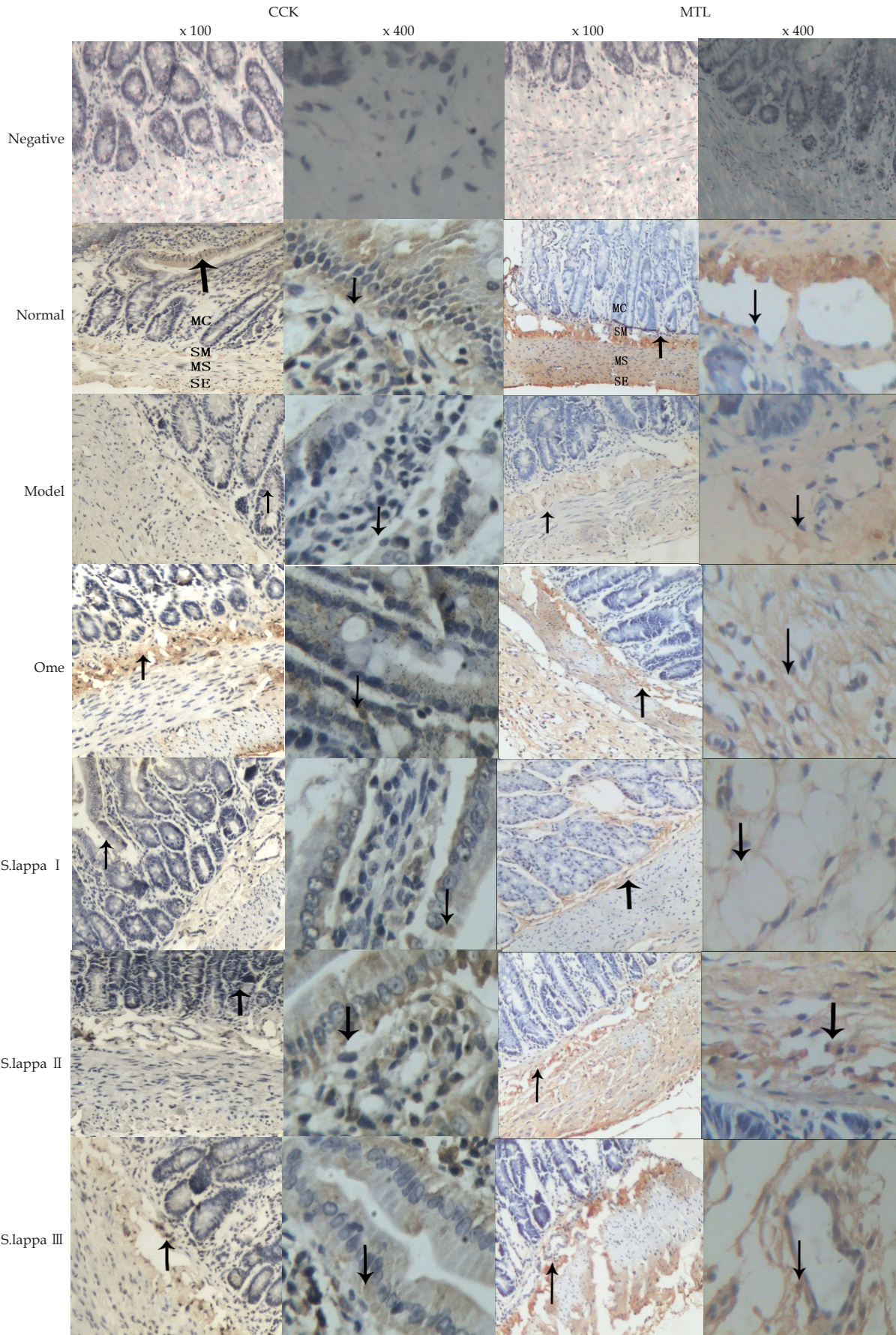


Figure 3 Effects of Saussurea lappa(S.lappa) decoction on protein expression of ethylic acid-induced duodenal ulcer in rats. Ome: 9mg/(kg. d) Omeprazole; S.lappa I : 12g/(kg. d); S.lappa II : 24g/(kg. d); S.lappa III: 48g/(kg. d). CCK: cholecystokinin; MTL: motilin. MC: mucosa; SM: submucosa; MS: muscularis; SE: serosa. (The arrows represent expression-positive cells).

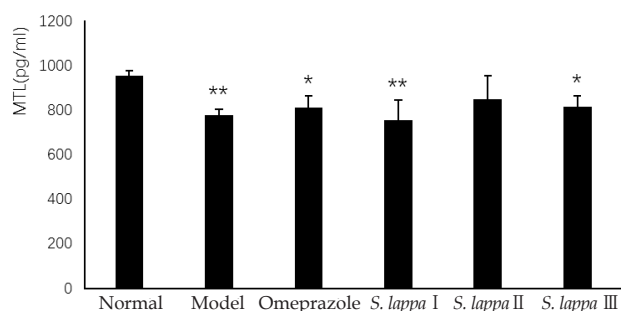


Figure 4 The effect of *Saussurea lappa* decoction on MTL in serum. $n = 4-6$, * $p < 0.05$, ** $p < 0.01$, vs normal.

CCK, an old known hormone, is synthesized by I-cells in the mucosal epithelium of the small intestine, secreted in the duodenum and widely distributed in the gastrointestinal tract especially in small intestine and central/peripheral nervous system and CCK-1 plays an important role in diverse physiological processes including gallbladder contraction, sphincter of Oddi relaxation, stimulation of pancreatic secretion, inhibition of acid secretion, and regulation of small intestine^[31]. The results of some studies also supported that enterohepatic circulation of bile acid is related to regular cycling of MMC^[20,21]. Yamahara *et al*^[32] reported that *S. lappa* has cholagogic effect, agreed with Chinese traditional medicine. Our finding that CCK-1 levels were higher in *S. lappa* and normal group than in model group suggests *S. lappa* could accelerate the secretion of CCK-1 to inhibit ulceration. On the one hand, CCK may reduce aggressive factor by inhibiting the secretion of gastric acid and enhance the motility of small intestine directly. On the other hand, it probably improves MMC activity by restoring enterohepatic circulation of bile acid due to a role for CCK-1 in gallbladder contraction and bile secretion.

Substantial evidence has shown that MTL, the 22 amino acid peptide hormone, is secreted by endocrine M cells in the proximal small bowel, participating in controlling smooth muscle contraction and improving peristalsis of small intestine^[22,23]. Further studies showed it affects gastrointestinal motility by increasing MMC, related to the onset of housekeeping contraction^[24,25]. In the present study, our result that the levels of MTL in small intestine and serum are both higher in *S. lappa* group than in model group indicates *S. lappa* may be helpful in secreting MTL to promote motility, consenting to study by Chen *et al*^[33]. In addition, the consistent consequence between MMC activity and the MTL level among groups suggested MTL can regulate MMC activity to improve intestinal motility.

In conclusion, the decoction of *S. lappa* can heal ethylic acid–induced DU in Wistar rats. The mechanisms are probably due to the enhancement of motility and the increase of MTL and CCK expression in duodenum to increase defensive factors and decrease aggressive factors. Thus, this provides a pharmacological basis for its clinical application in the treatment of chronic duodenal ulcer.

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Author contributions: Zhang TY performed the research, analyzed data and wrote the paper, Li HF designed the research, analyzed data, wrote and revised the manuscript, Wang P, Wang Q, Liang QK, Li YX, Wang YD, Ling XC, Mao LF contributed new reagents/analytic tools and performed the research.

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CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

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