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ORIGINAL ARTICLE

Xiaotan Tongfu granules contribute to the prevention of stress ulcers

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Abstract

AIM: To investigate the efficacy and potential mechanism of Xiaotan Tongfu granules (XTTF) in stress ulcers.

METHODS: One hundred sixty rats were randomly divided into 4 groups (n = 10) as follows: the model group (MP group), the control group (CP group), the ranitidine group (RP group) and the XTTF granule group (XP group). Rats in the MP group received no drugs, rats in the CP group received 0.2 mL of a 0.9% sodium chloride solution *via* oral gavage, and rats in the RP and XP groups received the same volume of ranitidine (50 mg/kg) or XTTF granule (4.9 g/kg). The cold-restraint stress model was applied to induce stress ulcers after 7 consecutive days of drug administration. Afterwards, rats were sacrificed at 0, 3, 6 and 24 h. Gastric pH was measured by a precise pH meter; gastric emptying rate (GER) was measured by using a methylcellulose test meal; myeloperoxidase activity (MPO), macrophage migration inhibitory factor (MIF), proliferating cell nuclear antigen (PCNA), and heat shock protein 70 (HSP70) were measured by immunohistochemical staining; and mucosal cell apoptosis was measured by transferase dUTP nick end labeling.

RESULTS: In the cold-restraint stress model, the development of stress ulcers peaked at 3 h and basically regressed after 24 h. Gastric lesions were significantly different in the RP and XP groups at each time point. Interestingly, although this index was much lower in the RP group than in the XP group immediately following stress induction (7.00 ± 1.10 *vs* 10.00 ± 1.79, $P < 0.05$. Concerning gastric pH, between the RP and XP groups, we detected a statistically significant difference immediately after stress induction (0 h: 4.56 ± 0.47 *vs* 3.34 ± 0.28, $P < 0.05$) but not at any of the subsequent time points. For GER, compared to the RP group, GER was remarkably elevated in the XP group because a statistically significant difference was detected (3 h: 46.84 ± 2.70 *vs* 61.16 ± 5.12, $P < 0.05$; 6 h: 60.96 ± 6.71 *vs* 73.41 ± 6.16, $P < 0.05$; 24 h: 77.47 ± 3.17 *vs* 91.31 ± 4.34, $P < 0.05$). With respect to MPO and MIF, comparisons between the RP and XP groups revealed statistically significant differences at 3 h (MPO: 18.94 ± 1.20 *vs* 13.51 ± 0.89, $P < 0.05$; MIF: 150.67 ± 9.85 *vs* 122.17 ± 5.67, $P < 0.05$) and 6 h (MPO: 13.22 ± 1.54 *vs* 8.83 ± 0.65, $P < 0.05$; MIF: 135.50 ± 9.46 *vs* 109.83 ± 6.40, $P < 0.05$). With regard to HSP70, HSP70 expression was significantly increased in the RP and XP groups at 3 and 6 h compared to the MP and CP groups. In addition, comparing the RP and XP groups also showed statistically significant differences at 3 and 6 h. The expression of PCNA was higher in the RP and XP groups 3 h after stress induction. Between these two groups, small but statistically significant differences were observed at all of the time points (3 h: 69.50 ± 21.52 *vs* 79.33 ± 15.68, $P < 0.05$;

6 h: 107.83 ± 4.40 *vs* 121.33 ± 5.71, $P < 0.05$; 24 h: 125.33 ± 5.65 *vs* 128.50 ± 14.49, $P < 0.05$) except 0 h. With regard to apoptosis, the apoptotic activity in the RP and XP groups was significantly different from that in the MP and CP groups. The XP group exhibited a higher inhibition of cell apoptosis than the RP group at 3 h (232.58 ± 24.51 *vs* 174.46 ± 10.35, $P < 0.05$) and 6 h (164.74 ± 18.31 *vs* 117.71 ± 12.08, $P < 0.05$).

CONCLUSION: The Xiaotan Tongfu granule was demonstrated to be similar to ranitidine in preventing stress ulcers. It exhibited multiple underlying mechanisms and deserves further study.

Key words: Stress ulcer; Xiaotan Tongfu granule; Inflammation; Heat shock protein 70; Proliferation and apoptosis; Gastric emptying rate

Core tip: Although the underlying mechanism of stress ulcers is commonly believed to depend on the balance between known aggressive factors and mucosal defense mechanisms, most clinical strategies still aim to inhibit gastric acid. In this study, we demonstrated that the Xiaotan Tongfu granule was similar to ranitidine treatment in reducing gastric lesions in a cold-restraint stress model. The underlying mechanisms may include acceleration of the gastric emptying rate, inhibition of local inflammation, promotion of cell proliferation and suppression of apoptosis. Our study indicated that multiple manipulations of the factors involved in inducing stress ulcers could be as effective as simple acid inhibition.

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INTRODUCTION

Stress ulceration (SU) has been conventionally regarded as an inevitable complication of the gastrointestinal tract in people experiencing abnormally high physiological stress (*e.g.*, trauma, surgery, organ failure, sepsis, or burn)[1]. Gastrointestinal bleeding is a life-threatening complication of SU and was observed in 64% of patients with SU, compared to only 9% of patients without SU, in a previous study[2]. It is believed that clinically significant gastrointestinal bleeding in critically ill patients is associated with increased mortality rates, lengthened intensive care unit stays and additional costs[3-5].

The development of stress ulcers is largely determined by the balance between known aggressive factors and defense mechanisms. The former usually include gastric acid[6], abnormal motility[7], and *Helicobacter pylori* infection[8,9], and the latter include heat shock protein[10], cellular regeneration[11], *etc*. SU prophylaxis (SUP) was thought to play a pivotal role in the care of critically ill patients, and it was reported that appropriate SUP could decrease mortality. At present, although multiple protocols are available for SUP, there are no universally accepted regimens[12]. Nevertheless, the evidence that the appropriate application of some pharmacologic agents, such as proton pump inhibitors, histamine-2 receptor antagonists, and sucralfate[13], could decrease the risk of bleeding has been long established.

Traditional Chinese medicine (TCM) has been demonstrated to be effective in the management of stress-related gastrointestinal disorders, including irritable bowel syndrome[14,15], and a number of studies have also indicated that TCM could exert measurable therapeutic effects on gastric ulcers in rats[16-18]. Based on these studies on TCM, the Xiaotan Tongfu (XTTF) granule (Table 1), which is primarily composed of a Xiao-cheng-qi decoction[19] and a Xiao-ban-xia decoction[20] (two ancient herbal formulas originating from the Treaty of Febrile and Miscellaneous written by Zhongjing Zhang in the years of 25-220 AD during the Eastern Han Dynasty), was used to treat gastrointestinal disorders in critically ill patients at our hospital. The rationale behind this treatment was that previous studies have indicated that the granule could improve the Acute Physical and Chronic Health Evaluation scores in patients experiencing gastrointestinal dysfunction (unpublished data). Considering this background, we speculated that the XTTF granule could be applied to the management of SU. In the present study, we investigated the efficacy of the XTTF granule in SU and the potential mechanisms involved.

MATERIALS AND METHODS

Animals

One hundred sixty male Sprague-Dawley rats weighing 200-220 g were purchased from Xipuer-Bikai Experimental Animal Co. LTD (Shanghai). The animals were housed in cages with wide mesh wire bottoms to prevent coprophagy, fed a standard laboratory diet and given free access to tap water. The cages were kept in a room with controlled temperature (22 ℃ ± 1 ℃), relative humidity (65%-70%) and day/night cycle (12:12 light/dark). All of the rats were handled according to the recommendations of the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. The protocol was approved by the Shanghai Medical Experimental Animal Care Commission.

Drug administration

The XTTF granule was manufactured by Tian Jiang Pharmacy Co. Ltd (Jiangyin, China) and supervised by the Changzheng Hospital of the Second Military Medical University with the assigned batch number 1011370. We established the granule under the guidance of TCM

Table 1 Ingredients and the corresponding percent of Xiaotan Tongfu granules

Chinese name	Common name	Latin name	Percent
Da Huang	Rhubarb	*Rhei Radix Et Rhizoma*	10%
Zhi Shi	Immature Bitter Orange	*Aurantii Fructus Immaturus*	10%
Ban Xia	Pinellia Tuber	*Pinelliae Rhizoma*	10%
Hou Pu	Magnolia Bark	*Magnoliae Officinalis Cortex*	6%
Bai Shao	White Peony Root	*Radix Paeoniae Alba*	10%
Xi Xin	Manchurian Wild Ginger	*Asari Radix Et Rhizoma*	4%
Huang Lian	Coptis Root	*Coptidis Rhizoma*	4%
Pu-Gong Yin	Dandelion	*Asari Radix Et Rhizoma*	10%
Bai-Hua-She-She Cao	Snake-needle Grass	*Hedyotis Diffusa*	10%
Fo Shou	Finger Citron	*Citri Sarcodactylis Fructus*	10%
Xiang Yuan	Citron Fruit	*Citri Fructus*	10%
Gan Cao	Licorice Root	*Glycyrrhizae Radix Et Rhizoma*	6%

related to stress ulcers[21], and some of the components were previously shown to be effective in the management of stress-related symptoms. For example, the Xiao-ban-xia decoction could elevate gastric emptying[22], which was delayed under stress conditions. In addition, the major component of the Xiao-cheng-qi decoction, *Rhei Radix Et Rhizoma*, was demonstrated to be effective in the prevention of stress ulcers *via* multiple mechanisms[23,24]. The rats were randomly divided into 4 groups (n = 10) as follows: the model group (MP group), the control group (CP group), the ranitidine group (RP group) and the XTTF granule group (XP group). Rats in the MP group received no drugs; rats in the CP group received 0.2 mL of a 0.9% sodium chloride solution *via* oral gavage; and rats in the RP and XP groups received the same volume of ranitidine (50 mg/kg)[25-27] or XTTF granule (4.9 g/kg, corresponding to twice that of an adult human dose), respectively. The administration frequency was twice daily and sustained for 7 d. On the 8th day, rats were starved for 24 h (free of water) and prepared for the stress experiment.

Induction of stress ulceration

The cold-restraint stress model used in the present study was originally devised by Senay *et al*[28] and modified by Wong *et al*[29]. Briefly, rats were restrained inside individual close-fitting tubular wire mesh cages and exposed to an ambient temperature of 4 ℃ for 3 h. Rats were anesthetized and sacrificed at 0, 3, 6 and 24 h after stress induction, and the stomachs were opened along the greater curvature. After measuring the mucosal lesions, sections of the tissues were fixed in 10% buffered formalin solution and stained for proliferating cell nuclear antigen (PCNA), heat shock protein 70 (HSP70), and macrophage migration inhibitory factor (MIF) *via* immunohistochemistry (IHC) and for apoptosis *via* transferase dUTP nick end labeling (TUNEL) staining.

Measurement of gastric ulcer index, pH and emptying rate

The severity of the mucosal lesions was determined using a magnifier (× 10) and rated for gross pathology according to the scale of ulcer scores as described by Dekanski *et al*[30] with a modification introduced by Martín *et al*[31]. For every group, 4 rats were used in the precise measurement of gastric pH, and the test was performed by 3 independent investigators to determine the mean pH. Gastric emptying (n = 6, 2 rats were used as a control in each group) was measured using a methylcellulose test meal, as previously described[32,33].

Measurement of myeloperoxidase activity in the gastric mucosa

Myeloperoxidase (MPO) activity was determined by the method described by Bradley *et al*[34] with some modifications[35]. The gastric mucosa was homogenized in a potassium phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide, and the supernatant was assayed for MPO activity. The sample was mixed with hydrogen peroxide and *O*-Dianisidine prepared in a potassium phosphate buffer solution. The end point absorbance of the mixture was measured at 460 nm using a spectrophotometer with horseradish peroxidase as a standard. The protein assay was conducted using the method described by Lowry *et al*[36].

Immunohistochemical staining for PCNA, HSP70 and MIF

Tissues were fixed in 10% formalin, embedded in paraffin, and processed by standard histological methods. From each paraffin block, 5-μm serial sections were sliced. IHC studies were performed with kits utilizing the avidin-biotin-peroxidase complex according to the manufacturer's instructions (Invitrogen, United States). Primary antibodies [anti-PCNA (rabbit polyclonal, dilution 1:50, BD Biosciences) anti-HSP70 (rabbit polyclonal, dilution 1:50, BD Biosciences, United States), and anti-MIF (rabbit polyclonal, dilution 1:100, BD Biosciences, United States)] were incubated at room temperature overnight in a humidified chamber. The positive results were stained brown and counted by the Image Pro Express system (Olympus, Japan) at × 400 magnification (BX51, Olympus, Japan); the method of calculation was introduced by Soslow *et al*[37].

Measurement of apoptotic cells in the gastric mucosa

Apoptosis measurement was detected by TUNEL staining according to the method of Gavrieli *et al*[38]. After digestion with proteinase K, the tissues were treated with H_2O_2 solution and washed with distilled water. The sections were then covered with TdT buffer containing TdT and biotinylated dUTP. The reaction was halted by washing the sections with a 3% H_2O_2 methanol solution at room temperature. After blocking the non-specific binding with normal diluted serum, sections were incubated with peroxidase-labeled streptavidin and stained with

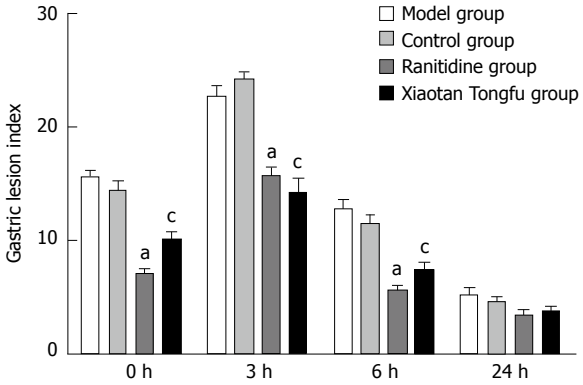


Figure 1 Results of the gastric lesion index (*n* = 6 for each group). At 0 h, ranitidine was demonstrated to be the most powerful agent in the inhibition of gastric lesions. The difference in the inhibition of gastric lesions between the ranitidine group and the Xiaotan Tongfu granule group was statistically significant ($P < 0.05$). However, at the subsequent time points, this difference vanished. $^{a}P < 0.05$ *vs* the model group; $^{c}P < 0.05$ *vs* the control group.

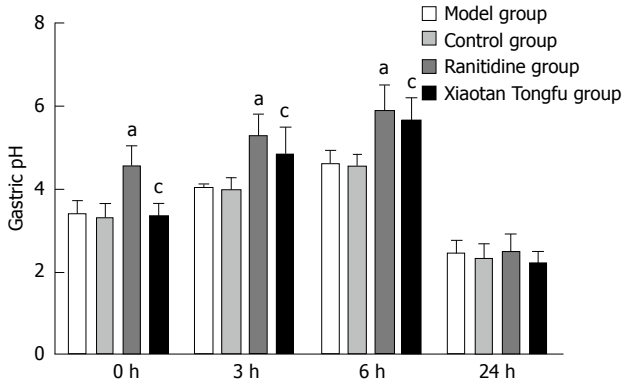


Figure 2 Results of gastric pH (*n* = 4 in each group). At 0 h, ranitidine was demonstrated to be the most powerful agent in increasing the gastric pH, and the increase in the gastric pH was significantly different between the ranitidine group (RP group) and the Xiaotan Tongfu granule group (XP group) ($P < 0.05$). In addition, there were no significant differences regarding the increase in the gastric pH among the XP, the model group (MP group), the control group (CP group) ($P > 0.05$). At 3 h and 6 h after stress induction, there were no differences in the gastric pH between the RP and XP groups. $^{a}P < 0.05$ *vs* the MP group; $^{c}P < 0.05$ *vs* the CP group.

diaminobenzidine-H_2O_2. Finally, the sections were counterstained with Mayer's hematoxylin. Sections treated with DNase I in buffer solution served as the positive control, whereas the negative control was prepared by omitting the TdT from the buffer solution. The positive cells were counted by the Image Pro Express system (Olympus) at × 400 magnification (BX51, Olympus). The apoptotic index was defined as the average number from 10 to 25 glands of each mucosal section.

Statistical analysis

All data were processed by SPSS 18.0 and presented as the mean ± SD. Comparisons between the different groups were evaluated by a one-way analysis of variance followed by the Bonferroni test. Values of $P < 0.05$ were considered to be statistically significant. To avoid subjective bias of the parameters measured in this study, observers were blinded to the sample sources at the time of assessment.

RESULTS

XTTF granule shows similar capabilities as ranitidine in reducing gastric lesions

As shown in Figure 1, gastric lesions developed in a time-dependent manner and peaked at 3 h after stress induction; at 24 h after stress induction, these lesions had regressed. In the MP and CP groups, no statistically significant differences in this index were detected either overall or at each of the individual time points ($P > 0.05$). In the RP and XP groups, gastric lesions were significantly different compared to the MP and CP groups at each time point ($P < 0.05$), except at 24 h after stress induction ($P > 0.05$). Interestingly, although this index was much lower in the RP group than in the XP group immediately after the stress (7.00 ± 1.10 *vs* 10.00 ± 1.79, respectively; $P < 0.05$), this difference was eliminated at 3 h (15.67 ± 1.97 *vs* 14.17 ± 3.125, respectively; $P > 0.05$), 6 h (5.50 ± 1.05 *vs* 7.33 ± 1.63, respectively; $P > 0.05$) and 24 h (1.67 ± 0.52 *vs* 1.50 ± 0.55, respectively; $P > 0.05$) after stress induction.

Gastric pH in the XP and RP groups is significantly lower than in the MP and CP groups

As shown in Figure 2, the fluctuation of gastric pH was restricted to a limited range, except for 24 h after stress induction. There were no significant differences observed between the MP and CP groups ($P > 0.05$). In the RP and XP groups, we detected a statistically significant difference immediately after stress induction (4.56 ± 0.47 *vs* 3.34 ± 0.28, respectively; $P < 0.05$) but not at any of the subsequent time points ($P > 0.05$). The gastric pH also recovered to normal levels 24 h after stress induction in these two groups, and no significant differences were observed among all of the groups ($P > 0.05$).

XTTF granule and ranitidine treatment accelerated the gastric emptying rate

It has been established that stress could produce a marked delay of gastric emptying in both humans and animals[39,40]. As shown in Figure 3, the gastric emptying rate (GER) was remarkably suppressed very shortly after stress induction and was gradually restored over time. This effect was obvious in the MP and CP groups, and no significant differences were observed between these groups ($P > 0.05$). Previous studies had shown that ranitidine could accelerate the GER in stress conditions[41,42], and our study echoed this conclusion. In addition, we were intrigued by the greater improvement in GER for the XP group because a statistically significant difference was detected immediately after stress induction (46.84 ± 2.70 *vs* 61.16 ± 5.12, respectively; $P < 0.05$), at 3 h (60.96 ± 6.71 *vs* 73.41 ± 6.16, respectively; $P < 0.05$) and at 6 h (77.47 ± 3.17 *vs* 91.31 ± 4.34, respectively; $P < 0.05$) compared to the RP group. This difference was sustained

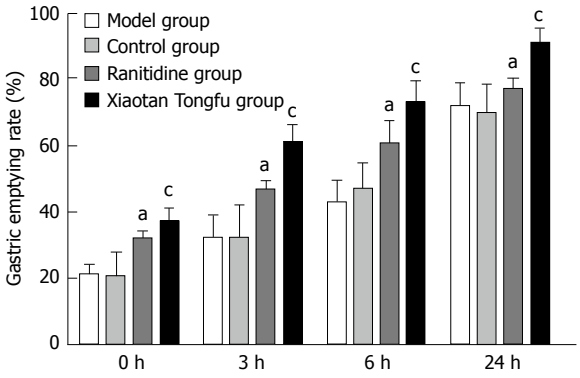


Figure 3 Results of the gastric emptying rate (n = 6, 2 rats were used as controls for each group). At 0 h, the Xiaotan Tongfu granule group (XP group) was superior in elevating the gastric emptying rate (GER); however, no significant difference was detected between the ranitidine group (RP group) and the XP group at this point ($P > 0.05$). At 3, 6 and 24 h after stress induction, the GER in the XP group was sustained at a high value and was significantly different compared to the RP group ($P < 0.05$). $^{a}P < 0.05$ *vs* the model group; $^{c}P < 0.05$ *vs* the control group.

at 24 h after stress induction ($P < 0.05$ compared to all of the groups).

XTTF granule and ranitidine inhibited local inflammation

Tissue MPO levels were correlated with the neutrophil levels and served as a marker of neutrophil infiltration[43]. MIF, a 12.5-kDa cytokine, has increasingly been recognized for its proinflammatory properties in the inflammatory process in SU[43,44]. In our study, as shown in Figure 4, the variation of local inflammation (MPO and MIF) resembled the gastric pH. No significant differences were observed between the MP and CP groups, but comparisons between the RP and XP groups revealed statistically significant differences at 3 h (18.94 ± 1.20 *vs* 13.51 ± 0.89, respectively; $P < 0.05$) and 6 h (13.22 ± 1.54 *vs* 8.83 ± 0.65, respectively; $P < 0.05$) after stress induction.

XTTF granule and ranitidine promoted the expression of HSP70

Numerous studies have suggested that HSP70 could provide protection against gastric ulcers *via* multiple mechanisms[45]. As shown in Figure 5, there was a measurable expression of HSP70 3 h after stress induction, and this expression peaked at 6 h. No significant differences regarding HSP70 expression were observed between the MP and CP groups at any of the time points, but HSP70 expression was significantly higher in the RP and XP groups at 3 and 6 h compared to the MP and CP groups ($P < 0.05$). In addition, comparison of the RP and XP groups also yielded statistically significant differences at 3 h (133.33 ± 35.53 *vs* 176.17 ± 9.37, respectively; $P < 0.05$) and 6 h (182.83 ± 38.78 *vs* 226.50 ± 18.84, respectively; $P < 0.05$) after stress induction.

XTTF granule and ranitidine promote cell proliferation and inhibit gastric mucosal cell apoptosis

As shown in Figure 6, cell proliferation varied in a time-dependent manner and increased gradually after stress induction. No significant differences were observed between the MP and CP groups ($P > 0.05$). In contrast with these groups, the expression of PCNA was higher in the RP and XP groups 3 h after stress induction ($P < 0.05$), with small but significant differences observed at all of the time points except 0 h (37.50 ± 10.91 *vs* 40.83 ± 1.56, respectively; $P > 0.05$) between these two groups. Peak apoptotic activity was observed at 3 h and returned to normal levels over time, as shown in Figure 6. There were no significant differences regarding apoptotic cells between the MP and CP groups, but the apoptotic activity in the RP and XP groups was significantly different from that in the MP and CP groups ($P < 0.05$). Treatment in the XP group led to a higher inhibition of cell apoptosis than in the RP group at 3 h (232.58 ± 24.51 *vs* 174.46 ± 10.35, respectively; $P < 0.05$) and 6 h (164.74 ± 18.31 *vs* 117.71 ± 12.08, respectively; $P < 0.05$), but 24 h after stress induction, no significant differences could be detected between either of the groups.

DISCUSSION

In the present study, the antiulcer effect of the Xiaotan Tongfu granule was established, and its efficacy was demonstrated to be similar to that of ranitidine. The cold-restraint stress model induced a series of pathological alterations and lesions in the stomach, which, when examined together with previous studies, suggested that SU is a process that results from multiple sources[46,47]. We concluded that although the XTTF granule was inferior to ranitidine in reducing gastric acid secretion immediately after stress induction, this did not impair its efficacy because the XTTF granule was superior in promoting a series of parameters, including inhibited local inflammation, increased GER, enhanced HSP70 expression, decreased cell apoptosis and elevated cell proliferation over time. The majority of these parameters have been demonstrated to contribute to ulcer prevention and healing[45], which was confirmed by our observations of gastric lesions measured at the designated time points. Based on these results, we speculate that any agents that can interfere with the above parameters either individually or collectively would be useful to ameliorate any complications due to stomach ulcers.

The underlying mechanism of SU was previously not thoroughly understood and was commonly believed to depend on the balance between known aggressive factors and mucosal defense mechanisms[47]. Previous studies indicated that some components in our decoction, for example, the *Magnoliae Officinalis Cortex*, *Coptidis Rhizoma* and *Glycyrrhizae Radix Et Rhizoma*, were effective in inhibiting gastric acid secretion by a potential mechanism of regulating the activity of various postsynaptic gastric receptors such as histamine H2[48,49]. It was interesting that the XTTF granule was less efficacious in reducing gastric acid secretion immediately after stress induction and resulted in more serious gastric lesions compared to ra-

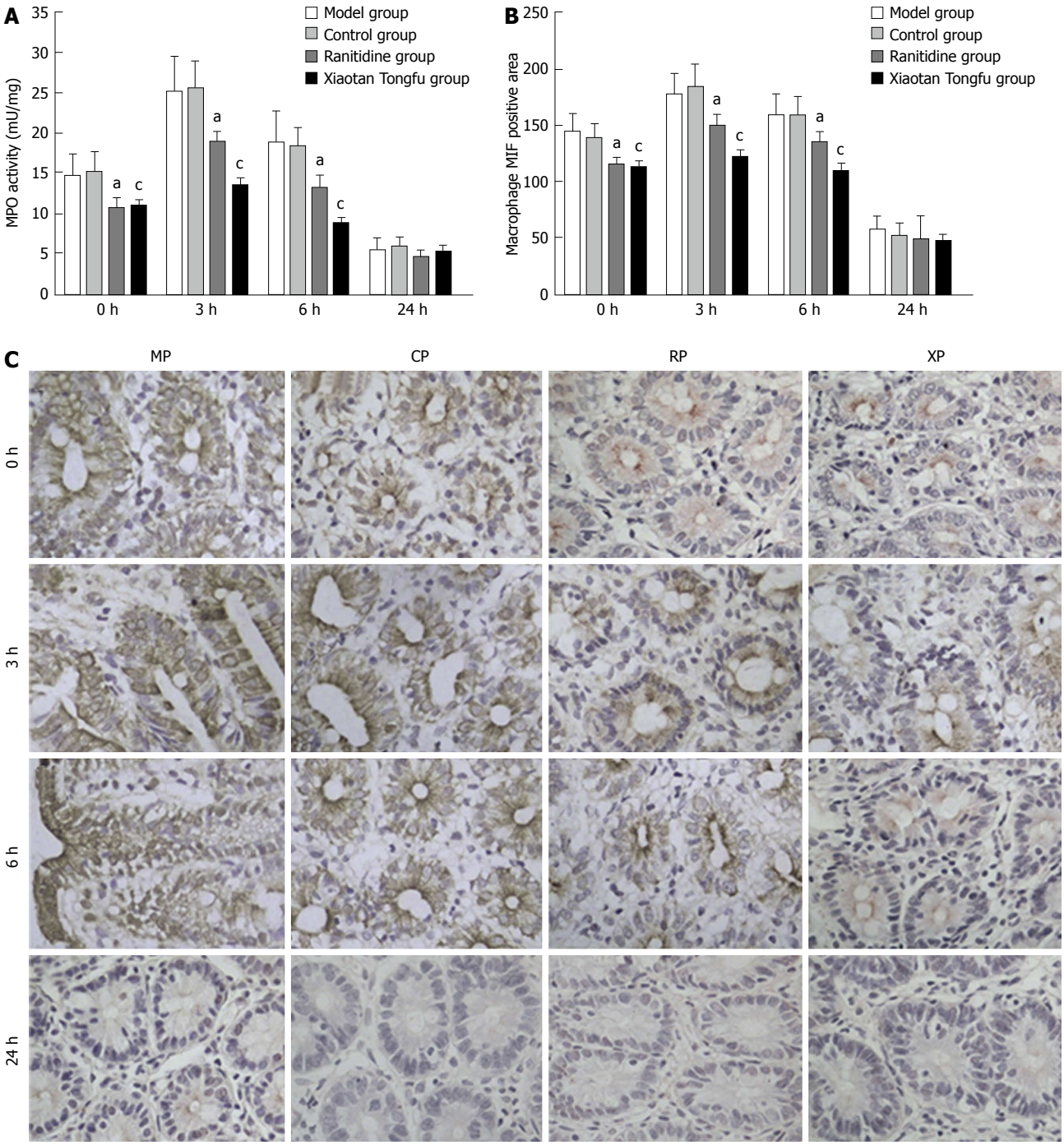


Figure 4 Measurement of myeloperoxidase activity and macrophage migration inhibitory factor (*n* = 6 for each group). A, B: The variation of myeloperoxidase activity (MPO) activity and migration inhibitory factor (MIF) was similar. At 0 h, no significant difference was detected between the ranitidine group (RP group) and the Xiaotan Tongfu granule group (XP group) ($P > 0.05$). However, 3 and 6 h after stress induction, these two indexes were inhibited in the XP group, which was a statistically significant difference compared to the RP group ($P < 0.05$). $^{a}P < 0.05$ *vs* the model group (MP group); $^{c}P < 0.05$ *vs* the control group (CP group); C: The immunohistochemical staining results of MIF show that it was expressed in the cytoplasm of gastric epithelial cells and lamina propria cells. Original magnification × 400.

nitidine. These results could be regarded as a footnote in that gastric acid is one of the most important factors in the formation of SU[50]. However, it should also be noted that not all clinically observed gastrointestinal bleeding can be prevented by manipulating the gastric pH[51]. The XTTF granule was shown to significantly promote GER, echoing the results in our previous study (unpublished observations) that concluded that the XTTF granule could enhance plasma motilin levels, which is important in gastric movement[52,53] in critically ill patients. Additionally, the Xiao-ban-xia decoction, which is an important component of the XTTF granule, has been demonstrated to be a regulative mediator of gastric motility[20]. An enhanced gastric emptying rate could remove acidic material and other irritants in the stomach[54], which is beneficial for ulcer prevention. Additionally, it was notable that

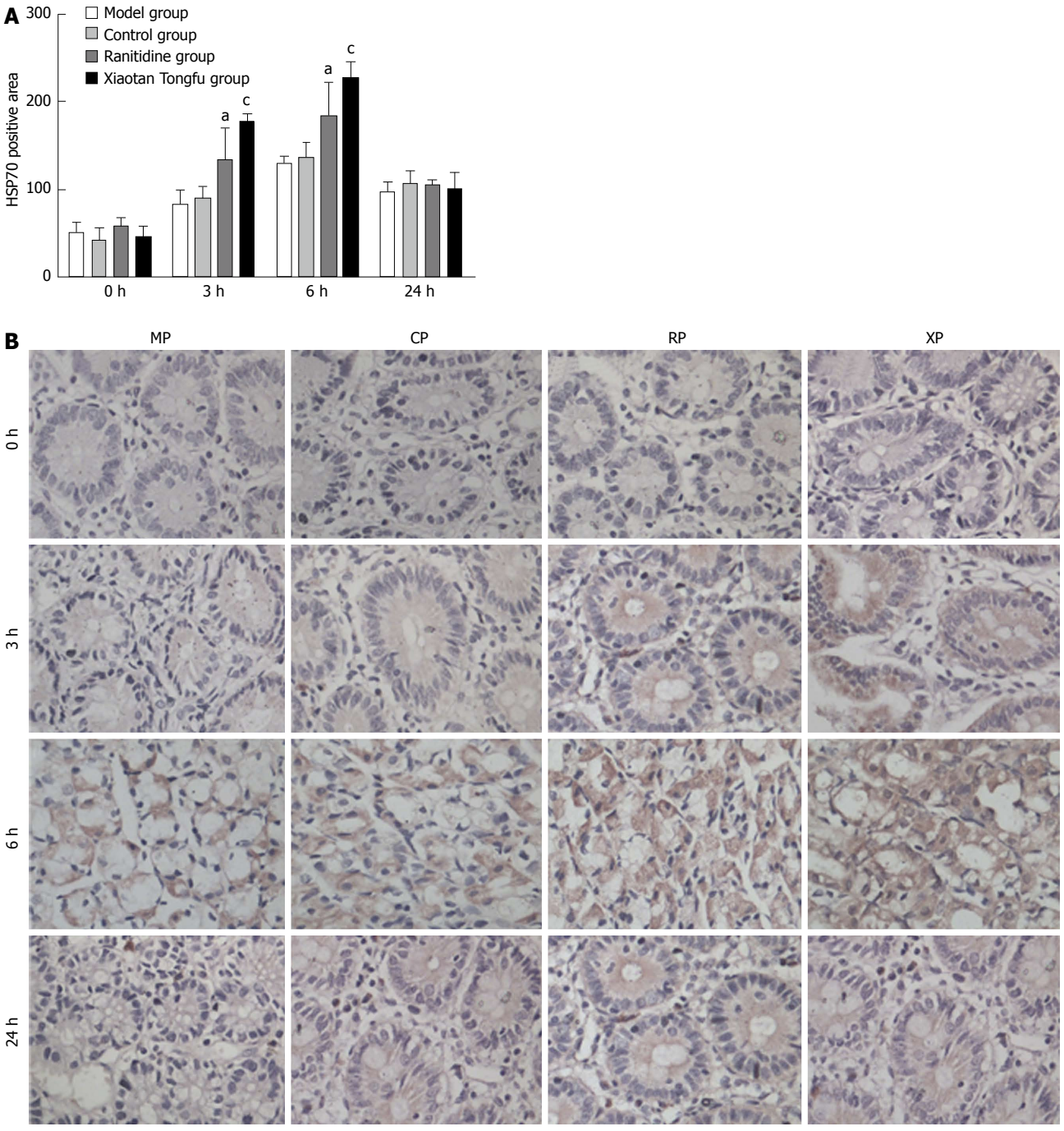
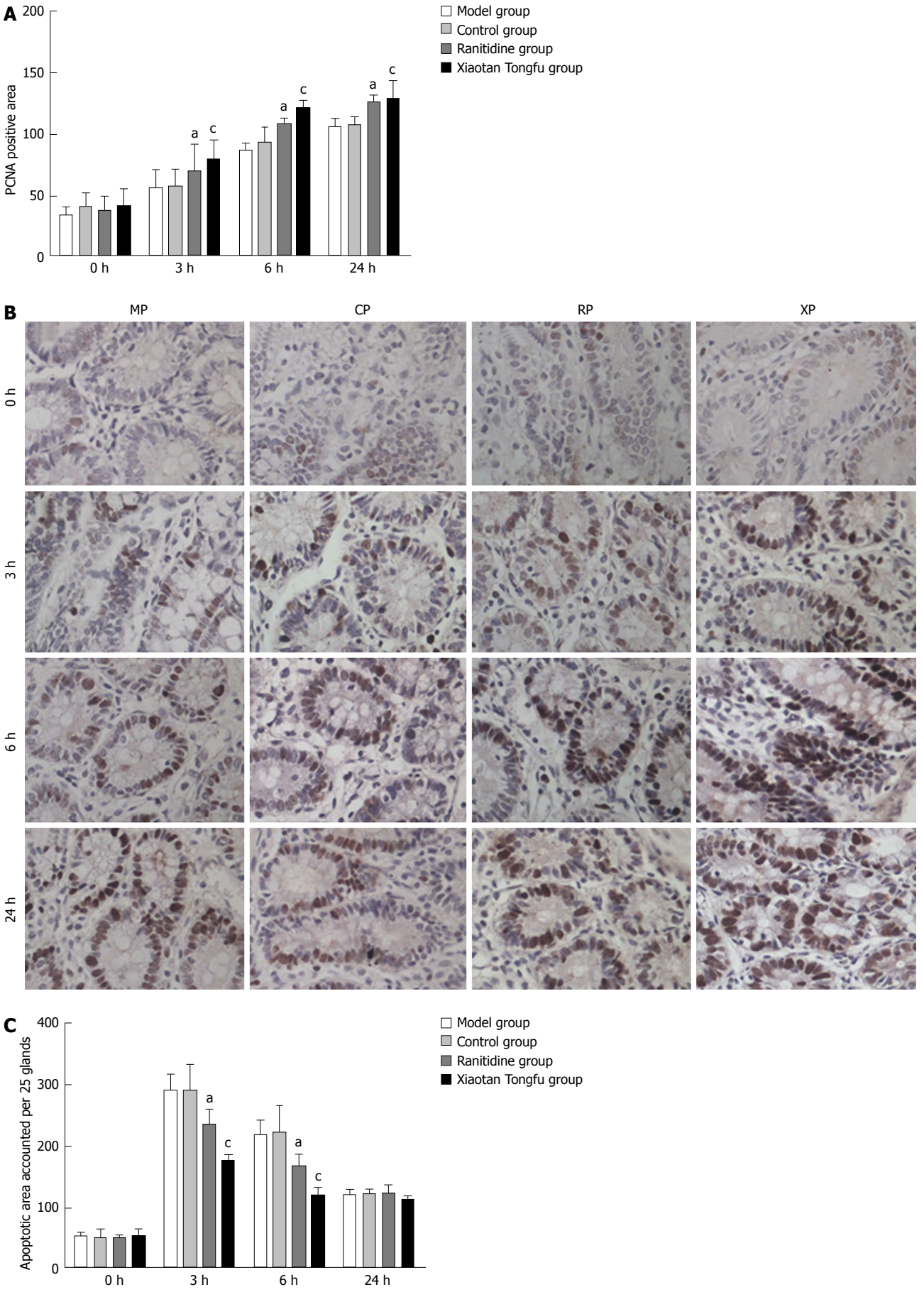


Figure 5 Immunohistochemical staining results for heat shock protein 70 (*n* = 6 for each group). A: There was a statistically significant difference in the protein expression levels between the ranitidine group (RP group) and the Xiaotan Tongfu granule group (XP group) groups at 3 and 6 h at the exact site of initial ulceration ($P < 0.05$). $^{a}P < 0.05$ *vs* the model group (MP group), $^{c}P < 0.05$ *vs* the control group (CP group); B: Strong heat shock protein 70 (HSP70) immunoreactivity was observed in the gastric surface epithelium primarily in the nuclei, but protein was also observed in the cytoplasm. Original magnification × 400.

some traditional Chinese herbal medicines were effective in preventing inflammation by various mechanisms, such as the inhibition of nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF-α), and interleukin-17[55,56]. Interestingly, in our study, the XTTF granule was able to alleviate local inflammation by decreasing MPO activity and restraining MIF expression. It is well known that neutrophil adherence within the gastric microcirculation and migration into the gastric tissue are major causes of gastric ulcers[54]. MIF has been suggested to play a pivotal role in this process, and anti-MIF treatment could have therapeutic value in SU[57]. Although the data indicating that the XTTF granule could inhibit MIF are limited, *Rhei Radix Et Rhizoma* (a main herb in the Xiao-cheng-qi decoction[19]) was previously shown to inhibit gastrointestinal inflammation by acting on TNF-α[24], a strong inducer of MIF secretion[58]. Furthermore, XTTF granule can also improve local microcirculation[22], thereby reducing neutrophil concentrations[59]. Other components, such as *Coptidis Rhizoma*, could ameliorate acute inflammation by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c
Model group
Control group
Ranitidine group
Xiaotan Tongfu group

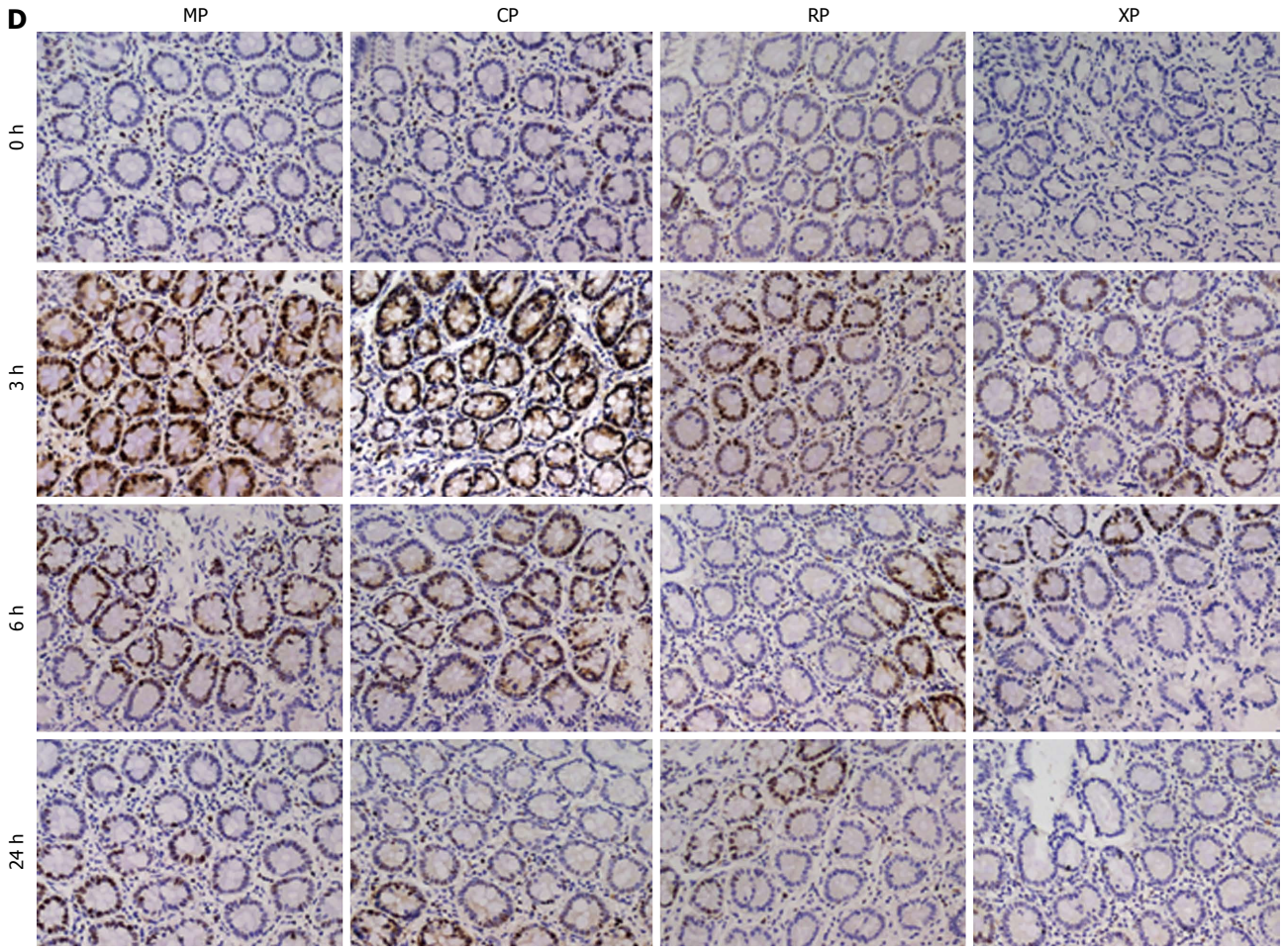

Figure 6 Measurement of cell proliferation and mucosal cell apoptosis (*n* = 6 for each group). A: The cell proliferation was significantly different between the ranitidine (RP) and Xiaotan Tongfu granule (XP) groups ($P < 0.05$) at 3 and 6 h; B: Proliferating cell nuclear antigen (PCNA) immunoreactivity was observed in the gastric surface epithelium, and this staining was focused in the nucleus; C, D: Strongly apoptotic cells were observed in the nucleus of the gastric surface epithelium. Similar to the cell proliferation, the cell apoptosis was significantly different between the RP and XP groups at 3 and 6 h ($P < 0.05$). Original magnification × 400. $^{a}P < 0.05$ *vs* the model group (MP group); $^{c}P < 0.05$ *vs* the control group (CP group).

inhibiting NF-κB-mediated nitric oxide and pro-inflammatory cytokine production[60]. Except these effects, the XTTF granule has also been shown to play a role in the manipulation of HSP70 expression, and although the data are still limited, the previous study did indicate that some herbal medicine constituents, such as *Glycyrrhizae Radix Et Rhizoma* (an herbal component in the Xiaocheng-qi decoction[19]), could promote HSP expression[61]. Interestingly, *Glycyrrhizae Radix Et Rhizoma* was also demonstrated to be effective in protecting gastric mucosa *via* gastric mucin[62]. Finally, the XTTF granule also inhibited cell apoptosis and promoted cell proliferation, which is related to the mucosal protection of some components such as *Magnoliae Officinalis Cortex*[62] and *Aurantii Fructus Immaturus*[63]. We speculate that all of these actions may contribute to tissue regeneration and reconstruction in the stomach[64].

It should be noted that the parameters manipulated by the XTTF granule in SU might not work individually, and these parameters could be connected in a complex relationship. For example, previous studies have shown that the aforementioned MIF inhibition effect of the XTTF granule could result in the elevation of nitric oxide levels[57], which are involved in HSP70 expression[65] and cell proliferation[66] during ulcer healing in the stomach. HSP70 could also exert its cytoprotective effect by interfering with the stress-induced apoptotic pathway[67,68]. Except that studies have indicated that ranitidine can inhibit gastric acid secretion[12], accelerate GER[33,42], and reduce apoptosis levels[69], our study showed that the effect of ranitidine on parameters such as promoting cell proliferation may also be attributed to the comprehensive network of SU. The XTTF granule was shown to prevent ulcers and promote healing by attenuating aggressive factors and enhancing defensive factors. Future studies, such as randomized controlled trials, are necessary to further confirm its efficacy.

This study has several limitations. First, although we demonstrated that the XTTF granule exerts measurable preventative effects on SU, whether the XTTF granule acts in a dose-dependent manner remains unknown. Second, pretreatment with the XTTF granule in rats scheduled to undergo stress may not correspond to clinical practice because the majority of patients are administered

pharmacological agents for SUP after stress. Additional studies are necessary to measure the efficacy of the XTTF granule in this scenario.

COMMENTS

Background

Stress ulcer prophylaxis plays a pivotal role in the care of critically ill patients. Recent studies indicated that traditional Chinese medicine (TCM) could exert measurable therapeutic effects on gastric ulcers. The Xiaotan Tongfu (XTTF) granule has been used for a long time to treat gastrointestinal disorders in critically ill patients. However, whether it could be applied to stress ulcers remained unknown.

Research frontiers

Emerging evidence suggests that TCM was effective in the management of stress-related gastrointestinal disorders, such as irritable bowel syndrome. In addition, a number of studies have also indicated that TCM could exert measurable therapeutic effects on gastric ulcers in rats. Stress ulceration was an inevitable complication of the gastrointestinal tract in animals experiencing abnormally high physiological stress. In this study, the authors demonstrated that a traditional Chinese herbal decoction could play an important role in the prevention of stress ulcers.

Innovations and breakthroughs

Although the underlying mechanism of stress ulcers was commonly believed to depend on the balance between known aggressive factors and mucosal defense mechanisms, most of the clinical strategies are still aimed at inhibiting gastric acid. This study focused on demonstrating the efficacy of a traditional Chinese herbal medicine used to treat stress ulcers. The study indicated that traditional Chinese herbal medicine was effective in preventing stress ulcers and that inhibiting gastric acid would not be the only strategy.

Applications

By confirming its efficacy and potential mechanisms in an animal study, the findings suggest that the XTTF granule could be regarded as a potential option for stress ulcer prophylaxis in the future.

Terminology

Stress ulceration refers to an inevitable complication of the gastrointestinal tract in people experiencing abnormally high physiological stress, which usually leads to gastrointestinal bleeding. The underlying mechanism of stress ulcers was commonly believed to depend on the balance between known aggressive factors and mucosal defense mechanisms. Therefore, any agents that can interfere with the above factors, either individually or collectively, could be used as a stress ulcer prophylaxis.

Peer review

The authors examined the efficacy and potential mechanisms of the XTTF granule in stress ulcers. This study revealed that the XTTF granule was similar to ranitidine treatment with regard to reducing gastric lesions in a cold-restraint stress model, and the underlying mechanisms may include acceleration of the gastric emptying rate, inhibition of local inflammation, promotion of cell proliferation and suppression of apoptosis. The results are interesting and may represent a potential option in the management of stress ulcers in the future.

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