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Effect of a Tibetan Medicine Rinqingchangjue on Gastric Hypomotility in Rats

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ABSTRACT

This basic research aimed to investigate the effects and mechanisms of Rinqingchangjue (RC) on gastric hypomotility in SD rats. A rat gastric hypomotility model was established by administrating rats with licorice via oral gavage. Then, rats were divided into five groups: Control group (no treatment), Positive group (cisapride 0.4 mg/mL), Low RC group (0.1 g/kg RC), Medium RC group (0.2 g/kg RC), and High RC group (0.4 g/kg RC). Changes in various indicators were measured to explore the mechanism of action of RC. After treatment with RC extract, the gastric residual rate and small intestinal propulsion rate in rats with gastric hypomotility were significantly improved. The serum stem cell factor (SCF) levels were significantly increased in the medium RC and High RC groups, indicating the promotive effects of RC. The protein expression of serotonin 5-hydroxytryptamine (5-HT) in the gastric antrum was slightly increased in the low RC and medium RC groups, while that in the positive group and high RC group was significantly increased. High dose RC treatment regulated the function of the digestive system in rats with gastric hypomotility. The expression of stem cell factor receptor (SCFR) in the low RC group was comparable to that of the control group, while RC at a dose of 0.2 g/kg and 0.4 g/kg significantly upregulated the expression of SCFR. The high RC group also showed slightly higher RC expression compared to the positive group (gastric fundus, p=0.657; gastric body; p=0.172). To conclude the RC extract significantly decreased the gastric residual rate in rats with gastric hypomotility and promoted the expression of 5-HT and SCFR in the gastric body, gastric fundus, and gastric antrum. These findings suggest that this Tibetan medicine may be used to enhance gastric motility.

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Authors' Contribution
NZ conductd literature review, performed data detection, conducted statistical analysis, and drafted the manuscript. HX, NZ, HR, D Dongzhi and D Drolma designed experiments. HX and NZ executed experiments. HR, D Dongzhi and D Drolma selected methodologies and execution. YP, PZ, and PY contributed to the statistical analysis and the creation of graphics. HX wrote and revised the manuscript, and provided technical support.

Key words
Rinqingchangjue, Gastric hypomotility, Gastric residual rate, Small intestinal propulsion rate, Stem cell factor, Stem cell factor receptor, 5-hydroxytryptamine

INTRODUCTION

Gastric hypomotility (GH) is defined as food retention caused by esophagogastroduodenoscopy (EGD) and is attributed to damage to the myenteric plexus surrounding the distal esophagus (Bi *et al*., 2021; Kaneshiro *et al*., 2018). It is also known as gastrointestinal dysmotility and is characterized by delayed gastric emptying and decreased small intestinal propulsion. The overall incidence of GH is 1.9% (Oikawa *et al*., 2020). Diabetes is reported as the most common cause of GH (29%); however, idiopathic GH, where the cause is unknown, is also prevalent (36%) (Soykan *et al*., 1998). Although clinical manifestations of GH are usually not severe and sometimes asymptomatic, they may lead to gastroesophageal reflux, gastroparesis, functional dyspepsia, biliary reflux gastritis, and habitual constipation, among other conditions (Yang *et al*., 2019).

With the development and intensification of competition, people experience increased stress and mental tension, which can enhance the excitability of the vagus nerve and contribute to the rising incidence of gastrointestinal motility disorders. However, the long-term use of Western medicine often leads to recurrent symptoms after discontinuation, and the induced adverse reactions limit its clinical efficacy. Traditional medicine, with fewer toxic side effects, aligns better with the prolonged and recurrent nature of GH and has gained renewed attention (Mao *et al*., 2021). Previous studies have suggested that the modified Banxia Houpu Decoction can improve gastrointestinal and pulmonary function in GH (Wei and Zhang, 2008). Additionally, Xiangsha Pingwei Decoction can be used as a therapeutic approach for GH (Lu an

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Huang, 2020).

Rinqingchangjue (RC) is a widely used Tibetan medicine for treating various diseases. It is characterized by the combination of multiple rare Tibetan herbs and contains various trace metal elements (Fang and Losang, 2012). The main components of RC include cinnabar, chebulae fructus, agarwood, sandalwood, musk, pearl, melia toosendan, saffron, and more than 140 other traditional Chinese medicine ingredients. It has been clinically used for treating various gastrointestinal inflammations (Chen *et al*., 2013). However, there are limited studies on its efficacy in treating gastric hypomotility. Therefore, in the present study, we aimd to investigate the effects and potential mechanisms of RC on gastric hypomotility in rats.

MATERIALS AND METHODS

Materials

Thirty SPF-grade male SD rats, aged 7-8 weeks, were purchased from Shanghai Kaixue Biological Technology Co., Ltd. RC was produced by Tibet Ganlu Tibetan Medicine Co., Ltd. (catalog No. C14003044416). Raw licorice was purchased from Anhui Jingquan Group Traditional Chinese Medicine Slice Co., Ltd. (catalog No. 20090301). Cisapride was produced by Shandong Qikang Pharmaceutical Co., Ltd. (National Drug Approval No. H20050853). The SCF ELISA kit was purchased from Beyotime. The serotonin (5-HT) APUD cell kit was obtained from Shanghai Jining Industrial Co., Ltd. The SCRF antibody was purchased from Abcam. Phenol red, xylene, ethanol of different concentrations, PBS, 0.3% H_2O_2, methanol, and other reagents were purchased from Solarbio Life Sciences Co., Ltd. According to previous studies, RC was extracted using the petroleum ether mehtod. The extraction process was as follows: RC fine powder was extracted three times with petroleum ether at a volume-to-weight ratio of 6:1, each time for 1 hour, and the petroleum ether was recovered under reduced pressure at 40–50 °C to obtain the petroleum ether extract of RC.

Establishment of gastric hypomotility model

The gastric hypomotility model was established based on the study by Ye *et al*. (2018). Rats were randomly divided into 5 groups: Control group, positive group, low RC group, medium RC group, and high RC group. The gastric hypomotility model was established by administering SD rats with prepared licorice decoction at a dosage of 10 g/kg via oral gavage for 5 consecutive days. The control group received 0.9% saline solution + 10 g/kg licorice decoction. The positive group received 10 g/kg licorice decoction + 0.4 mg/mL cisapride. The low (0.1 g/kg), medium (0.2 g/kg), and high (0.4 g/kg) RC groups received different concentrations of RC extract + 10 g/kg licorice decoction for 5 consecutive days (Rezeng *et al*., 2016).

Measurement of gastric residual rate

After the 5th administration, rats were fasted for 12 h but allowed access to water. Then, 0.002 g/mL phenol red solution was administered by gavage to determine the gastric residual rate. After 20 min of gavage, the rats were euthanized, and the stomachs were removed. The 0.002 g/mL phenol red solution was added to a 0.1 mol/L NaOH solution at a ratio of 1:10. The resulting solution was then diluted in water at a ratio of 1:5, and the absorbance at 560 nm was measured and used as the standard solution. The stomachs were immersed in a 0.1 mol/L NaOH solution, thoroughly rinsed, and the supernatant was collected. The supernatant was diluted in water at the same ratio, and the absorbance at 560 nm was measured as the test solution. The gastric residual rate (%) was calculated as follows: Gastric residual rate (%) = Test solution OD value/ Standard solution OD value × 100%.

Measurement of gastric motility

The solution containing 2 mL with an equal amount of 0.002 g/mL phenol red was administered by gavage. After 20 min, rats were euthanized by cervical dislocation. The abdomen was opened, and the pylorus and duodenum were ligated. The small intestine was then removed. The length of phenol red staining in the small intestine was measured to determine the small intestinal propulsion rate: Small intestinal propulsion rate (%) = Distance of phenol red progression/ Total length of the small intestine (cm) × 100%. The distance of phenol red progression refers to the distance from the leading edge of phenol red to the pylorus. The total length of the small intestine refers to the portion from the pylorus to the distal ileocecal valve, and its length was measured.

Measurement of serum SCF levels

The serum samples of each rat were processed according to the instructions of the ELISA kit. The absorbance values were measured at 492 nm wavelength using a plate reader, and the corresponding concentrations were determined based on the standard curve.

Immunohistochemical detection of SCFR and 5-HT expression

Paraffin-embedded gastric tissue sections were prepared and processed. The sections were deparaffinized, rehydrated, washed with distilled water and PBS, subjected to sodium citrate antigen retrieval, and underwent H_2O_2 and

serum blocking. Then, the sections were then incubated with primary antibodies against 5-HT (Abcam, 1:1000) and SCFR (Abcam, 1:4000) overnight. Then, biotinylated and HPR-labeled antibodies were applied using the SP-9001 Rabbit SP kit (Zhongshan Golden Bridge) for 2 h. Diaminobenzidine (DAB) solution was used for color development. The sections were counterstained, dehydrated, cleared, mounted, and observed and photographed under a microscope. ImageJ Pro software was used for relative expression analysis.

Statistical analysis

Data analysis was performed using SPSS 18.0 statistical software. Experimental data are presented as mean±standard deviation. Group comparisons were conducted using one-way ANOVA analysis, and a *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Gastric residual rate and small intestinal propulsion rate

Gastric residual rate is one of the indicators used to assess gastrointestinal motility disorders. As shown in Table I, the results of gastric residual rate (%) indicated that the control group had gastric hypomotility, with a residual rate of 72.89%, indicating inhibited gastric emptying. The positive group (treated with cisapride) showed a reduced gastric residual rate of 49.60%, suggesting significantly promoted gastric emptying compared to the control group ($p<0.01$). Low RC had no significant effect on the gastric residual rate ($p>0.05$). Medium RC decreased the gastric residual rate, but the difference was not significant compared to the control group ($p>0.05$). However, high RC demonstrated a significant effect on gastric residual rate compared to the control group ($p<0.01$).

The results of small intestinal propulsion rate, as shown in Table I, indicated that the cisapride treatment effectively enhanced the inhibitory effect on gastric motility compared to the control group ($p<0.01$). The low and medium RC groups showed increased small intestinal propulsion rate, but the difference was not significant compared to the control group ($p>0.05$). The positive group and the high RC group exhibited a significant increase in small intestinal propulsion rate, with a significant difference ($p<0.05$). Therefore, high dose RC was effective in alleviating gastric hypomotility in rats.

Serum SCF

Serum SCF levels were measured using ELISA, and the results are shown in Figure 1. Compared to the control group, the positive group (cisapride treatment) showed increased levels of SCF in the serum, with a significant difference ($p<0.01$). The low RC group exhibited increased SCF levels, but the difference was not significant ($p>0.05$). The medium and high RC groups demonstrated a significant increase in the serum SCF levels, indicating a strong promoting effect ($p<0.01$).

Table I. Gastric residual rate and small intestine propulsion rate in each group.

	Gastric residual rate (%)	Small intestine propulsion rate (%)
Control	72.89±5.07	60.82±0.55
Positive	49.60±3.23b**	72.11±1.26**
Low RC	73.08±2.95	60.38±0.87
Medium RC	62.95±4.65	62.98±1.28
High RC	56.56±4.43*	66.64±2.90*

Compare to control group, *$p<0.05$, **$p<0.01$.

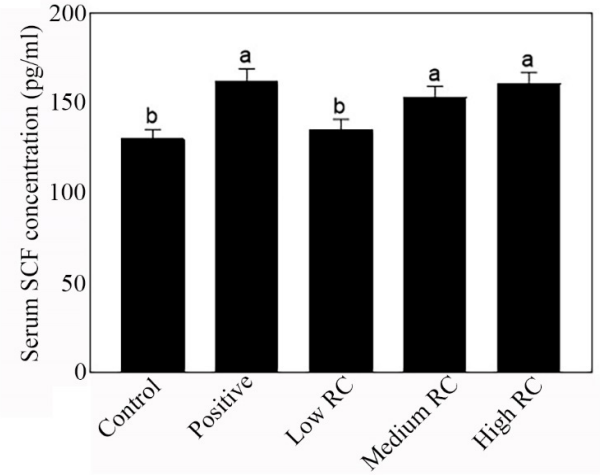

Fig. 1. Effect of RC on serum SCF levels in rats.

Gastric antrum 5-HT protein

Immunohistochemical detection of 5-HT protein in the gastric fundus tissue is shown in Figures 2 and 3. Compared to the control group, the low and medium RC groups showed an increased expression of 5-HT protein. The difference was significant in both the low ($p<0.05$) and medium ($p<0.01$) RC groups. The positive group (cisapride treatment) and the high RC group exhibited a significant increase in 5-HT protein expression in the gastric antrum, with a highly significant difference ($p<0.01$). In addition, the 5-HT expression in the gastric body tissue was measured. The expression of 5-HT in the control group was low. Compared to the control group, the positive group (cisapride treatment) showed a significant increase in 5-HT levels ($p<0.01$). The low RC group had

a significant difference compared to the control group (p<0.05). Medium and high doses of RC had a significant promoting effect on 5-HT release (p<0.01). The high RC group showed a significant increase in the 5-HT expression in the gastric body (p<0.01). The results of 5-HT protein expression in the gastric antrum, as shown in Figure 2, indicated that the low and medium RC groups exhibited an increased expression of 5-HT protein, but the difference was not significant (p>0.05). However, the positive group (cisapride treatment) and the high RC group showed a significant increase in 5-HT protein expression in the gastric antrum, with a highly significance (p<0.01). Thus, high dose RC was effective in regulating the function of the digestive system in rats with gastric hypomotility.

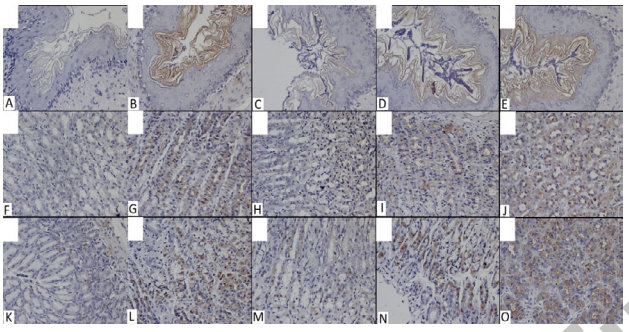

Fig. 2. Effect of RC on 5-HT protein expression in rat gastric tissues: A, B, C, D and E show the stomach bottom of the Control, Positive, Low RC, Medium RC, and High RC group, respectively. F, G, H, I and J show the gastric body of the Control, Positive, Low RC, Medium RC, and High RC group, respectively. K, L, M, N and O show the gastric antrum of the Control, Positive, Low RC, Medium RC, and High RC group, respectively.

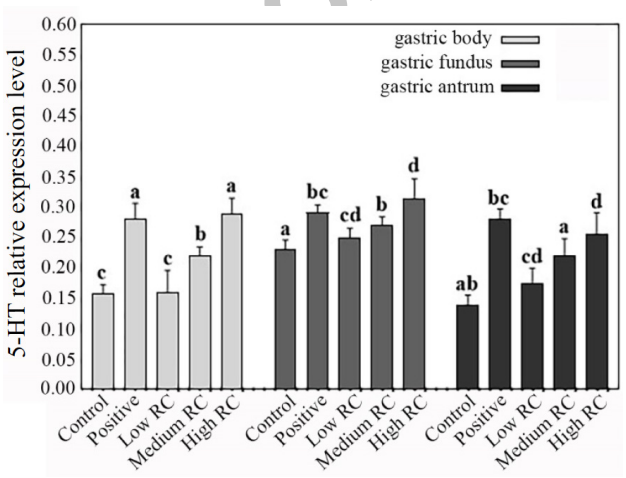

Fig. 3. Effect of RC on 5-HT protein expression in rat stomach.

Gastric antrum SCFR protein

Figures 4 and 5 show the immunohistochemical detection of SCFR protein expression in the gastric antrum of rats. Compared to the model rats, the low RC group showed an increased expression of SCFR protein in the gastric antrum, but the difference was not significant (p>0.05). However, the positive group, as well as the medium and high RC groups exhibited a significant increase in SCFR protein (p<0.01). We further examined the expression of SCFR in the gastric fundus and gastric body to verify whether RC regulated the function of the gastrointestinal smooth muscle by regulating the content of SCFR. In the control group, a lower level of SCFR was observed around the myenteric plexus, while the positive group showed a significant increase in the abundance of brownish-yellow stianing of SCFR. The expression of SCFR in the low RC group was similar to that of the control group, while the medium and high doses of RC significantly promoted the expression of SCFR. The high RC group showed slightly higher expression of SCFR compared to the positive group, with p-values of 0.657 (gastric fundus) and 0.172 (gastric body), respectively.

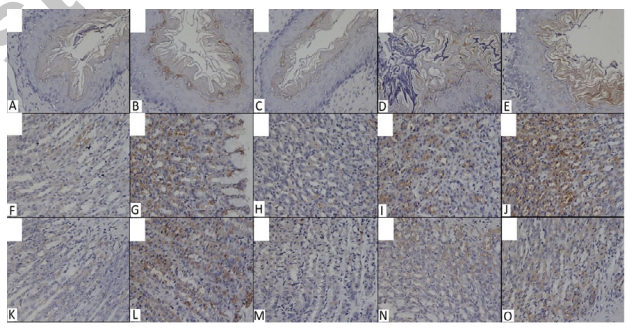

Fig. 4. Effect of RC on SCFR protein expression in rat gastric tissues: A, B, C, D and E show the stomach bottom of the Control, Positive, Low RC, Medium RC, and High RC group, respectively. F, G, H, I and J show the gastric body of the Control, Positive, Low RC, Medium RC, and High RC group, respectively. K, L, M, N and O show the gastric antrum of the Control, Positive, Low RC, Medium RC, and High RC group, respectively.

DISCUSSION

RC, as a rare compound Tibetan medicine, primarily consists of pearl, cinnabar, sandalwood, borneol, agarwood, fruits of Terminalia chebula, calculus bovis, artificial musk, and saffron. It has been recorded in the “Four Medical Classics” for its effects in promoting physical health and enhancing immune function. It has shown unique therapeutic effects and clinical safety evaluations in the treatment and intervention of gastrointestinal

inflammation, gastric ulcers, cervical cancer, and asthma (Li *et al*., 2022; Chen and Zhou, 2020; Yang *et al*., 2019; Wang, 2020).

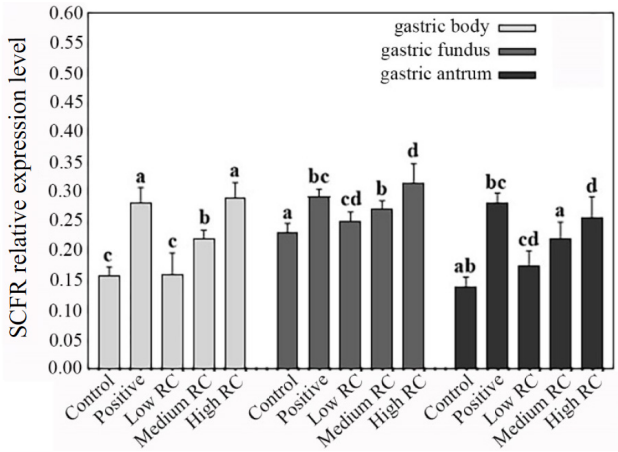

Fig. 5. Effect of RC on SCFR protein expression in rat stomach.

Previous studies have suggested that upregulation of the SCF/SCFR signaling pathway is one of the mechanisms involved in improving gastrointestinal motility (Song *et al*., 2023; Han, 2020). The upregulation of calcium channel proteins IP3R, RyR, and PLC in the Cajal interstitial cells (ICCs) have been proposed to mediate the increased expression of SCFR (Han, 2020). The 5-HT in the myenteric plexus is a factor that regulates gastrointestinal smooth muscle movement, and alterations in 5-HT content reflect changes in the number of 5-HT APUD cells (Tao *et al*., 2017). A decrease in the number of 5-HT APUD cells leads to reduced 5-HT secretion and weakened gastric smooth muscle function. In this study, we found that RC significantly increased the expression of SCFR and 5-HT in the gastric fundus and gastric body of rats with gastric hypomotility, leading to improved gastric emptying capacity. Therefore, we preliminarily propose that RC can alleviate symptoms of gastric hypomotility by upregulating the levels of SCFR and 5-HT, thereby enhancing smooth muscle contractility.

DGIM often occurs in combination with other diseases, such as DGIM combined with exacerbation of pulmonary heart disease (Lu *et al*., 2021), and DGIM combined with diabetes (Pop-Busui *et al*., 2017). The treatment of gastrointestinal motility disorders alone is no longer sufficient to meet the needs of current patients. Therefore, the clinical treatment of DGIM presents new challenges, and further research is required.

Endocrine cells are located in the mucosa or submucosa of the entire stomach, intestines, and pancreas. They secrete various hormones, including 5-HT, somatostatin, glucagon-like peptide, and gastrin. In the gastrointestinal tract, 5-HT acts as both a neurotransmitter and an endocrine/paracrine signaling molecule. Cells producing 5-HT are considered to be major chemosensory cells in the gastrointestinal tract (Cheng *et al*., 2020), responsible for over 90% of the synthesis of endogenous 5-HT (Ahlman and Nilsson, 2001). 5-HT mediates various functions in the gastrointestinal tract, including intestinal reflex activity and inflammatory processes (Bellono *et al*., 2017; Spohn *et al*., 2017; Gershon, 2013). Therefore, quantifying the distribution of 5-HT throughout the gastrointestinal tract may provide essential information to enhance the treatment of gastrointestinal diseases. Immunohistochemical results in this study showed that high-dose treatment of RC significantly increased the small intestinal propulsion rate and the expression of 5-HT protein in the gastric antrum of rats ($p<0.01$), indicating that high-dose RC treatment can regulate the function of the digestive system in rats with gastric hypomotility. 5-HT cells are distributed throughout the stomach and intestinal regions, and the 5-HT secreted by enterochromaffin cells plays an important role in controlling gastric motility (Gershon, 2013). Additionally, the various actions of gastrointestinal 5-HT cells have been demonstrated or proposed to affect the stomach (Diwakarla *et al*., 2017; Martin *et al*., 2017).

Patients with long-term diabetes often suffer from gastrointestinal motility disorders, including early satiety, postprandial bloating, abdominal discomfort, and recurrent vomiting. However, the pathogenesis of gastrointestinal motility disorders is still unclear, and effective treatments are limited. In recent years, some studies have indicated that ICCs play a crucial physiological role in coordinating gastric contraction and are an essential aspect of gastric motility (Wang *et al*., 2005; Hirst and Edward, 2006). Furthermore, the loss of ICCs has been observed in the stomachs of diabetic animals and patients and is considered to contribute to the development of motility disorders (Ordög *et al*., 2000; Grover *et al*., 2012). The results of this study showed a decreased expression of ICCs (SCFR protein) in the gastric antrum of rats with gastric hypomotility, while medium and high doses of RC significantly increased the expression of SCFR protein in the gastric antrum. Furthermore, it was found that the serum SCF content significantly increased in rats treated with medium and high doses of RC compared to the model group rats. It is possible that the activation of SCFR protein located on the surface of ICCs through the interaction with SCF is involved, where SCF exists as both a transmembrane protein (M-SCF) and a soluble protein (S-SCF). The SCF/SCFR signaling pathway is known to be essential for maintaining ICC phenotype,

survival, proliferation, and differentiation (Torihashi *et al*., 1999; Tong *et al*., 2010). Previous studies have shown a significant decrease in SCF and SCFR expression in diabetic animals (Horváth *et al*., 2006; Lin *et al*., 2010; Xu *et al*., 2012). Fortunately, some studies have indicated that treatments can increase the expression of SCF and SCFR in rat models (Wang *et al*., 2008; Lu *et al*., 2013). Our immunohistochemical study also provided evidence that RC treatment can regulate the SCF/SCFR pathway and restore the expression of ICCs in the gastric antrum of rats. Additionally, data indicate that the expression of SCF, particularly M-SCF, induces prolonged activation and longer lifespan of SCFR (Xu *et al*., 2012; Miyazawa *et al*., 1995). Chen *et al*. (2013) demonstrated a significant correlation between the expression of S-SCF and M-SCF and SCFR expression (Chen *et al*., 2013). Consistent with these observations, we found that the higher the expression of M-SCF in gastric antrum tissue, the higher the expression of SCFR. Therefore, it is likely that the improvement of gastric motility in rats by Rinqingchangjue is associated with the enhancement of the SCF/SCFR pathway.

CONCLUSION

In conclusion, the results of this study demonstrate that Rinqingchangjue can increase the small intestinal propulsion rate and the expression of 5-HT in rats with gastric hypomotility, possibly through the enhancement of the SCF/SCFR pathway. Rinqingchangjue may have therapeutic potential in the restoration of gastric motility abnormalities. However, this study is a basic experiment and lacks clinical sample data support. There are significant differences between animals and humans, and further research is needed.

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DECLARATIONS

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Ethics approval and consent to participate

The ethic approval was obtained from the Ethic Committee of The Medical School of Xizang University.

Consent for publish

All of the authors have Consented to publish this research.

Availability of data and materials

The data are free access to available upon request.

Statement of conflict of interest

The authors have declared no conflict of interest.

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