



## Original article

## Inhibition of autophagy of Cajal mesenchymal cells by gavage of tong bian decoction based on the rat model of chronic transit constipation

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## ABSTRACT

The objective of this research was to study the effect of tong bian decoction on colon transport function of interstitial cells of Cajal (ICC) in chronic transit constipation (CTC) and the inhibition of autophagy of ICC, so as to achieve the free movement of the bowels. In this research, the experimental rats were divided into normal group (NG) and model group (MG) by random method, and the rat model of CTC was constructed by subdivision circulatory increasing operation gavage method of rhubarb. After the successful establishment of the model, the rats were divided into normal group, MG, tong bian decoction gavage group, mosapride group and normal recovery group. Then, rats in the NG and the MG were killed at the same time, and rats in the tong bian decoction gavage group, mosapride group and normal recovery group were killed at the same time. In this study, the transport function of colon of rats in each group was detected by activated carbon method, and the number of fecal residues in the colon was observed. The mRNA expression of c-kit gene in intestinal tissue of rat was detected by real-time quantitative polymerase chain reaction (RT-qPCR). In addition, the changes of ICC in rats treated with different drugs were detected by immunohistochemical method. The results revealed that in the tong bian decoction gavage group, the water content in the feces of rats was remarkably increased ( $P < 0.05$ ), the amount of residual feces in the colon was remarkably reduced ( $P < 0.01$ ), the percentage of carbon powder propulsion in small intestine was remarkably increased ( $P < 0.01$ ), the staining area of ICC positive cells in colon tissue was remarkably increased ( $P < 0.05$ ), and the expression of c-kit mRNA was remarkably increased ( $P < 0.01$ ). It can be concluded that the tong bian decoction could effectively enhance the colon transport function in the rat model of CTC. This laxative mechanism promotes the regeneration and repair ability of ICC by inhibiting the autophagy of ICC, and provides power for the large intestine, so as to achieve the free movement of the bowels. Therefore, the results of this study have certain guiding meaning for the treatment of CTC with traditional Chinese medicine.

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## 1. Introduction

Chronic constipation is a common clinical symptom, and the causes of chronic constipation can be divided into two types: functional and organic causes (Gallo et al., 2017). Functional etiology

refers to the lack of organic etiology, no structural abnormalities or metabolic disorders, and the exception of irritable bowel syndrome chronic constipation. Organic causes can be caused by gastrointestinal diseases, systemic diseases involving the digestive tract such as diabetes, scleroderma, nervous system diseases, and many drugs can also cause constipation. The main clinical manifestations of the two causes are long defecation cycle, intermittent defecation, dry and hard fecal quality, difficulty in excretion and other symptoms (Yao et al., 2017; Xiao et al., 2017; Li et al., 2019). Where, slow transmit constipation (STC) is the most common (Son et al., 2018; Additionally, 2017). STC is mainly characterized by poor colon peristalsis and a large delay in excretion. The immediate cause of the disease is unknown. Studies have found that with the occurrence of STC, the clinical parameters of factors such as colon ganglion cells and ICC all change in vivo (Jiang

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et al., 2017). A large number of relevant studies have revealed that the causes of STC are closely related to the distribution and form of ICC as well as the ganglion cells in the colon, and it has been found that the reduction of the number of these two types of cells and the changes in cell morphology can lead to the occurrence of STC in patients and the obstruction of gastrointestinal functions. Therefore, the role of ICC in the pathogenesis of STC has attracted more and more attention (Zhang et al., 2018).

Currently, laxatives are mostly used for the treatment of STC constipation, which is harmful to human body. However, Chinese traditional medicine is mild to the body, and the human body has a good tolerance to Chinese traditional medicine (Chen et al., 2017). Therefore, it is of great meaning to study the mechanism of combining traditional Chinese medicine with the treatment of CTC to explore the mechanism of STC. From the perspective of Chinese medicine, the location of constipation is mainly in the large intestine, but it is also closely related to the dysfunction of the lungs, liver, and other organs. For different patients, different pharmacies should be provided for recuperation. For the constipation patients with deficiency of vital energy, tonic drugs should be taken and supplemented by the tea that moistens intestinal tract. For the patient with qi constipation, stagnation drug should be given. For patients with cold constipation, warming laxatives should be given, and for patients with hot constipation, aperient for clearing heat should be given (Shen et al., 2017). Application of Chinese medicine in clinical treatment of constipation is good, the efficiency can reach 90% or more. However, the mechanism of tong bian decoction in treating constipation is not clear (Shen, 2017).

Based on the above conditions, a CTC rat model was constructed to analyze the inhibitory effect of Chinese herbal tong bian decoction on autophagy of ICC. The rat model of CTC was constructed by subdivision circulatory increasing operation gavage method of rhubarb. After the successful establishment of the model, the rats were divided into normal group, MG, tong bian decoction gavage group, mosapride group and normal recovery group. The rats in the experiment were then sacrificed. Finally, the transfer function of the colon was detected by activated carbon method, the number of fecal residue particles in the colon was observed, and the changes of c-kit mRNA gene expression were detected by real-time quantitative PCR method.

## 2. Materials and methods

### 2.1. Modeling of experimental animals

A total of 50 female SD rats of 6 weeks old, with an average body weight of  $210 \pm 5$  g, all purchased from Shanghai Slack Laboratory Animals Co., Ltd. They were kept in the laboratory, where the room temperature (RT) was kept at  $16\text{--}5$  °C and the relative humidity was kept at 40–60%. Feeding was provided by a biological company and the model was started after 7 days. Fifty SD rats were induced into STC model.

Firstly, the rats were raised in cages and the model was built after a week. Model rats were weighed every day, and each group was given laxative gavage according to their weight (rhubarb subdivision). Before each gavage, the temperature of the rhubarb solution should be controlled at about 30 °C to prevent other damage to the rats caused by excessive temperature. The first dose of rhubarb subdivision was 130 mg/kg·d, followed by daily gavage in 100 mg/kg·d increments, and the rats were observed. When nearly half of the rats started diarrhea, the sub-dose was administered until more than 90% of the rats stopped diarrhea. This was followed by daily increments of 100 mg/kg·d. When more than half of the rats

started diarrhea, the dose was maintained until the diarrhea was stopped in more than 90% of the rats, and the dose was increased. Rhubarb subdivided gavage was performed by circulating rhubarb three times, and when more than 90% of the rats stopped diarrhea in the third time, the rats were given gavage at this dose for 7 days, and then stopped gavage rhubarb. According to the above methods, 40 rats in the MG were induced into CTC model, which means the model was completed. The experimental modeling lasted for 100 days.

### 2.2. Experimental grouping and treatment methods

According to Section 2.1, after successful modeling, the rats required in this experiment were divided into 5 groups, that is, normal group, MG, tong bian decoction gavage group, mosapride group, and normal recovery group. The intervention methods for 5 groups of rats were as follows.

Normal group: the rats were fed with normal diet and given free drinking water every day. They were fed at the same time with the MG and performed death treatment together with the MG.

MG: after successful modeling according to the method in Section 2.1, the rats in this group were performed death treatment together with the NG for 10 days. It was forbidden to feed the rats in the MG, but drinking water was not prohibited, and death treatment was carried out after 12 h. And activated carbon gavage was used to detect the transmission function of the colon, observe the changes of c-kit mRNA gene expression from the colon tissues and the number of fecal particles left in the colon.

Tong bian decoction gavage group: after successful modeling according to the method in Section 2.1, the rats were given lavage of defecation soup every two weeks. The tong bian decoction dose was 15 mL for 1 month, a total of 4 weeks.

Mosapride group: after successful modeling according to the method in Section 2.1, the rats were given mosapride lavage every two weeks. The mosapride dose was 1.60 mg/kg for 1 month, a total of 4 weeks.

Normal recovery group: after successful modeling according to the method in Section 2.1, no drugs were given, natural feeding, unlimited food and water were allowed.

### 2.3. Detention of general observation index

General observation is the observation of the rat's fur luster, mental state and range of motion. The weight of the rats was counted every 3 days in the first month and every 7 days after that.

Indicators of water content in rat feces: rat feces should be collected every 3 days, with a total of about 10 grains of wet heavy feces. The collected manure was then dried in a drying box. The drying temperature of the drying box was set as 100 °C, the drying time was set as 20 min, and the sample weight change was less than 0.5 g as the limiting condition. When the drying reached the standard, the feces were quickly taken out and weighed to calculate the moisture content of the feces.

Number of fecal cases in colon: the rats were treated with death, and the number of feces in the whole colon was counted by laparotomy.

Carbon terminal propulsion experiment: it detects intestinal transit function. The entire intestine was removed from the pylorus to the end of the rectum and placed in the tray. The length from the pylorus to the anus was the total length of the intestine. In a relaxed state, the length of the intestinal tract and the activated carbon suspension were measured to calculate the propulsion length of the activated carbon suspension and the propulsion length of the intestinal tract (Shen et al., 2017). The calculation formula is as follows.

$$C_T(100\%) = \frac{L_{TY}}{L} \times 100\% \quad (1)$$

Among them,  $C_T$  represents the percentage of carbon terminal propulsion;  $L_{TY}$  indicates the distance (cm) between the carbon front end and the pylorus; and  $L$  represents the total length of the intestines.

#### 2.4. Specimen collection

The tong bian decoction gavage group and the mosapride group were both drug administration groups. At the end of one month, the rats in the treatment group were treated to death at the same time as those in the normal recovery group. Specifically, the rats were forbidden to eat for 12 h without forbidding water, followed by 1 h gavage with 5 mL 10% activated carbon solution, and the rats were killed by cervical dislocation. A quick laparotomy was performed to extract 150 mg of fresh intestinal wall tissue 2 cm from the cecum. After it was washed quickly in PBS, it was immediately put into 750 mg RNAlater, that is, RNA stabilizer, and stored at 4 °C for experimental observation.

#### 2.5. Immunohistochemical detection of ICC

After fixing the colon tissue of rat with 10% paraformaldehyde solution, the paraffin blocks wrapped in the tissue were cut into sections with a thickness of 4  $\mu$ m with a microtome. After baking at 70 °C for 2 h, conventional xylene solution was adopted for dewaxing, and then 100–60% gradient ethanol solution was used for tissue rehydration. After rinsing with PBS, the tissue was treated with high pressure repairing antigen by adding citrate buffer. 3% hydrogen peroxide solution was added, cultivated at RT for 10 min, and washed with PBS. Primary antibody was added and cultivated in a wet box for 2 h, then washed with PBS. The secondary antibody was added dropwise, cultivated at RT for 30 min, and rinsed with PBS. The DAB coloring solution was added dropwise, and the color was developed for 5 min and rinsed with pure water. Hematoxylin was used for counterstaining, and after the tap water was returned to the blue, the sample was sealed with a neutral gum. The staining status of ICC positive cells was observed under an optical microscope, and the area of ICC positive cells was detected by Image-pro plus6.0 software.

#### 2.6. Detection of c-kit-mRNA by real-time quantitative PCR

In this study, 160 mg colonic tissue was extracted, and RNA was extracted by soaking colonic tissue in BNA later liquid with Trizol method. The detailed steps for extracting RNA were as follows. Firstly, in the full dissolution stage, the colon tissue treated with DEPC was cut to pieces with surgical scissors, and 1.5 mL of Trizol liquid was added. The colon tissue fragments were fully mixed with Trizol liquid until the colon tissue fragments could not be observed by naked eyes. Secondly, in the centrifugation stage, the mixed solution was allowed to stand for 15 min at RT, and 0.2 mL of chloroform was added to the solution. The solution was transferred to the centrifugal tube to be covered tightly, and placed in the magnetic agitator for full oscillation for 30 s and then allowed to stand for 5 min. The solution was centrifuged for 15000r/min at a temperature of 5 °C for 15 min. After centrifugation, the supernatant in the centrifuge tube was transferred to a new centrifuge tube, and 0.8 mL of isopropanol was added to the new centrifuge tube. After standing at RT for 15 min, centrifugation was performed at a speed of 15,000 r/min and a temperature of 5 °C for 15 min. The centrifuge tube was removed, the supernatant was sucked away by a straw, and 0.8 mL of 75% ethanol solution was added to the centrifuge tube. After uniform mixing,

centrifugation was performed at a speed of 15000r/min and a temperature of 5 °C for 10 min. The tube was removed, and the supernatant was removed and dried for 10 min. Finally, in the extraction phase, 25  $\mu$ L of RNA-free enzyme solution was used to obtain the total RNA from the colon.

In this experiment, RNA was mixed with DEPC solution, 2  $\mu$ L of RNA and 98  $\mu$ L of DEPC solution were taken, and the absorbance of RNA samples at 260 nm and 280 nm was compared by ultraviolet spectrophotometer.

#### 2.7. Data statistics method

SPSS 20.0 was adopted to process the experimental data, which were represented by mean  $\pm$  standard deviation ( $\bar{X} \pm s$ ).  $P < 0.05$  was considered as the difference, indicating statistical meaning. For Ct value, the  $2^{-\Delta\Delta Ct}$  method was adopted, namely, the gene expression rate of normal rats was set to 1 as the reference, and the data were finally analyzed in multiple relation.

### 3. Results and discussion

#### 3.1. General analysis

The modeling time of this experiment was 100 days, among which the gavage took 90 days, and the gavage observation took 10 days. Three of the rats died during the establishment of the model, and it was found by autopsy that the death of the rats was due to the improper operation of gavage, which damaged the organs of the rats. Fig. 1 shows the weight change curves of mice in the NG and the MG. It can be observed from Fig. 1 that in the first 45 days, the weight growth rate of mice in the NG and the MG was almost the same. From day 58, the weight gain of the mice in the MG began to slow down, slower than the NG of mice. Over time, weight gain and change in the NG was remarkably higher than that in the MG. Through observation of the mice, it was found that the mice in the MG were depressed in spirit, had reduced food intake, drank too much water and made more urine, which presented yellow color. Two mice had blood in their stools, and another one had anal prolapse. No laxative drug residue was found in the colon tissue extraction of the two groups of mice.

#### 3.2. Analysis of changes in water content of rat feces

In this research, a SCT-type constipation rat model was constructed by the tong bian decoction enema. Three cycles of water

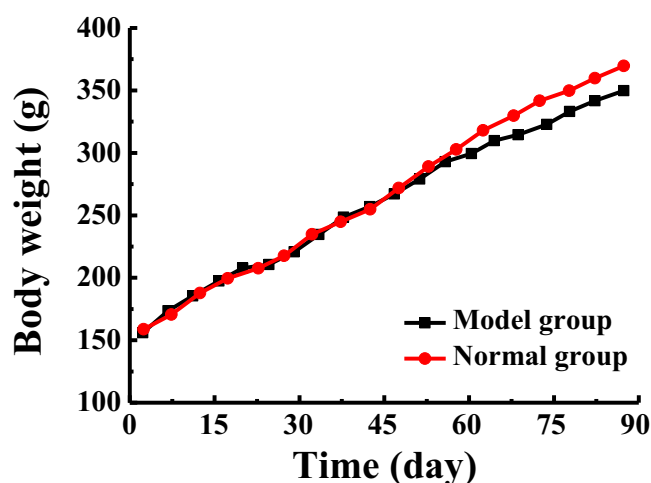


Fig. 1. Body weight changes in rats in the NG and MG.

content in rat feces were successfully completed in the experiment. After the MG stopped enema for 10 days, it was found that the fecal water content of rats was remarkably decreased compared with that of the normal group, so the difference in fecal water content was statistically remarkable. Changes in water content of rat feces within 100 days were detected, and the results were revealed in Fig. 2. It can be observed that the water content in the feces of normal rats revealed a waveform change with the passage of time, while the water content in the feces of rats in the MG was in a stable state.

As can be observed from Fig. 3, there was no remarkable difference in the water content in the feces of rats of each group before the drug intervention ( $P > 0.05$ ). 7d after the intervention of tong bian decoction and Mosapride, the water content in feces of rats in the laxative decoction group was remarkably higher than that in the normal recovery group ( $P < 0.05$ ), and the water content in feces of rats in the Mosapride group was also remarkably higher than that in the normal recovery group ( $P < 0.01$ ). At 15d to 30d after intervention, the fecal water content of rats in the tong bian decoction group and the Mosapride group was remarkably higher than that in the normal recovery group ( $P < 0.05$ ). Therefore, the feces of STC rats were still dry, indicating that the water content in the feces of STC rats could not naturally return to normal.

### 3.3. Analysis of the number of fecal pellets in the colon

The number of fecal particles in colon of rats in 5 groups was compared, as revealed in table 1 and Fig. 4. Compared with the rats in the normal group, the colons in the MG were larger and more circuitous, which provided more conditions for fecal residue. Therefore, there was a remarkable difference in the number of fecal particles, which was statistically remarkable ( $P < 0.01$ ). By comparing the number of fecal particles in the colon of rats in the tong bian decoction gavage group, mosapride group, and the MG, it was found that the number of fecal particles in the colon of the tong bian decoction gavage group and the mosapride group was remarkably less than that of the MG, so it was statistically remarkable ( $P < 0.01$ ). There was no remarkable difference in the number of fecal particles between the MG and the normal recovery group, so there was no statistical meaning between the two groups ( $P > 0.05$ ). However, there was a statistically remarkable difference in the number of fecal particles between the tong bian decoction gavage group and the normal recovery group ( $P < 0.01$ ). There were remarkable differences in the number of fecal particles in the colon

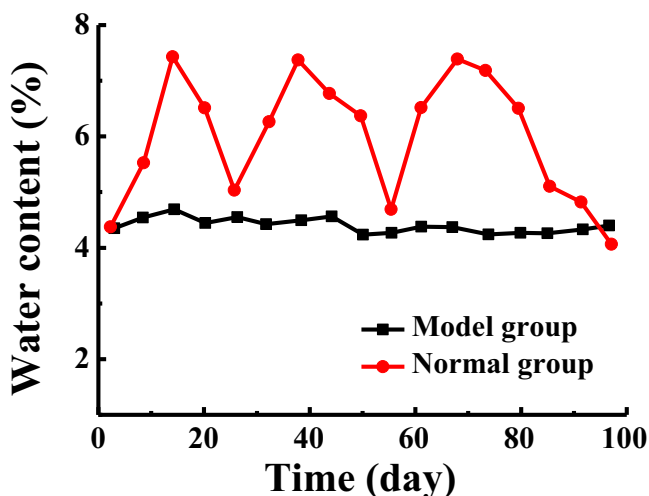


Fig. 2. Changes of fecal water content in rats in the NGand MG.

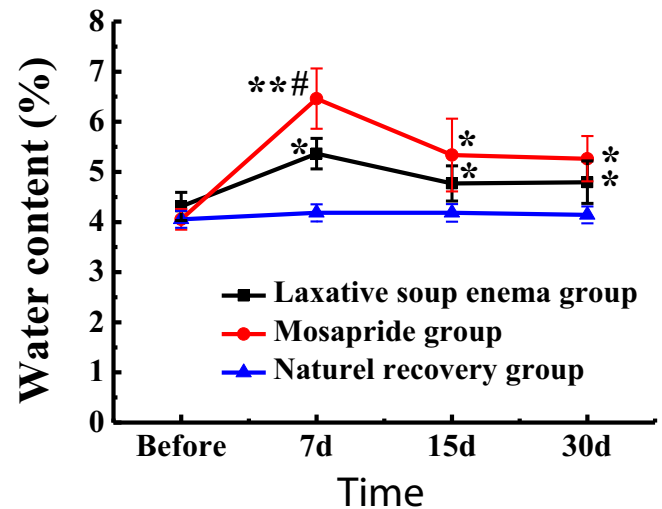


Fig. 3. Changes of water content in feces of different groups. Note: \* indicated a remarkable difference compared to the normal recovery group,  $P < 0.05$ ; \*\* indicated that there was an extremely remarkable difference compared with the normal recovery group ( $P < 0.01$ ); and # indicated that there was a remarkable difference compared with the Tong Bian Decoction group,  $P < 0.05$ .

between the mosapride group and the normal recovery group ( $P < 0.01$ ). However, there was no difference in the number of colonic fecal particles between the tong bian decoction gavage group and the mosapride group, so there was no statistical meaning between the two groups ( $P > 0.05$ ).

### 3.4. Analysis of intestinal transport function by carbon terminal propulsion experiment

According to the statistical analysis of the experimental results, compared with the normal group, the percentage of carbon terminal propulsion was remarkably reduced in the MG, so the difference between the two groups was statistically remarkable ( $P < 0.01$ ). Compared with the MG, the percentage of carbon terminal propulsion in the tong bian decoction gavage group and the mosapride group was remarkably increased, so the difference was statistically remarkable ( $P < 0.01$ ). Compared with the normal recovery group, the percentage of carbon terminal propulsion in the mosapride group and the tong bian decoction gavage group had statistical meaning ( $P < 0.01$ ). There was no remarkable difference in the percentage of carbon terminal propulsion in the mosapride group and the tong bian decoction gavage group ( $P > 0.05$ ), so there was no statistical meaning. Compared with the MG, there was no remarkable difference in the percentage of carbon terminal propulsion in the normal recovery group ( $P > 0.05$ ). Table 1 shows the comparison of colon length, carbon terminal propulsion distance, and propulsion percentage in different groups of rat models. Statistically speaking, gavage of tong bian decoction can provide power to the intestines and increase the effect of laxation (Luo et al., 2017). However, the intestinal transport function of rats in the normal recovery group was not improved, indicating that without the intervention of drugs, intestinal transport function of rats with constipation could recover and return to normal. The results also revealed that the stability of the model was good.

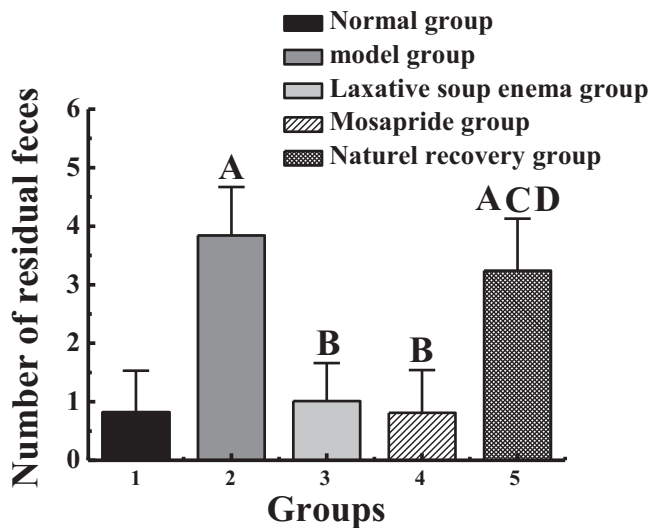
### 3.5. Comparison of the area of positive ICC cells in the colon tissue of rats

The staining areas of positive ICC cells in colon tissues of rats in different groups were compared, as revealed in Fig. 5. After com-

**Table 1**  
Colon length, carbon terminal propulsion distance, and propulsion percentage in different groups of rat models ( $\bar{X} \pm s$ ).

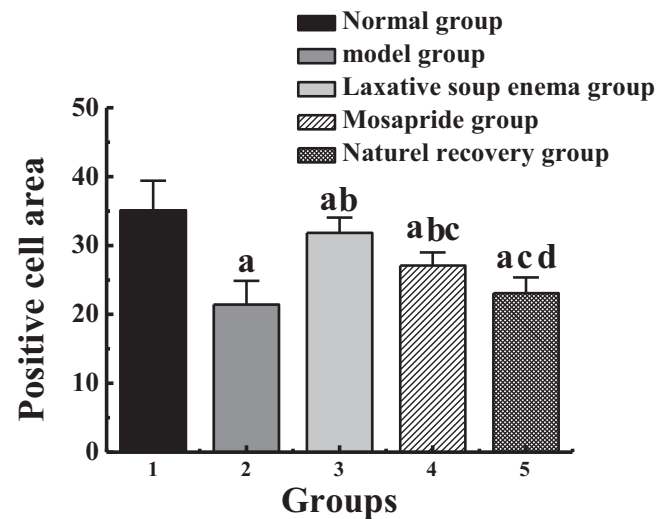
Group class	Number of samples	Total colon length/cm	Carbon terminal propulsion distance/cm	Propulsion percentage
Normal group	10	124.03 ± 6.11	78.63 ± 5.69	68.31 ± 7.71
MG	10	118.19 ± 4.16	60.35 ± 4.63 <sup>A</sup>	51.12 ± 4.82 <sup>A</sup>
Mosapride group	10	134.08 ± 8.82	93.12 ± 10.59 <sup>B</sup>	69.57 ± 6.41 <sup>B</sup>
Tong bian decoction gavage group	10	113.52 ± 6.09	76.53 ± 7.48 <sup>B</sup>	67.31 ± 4.72 <sup>B</sup>
Normal recovery group	10	134.62 ± 7.13	75.67 ± 4.92 <sup>CD</sup>	56.34 ± 5.63 <sup>CD</sup>

Note: A indicated that there was an extremely remarkable difference after comparison with the normal group,  $P < 0.01$ ; B indicated an extremely remarkable difference after comparison with the MG,  $P < 0.01$ ; C indicated an extremely remarkable difference compared with the tong bian decoction gavage group,  $P < 0.01$ ; and D indicated an extremely remarkable difference after comparison with the Mosapride group,  $P < 0.01$ .



**Fig. 4.** The number of fecal residues in different groups of rats. Note: A indicated that there was an extremely remarkable difference compared with the normal group,  $P < 0.01$ ; B indicated an extremely remarkable difference compared with the MG,  $P < 0.01$ ; C indicated an extremely remarkable difference compared with the tong bian decoction gavage group,  $P < 0.01$ ; and D indicated an extremely remarkable difference compared with the Mosapride group,  $P < 0.01$ .

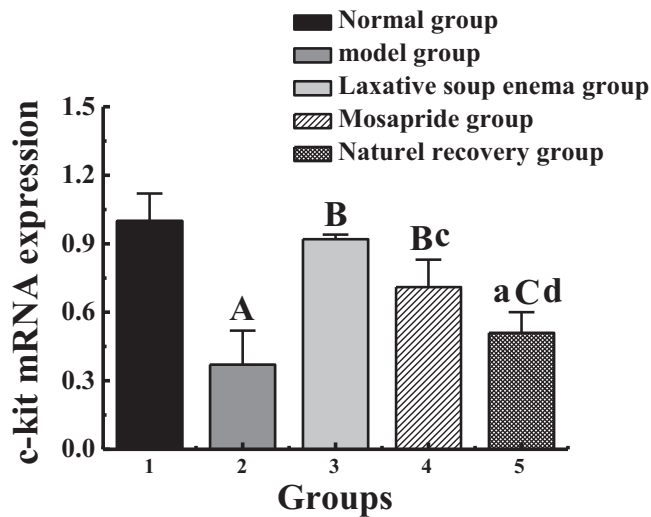
parison with normal rats, the staining area of positive ICC cells in the colon tissues of the MG, the tong bian decoction gavage group, the Mosapride group, and the normal recovery group was remarkably reduced ( $P < 0.05$ ). After comparison with the MG, the staining area of positive ICC cells in the colon tissues of both the tong bian decoction gavage group and the Mosapride group was remarkably increased ( $P < 0.05$ ), but there was no remarkable difference from the normal recovery group ( $P > 0.05$ ). After comparison with the tong bian decoction gavage group, the staining area of positive ICC cells in the colon tissues of both the Mosapride group and the normal recovery group was remarkably reduced ( $P < 0.05$ ). After comparison with Mosapride group, the staining area of positive ICC cells in the colon tissues of the normal recovery group was remarkably reduced ( $P < 0.05$ ). ICC is a major cell in the gastrointestinal tract that emits and transmits slow waves. It is an intermediate medium in the enteric nervous system that regulates the activity of intestinal smooth muscle. Previous studies have revealed that the number of ICC in colon and other tissues of patients with CTC is remarkably reduced after comparison with that of normal people (Lee et al., 2010), which is consistent with the result that the positive ICC staining area in intestinal tissues of the rat model of CTC constructed in this study is remarkably lower than that of the normal group. However, in this study, the number of ICC in the intestinal tissues of rat models was increased after the administration of tong bian decoction, which indicated that tong bian decoction could effectively promote the activity of intestinal smooth muscle and thus improve constipation.



**Fig. 5.** Stained area of positive ICC cells in colon tissue of different groups of rats. Note: a indicated that there was an extremely remarkable difference after comparison with the normal group,  $P < 0.05$ ; b indicated a remarkable difference after comparison with the MG,  $P < 0.05$ ; c indicated a remarkable difference after comparison with the tong bian decoction gavage group,  $P < 0.05$ ; and d indicated a remarkable difference after comparison with the Mosapride group,  $P < 0.05$ .

### 3.6. Comparison of differences of c-kit mRNA expression in colon tissue of rats

Fig. 5 shows the dissolution and amplification curves of real-time quantitative PCR products. Fig. 6 shows the changes of c-kit mRNA expression in colon tissues of rats of different groups. The relative quantitative method adopted the method of  $2^{-\Delta\Delta Ct}$ , and the gene expression rate of normal rats was recorded as 1, the comparison was performed with reference to GAPDHA, and the gene expression rate of other groups was calculated. As can be observed from Fig. 6, the expression of c-kit mRNA in the MG was remarkably lower than that in the NG ( $P < 0.01$ ), while the expression of c-kit mRNA in the normal recovery group was remarkably lower than that in the NG ( $P < 0.05$ ). The expression of c-kit mRNA in the tong bian decoction group and the Mosapride group was remarkably higher than that in the MG ( $P < 0.01$ ). The expression of c-kit mRNA in the normal recovery group was remarkably lower than that in the tong bian decoction group ( $P < 0.01$ ), and the expression of c-kit mRNA in the Mosapride group was remarkably lower than that in the tong bian decoction group ( $P < 0.05$ ). The expression of c-kit mRNA in the normal recovery group was remarkably lower than that in the Mosapride group ( $P < 0.05$ ). This laxative mechanism promotes the regeneration and repair ability of ICC by inhibiting the autophagy of ICC, and provides power for the large intestine, so as to achieve the free movement of the bowels (Chai et al., 2017). Although mosapride could also treat STC, its mechanism of action was independent of the regulation of ICC function.



**Fig. 6.** Changes of c-kit mRNA expression in colon tissues of rats in different groups. Note: a indicated a remarkable difference compared to the normal group,  $P < 0.05$ ; A indicated that there was an extremely remarkable difference after comparison with the normal group,  $P < 0.01$ ; B indicated an extremely remarkable difference after comparison with the MG,  $P < 0.01$ ; c indicated that there was a remarkable difference compared to the tong bian decoction group ( $P < 0.05$ ); C indicated that there was an extremely remarkable difference after comparison with the tong bian decoction group ( $P < 0.01$ ); d indicated a remarkable difference after comparison with the Mosapride group,  $P < 0.05$ .

#### 4. Conclusion

The effect of tong bian decoction on colon transport function of ICC in CTC and the inhibition of autophagy of ICC were studied. The changes of water content in feces of rats revealed that the feces of constipated rats were dry and the water content in feces of STC rats could not return to normal. The data of colon detection by activated carbon method revealed that the lavage of tong bian decoction could provide power for the intestine, and the lavage of tong bian decoction could effectively enhance the colon transport function and increase the laxative effect in the rat model of CTC. Real-time quantitative PCR was used to detect the changes in c-kit mRNA gene expression, and it was found that the laxative mechanism of lavage with tong bian decoction promoted the regeneration and repair ability of ICC by inhibiting the autophagy of ICC, provided power for large intestine, so as to achieve the purpose of laxation. Although mosapride could also treat STC, its mechanism of action was independent of the regulation of ICC function.

The experimental results verify the mechanism of traditional Chinese medicine in treating constipation and regulating intestinal

dynamics, which lays a certain foundation for traditional Chinese medicine in treating constipation and has certain guiding meaning for traditional Chinese medicine in treating CTC.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### References

- Additionally. 2017. Yangyin runchang decoction improves intestinal motility in mice with atropine/diphenoxylate-induced slow-transit constipation. *Evidence-Based Complem. Altern. Med.*, 2017(5), pp. 1–10.
- Chai, Y., Huang, Y., Tang, H., et al., 2017. Role of stem cell growth factor/c-Kit in the pathogenesis of irritable bowel syndrome. *Experim. Therap. Med.* 13 (4), 1187–1193.
- Chen, C.H., Chen, T.H., Wu, M.Y., et al., 2017. Far-infrared protects vascular endothelial cells from advanced glycation end products-induced injury via PLZF-mediated autophagy in diabetic mice. *Sci. Rep.* 7, 40442.
- Gallo, C., D'Ippolito, G., Nuzzo, G., et al., 2017. Autoinhibitory sterol sulfates mediate programmed cell death in a bloom-forming marine diatom. *Nat. Commun.* 8 (1), 92–93.
- Jiang, Q., Garcia, A., Han, M., et al., 2017. Electrostatic stabilization plays a central role in autoinhibitory regulation of the Na<sup>+</sup>, K<sup>+</sup>-ATPase. *Biophys. J.* 112 (2), 288–299.
- Lee, J.I., Park, H., Kamm, M.A., et al., 2010. Decreased density of interstitial cells of Cajal and neuronal cells in patients with slow-transit constipation and acquired megacolon. *J. Gastroenterol. Hepatol.* 20 (8), 1292–1298.
- Li, C.Y., Wu, S.L., Sun, L.X., et al., 2019. Protective effect of Zengye Decoction on submandibular glands in nonobese diabetic mice. *Chin. J. Integrat. Med.* 25 (1), 47–52.
- Luo, D., Cao, Q., Lin, G., et al., 2017. Character and laxative activity of polysaccharides isolated from *Dendrobium officinale*. *J. Funct. Foods* 34, 106–117.
- Shen, Y.Y., 2017. Traditional Chinese medicine-based nursing care in children with constipation: nursing effect and impact on anti-diarrhea factors. *World Chin. J. Digestol.* 25 (17), 1610.
- Shen, M., Cui, Y., Hu, M., et al., 2017. Quantifying traditional Chinese medicine patterns using modern test theory: an example of functional constipation. *BMC Complem. Altern. Med.* 17 (1), 44.
- Son, Y.O., Pratheeshkumar, P., Divya, S.P., et al., 2018. Withdrawal: Nuclear factor erythroid 2-related factor 2 enhances carcinogenesis by suppressing apoptosis and promoting autophagy in nickel-transformed cells. *J. Biol. Chem.* 293 (40), 57–61.
- Xiao, W., Lee, X., Hu, Y., et al., 2017. An experimental investigation of kinetic fractionation of open-water evaporation over a large lake. *J. Geophys. Res. Atmosph.* 122 (21), 12–22.
- Yao, C.J., Chow, J.M., Chuang, S.E., et al., 2017. Induction of Forkhead Class box O<sub>3a</sub> and apoptosis by a standardized ginsenoside formulation, KG-135, is potentiated by autophagy blockade in A549 human lung cancer cells. *J. Ginseng Res.* 41 (3), 247–256.
- Zhang, S., Xie, Y., Wang, J., et al., 2018. Simultaneous determination of six bioactive components of total flavonoids of *Scorzonera austriaca* in rat tissues by LC-MS/MS: application to a tissue distribution study. *Revista Brasileira De Farmacognosia* 28 (2), 79–81.