

# The effect on Tiaoshen Jianwei Decoction to the motility and SP content in gastrointestinal of functional dyspepsia rat

Wenzhi Liu<sup>1</sup>, Tiantian Bian<sup>1</sup>, Feng Li<sup>1</sup>, Junyan Li<sup>1,\*</sup>

<sup>1</sup>Department of Medical College of Qingdao University, Qingdao, China, 266021

<sup>2</sup>Department of First Sanatorium of Jinan Military Region at Qingdao, Qingdao, China, 266071

**Abstract:** To explore the therapeutic mechanism of Tiaoshen Jianwei Decoction to functional dyspepsia (FD). Forty rats were randomly divided into four groups (control group, model group, Mosapride group, Tiaoshen Jianwei Decoction group). All of the rats expect for in control group were induced the FD rat models by clamping their tails. The rats were given relevant medicine therapy and deal with them in 14 days. Then observed their pathological sections by HE staining method, measured and calculated the gastric emptying rate and promoting ratio of small intestine of rats, detected SP level in gastrointestinal by enzyme-linked immunosorbent assay (ELISA) method. As a result, compared with the control group, the rate of gastric emptying and promoting ratio of small intestine of rats were decreased ( $P < 0.05$ ) and the SP content in gastrointestinal was decreased obviously ( $P < 0.01$ ) in model group. After treatment with Tiaoshen Jianwei Decoction, the rate of gastric emptying and promoting ratio of small intestine of rats were increased ( $P < 0.05$ ) and the SP content was increased obviously ( $P < 0.01$ ). Tiaoshen Jianwei Decoction group compared with the control group and Mosapride group was not significant difference ( $P > 0.05$ ). According to the results of this study we can conclude that Tiaoshen Jianwei Decoction can regulate the levels of SP in gastrointestinal and recover the function of gastrointestinal motility so as to treat FD.

**Keywords:** Functional dyspepsia; SP; Gastrointestinal motility; Rat

Received 29 January 2015, Revised 5 March 2015, Accepted 11 March 2015

\* Corresponding Author: Junyan Li; jyli1972@126.com

## 1. Introduction

Functional dyspepsia (FD), means that has excluded organic disease by checking, is a group of clinical syndrome that take postprandial fullness discomfort, early satiety, belching, nausea, vomiting, epigastric pain, epigastric burning sensation as the main symptoms, and is a kind of functional gastrointestinal disease common and multiple in clinic. Its pathogenesis is complex, at present, which was considered that is mainly related to genetic susceptibility, gastrointestinal motility dysfunction, visceral hypersensitivity, helicobacter pylori infection, brain-gut axis dysfunction and social psychology factors. Traditional Chinese medicine believes that the diseased part is the stomach, the two dirty of liver and spleen were involved, so many doctors treated it as the aspect of liver and adopted the method of dispersing stagnated liver qi for relieving qi stagnation in clinic, as a result obtained the good curative effect. Zhou Fusheng [1] putted forward the theory of relevance between heart (mind) and stomach. He considered that there is correlation between regulation function of mind and gastrointestinal functional disorders, which is consistent with the social psychological factors that western medicine believed. This experiment mainly researches the effect on Tiaoshen Jianwei Tang to gastrointestinal motility and substance P (SP) of functional dyspepsia rats.

## 2. Materials and Methods

### 2.1. Animals

Forty SPF 8 week-old male Wistar rats, weighing 180 - 220g, were provided by the experimental animal center of Shandong Lukang whose license number is SCXK (Lu) 20130001. Animal were putted in laboratory and adapted to the environment for a week, drinking and eating free, at 21 to 25°C, for natural light.

### 2.2. Drugs

Mosapride tablets were made in Dainippon Sumitomo Pharma Co.Ltd (Japan). Chaihu Shugan San and Tiaoshen Jianwei Tang were purchased from Beijing Tongrentang pharmacy. Tiaoshen Jianwei Tang, is based on Chaihu Shugan San, composed of Chaihu 12g, Chenpi 12g, Chuanxiong 9g, Baishao 12g, Zhiqiao 9g, Xiangfu 9 g and Gancao 3g, Fushen 20g, Yuanzhi 9g, Hehuanpi 12g, Muxiang 9g. The traditional Chinese medicine was after soaking in the water by 1:5 volume ratio for 30 min, decocting for two times, Filtrating, removing slag to extracting juice, merging filtrate heating evaporation. Tiaoshen Jianwei Decoction was made into 1.04 g/ml water extract (containing crude drug 1.04 g/ml), saved at 4°C. Mosapride was made into 0.135 mg/ml mixed suspended solution saved at 4°C. Preparation of semisolid paste of carbon nutritional: sodium carboxymethyl cellulose 10g, milk powder 16g, sugar

8g, starch 8g, activated carbon 2g, add 250 ml distilled water was stirred evenly, formulated as 300 ml black semisolid paste saved in refrigerator and restored room temperature when it was used [2].

### 2.3. The main reagent and instrument

Bioprep -24 animal tissue grinder (Aosheng, Hangzhou, China), HF2000 automatic enzyme mark instrument (Huaan Maike, Beijing, China), TDZ4-WS Centrifuge (Xiangyi, Hunan, China), HS-100 constant temperature box (Taisite, Nanjing, China), BX41 microscope and BX51 microscope (Olympus, Japan), -80°C refrigerator, Slicing machine, electronic balance etc. ELISA detection kit: SP (item number: KEG007, batch number: RDYY387) was purchased from R&D reagent company in America.

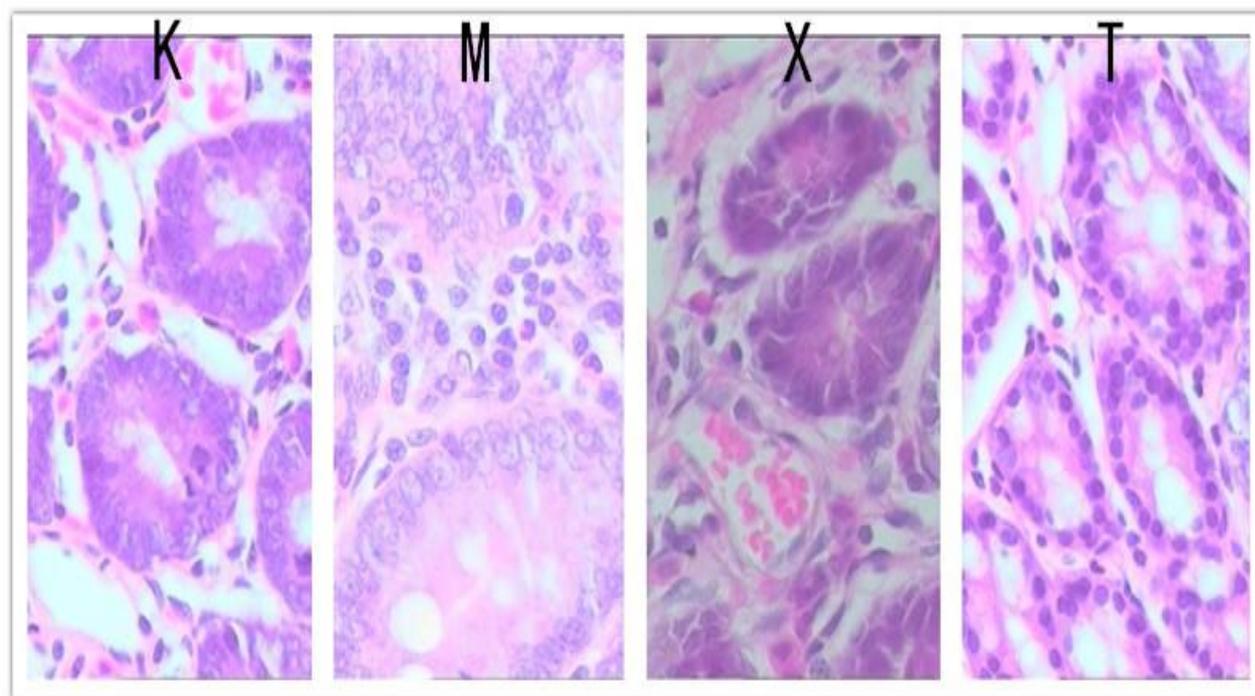
### 2.4. Animal grouping

The rats were randomly divided into four groups that are blank control group, model group, mosapride group, Tiaoshen Jianwei Decoction group. They were reared in the plastic cages whose bottoms are made

from steel wire net, 10 rats in each group, 5 rats in each cage and 2 cages in each group.

### 2.5. Copy model

The method that clamping the rat-tails to anger them was used [3]. The tails of rats in each group, except for the blank control group, were nipped on their distal 1/3 part, not to break the skin for the degree, to rage and scuffle to others aggressively so as to rile the whole cage of rats, and every time they were stimulated continuously for 30 min. With the intensification of the fight, the rats may be scratched, with 0.5% iodophor into their injured site to avoid inflammatory interference. They were stimulated a time every 3h, 4 times a day. The rats present the reaction of tension and anxiety and their food intake was significantly reduced after 7 days of continuous stimulation, which consistent with the literature, indicating that the model building was successful. During the period of making model, rats were allowed free access to food, drinking water. We daily observed, measured and recorded the general condition of rats.



**Figure 1. Pathological slices stained with HE of small intestinal tissue (400 times).**

**Note:** K: control group, M: model group, X: western medicine group, T: Tiaoshen Jianwei Tang group

### 2.6. Given drugs

The dose reference basis and technology of experimental animal science by the method of surface area dose [4]. Mosapride group were given Mosapride 1.35 mg/kg; Tiaoshen Jianwei Decoction group were given Tiaoshen Jianwei Decoction 10.4 g/kg, the blank control group and model group to the corresponding

volume of normal saline (10 ml/kg), All of the rats were lavaged one times a day, given medication starting the seventh days after building a model, continued to give poison for 14 d.

### 2.7. Tissue collection and index detection

We observed the rat's daily spirit, the hair colour

and the stool, measured their weight, eating, drinking quantity and so on. After the last administration fasted but watered 24 h, the rats were lavaged charcoal semisolid paste 1 ml/100 g and injected 10% chloral hydrate 0.35 ml/100 g into abdominal cavity after 30 min. Quickly opened the abdominal cavity, ligated the gastric cardia and pylorus, removed the stomach and weighted it after making it dry by filter paper. The appetizer was sheared along the greater curvature of the stomach, stomach contents were washed away by 4°C saline. The net weight of stomach was weighted after it was dry. Then we measured the total length of small intestine (the distance of pylorus to ileocecal) and charcoal semisolid paste in the intestinal propulsive distance (the distance of the pylorus to the most distal of charcoal semisolid paste). The rate of gastric emptying =  $[1 - (\text{stomach weight} - \text{net weight}) / \text{intra-gastric volume}] \times 100\%$ . The small intestinal propulsion ratio = propulsive distance of carbon powder in the intestinal (cm)/small intestine length (cm)  $\times 100\%$ .

Cut separately gastric antrum and upper jejunum organization 0.5 cm  $\times$  0.5 cm, they were flushed by 0.9% physiological saline and fixed 24 - 48 h in 4% paraformaldehyde. Fixed block, dehydrated, flushed to transparent, dipped wax, embedded, sliced, fished slices, spreaded, it was spend the night at 60°C in the drying box and then was carried out HE staining.

Gastric antrum and upper jejunum organization 0.5 cm  $\times$  0.5 cm were cut respectively, flushed by 0.9% physiological saline and saved in the  $-80^{\circ}\text{C}$  refrigerator and for using. Grinding and centrifugating for supernatant liquid, we measurement SP content in gastrointestinal according to operation steps of instructions of the ELISA kit of SP.

## 2.8. Statistical analysis

Statistical analysis was performed using SPSS17.0 software. Data were shown as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), multiple groups were analyzed by variance analysis, the two groups were compared by LSD-t test. The level of significance for the analysis was set at  $P < 0.05$ .

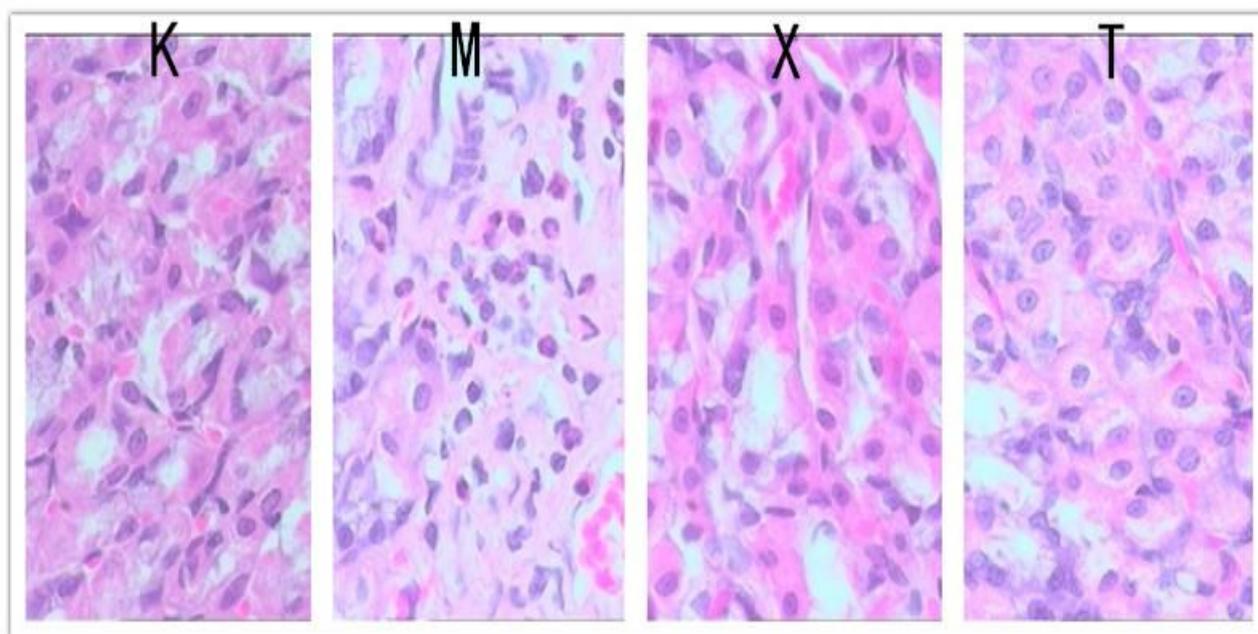
## 3. Results

### 3.1 The general situation

The rats in model group standed to fight as soon as moving or beating cage, means they were in the anxious and tension state. They binge eating at early days, then drinking and eating significantly reduce, weight loss, hair haggard and rough and response slightly slow. The rats in Tiaoshen Jianwei Decoction group, mosapride group after treatment, symptoms improved significantly, reaction was sensitive, eating and drinking quantity increases gradually, the body weight was slightly lower than the control group, hair shiny and compliance.

### 3.2 Pathological findings

On the one hand, ther was not obvious inflammatory, ulcer, tumor and other organic change were fond after cut the gastrointestinal. On the other hand, no erosion, atrophy, metaplasia, atypical hyperplasia and other organic lesions were found by HE staining in gastric antrum and small intestine mucosa. Rats in model group with moderate to severe infiltration of lymphoid and plasma cells and a few infiltration of neutrophils and the other group with mild infiltration of lymphocytes in small intestine and gastric antrum tissue(it is shown as Figure 1 and Figure 2).



**Figure 2. Pathological slices stained with HE of Gastric antrum (400 times).**

**Note:** K: control group, M: model group, X: western medicine group, T: Tiaoshen Jianwei Tang group

### 3.3 The effects on Tiaoshen Jianwei Decoction to the rate of gastric emptying and the promoting ratio of small intestine in rats

Compared with the control group, the rate of gastric emptying and the promoting ratio of small intestine of rats in model group were decreased ( $P < 0.05$ ). Compared with the model group, the rate of gastric emptying and the promoting ratio of small intestine of

rats in Tiaoshen Jianwei Decoction group were increased, the difference was statistically significant ( $P < 0.05$ ). Compared with the control group and Mosapride group, it is not significant difference ( $P > 0.05$ ). The results show that Tiaoshen Jianwei Decoction can promote gastrointestinal motility (it is shown as Table 1).

**Table 1** The gastric emptying rate and promoting ratio of small intestine of rats (n=10,  $\bar{x} \pm s$ )

grouping	rate of gastric emptying (%)	promoting ratio of small intestine
control group	44.71 ±11.02	81.82 ±6.05
model group	24.7 ±6.41*	67.05 ±7.83*
Mosapride group	40.45 ±8.00 <sup>Δ</sup>	82.89 ±4.67 <sup>Δ</sup>
Tiaoshen Jianwei Decoction group	43.95 ±11.93 <sup>Δ</sup> #	84.65 ±7.42 <sup>Δ</sup> #

**Note:** Compared with control group \* $P < 0.05$ , compared with model group <sup>Δ</sup> $P < 0.05$ , compared with Mosapride group #  $P > 0.05$ .

### 3.4 Expression of SP in rat gastrointestinal

The content of SP in gastrointestinal of the model group is the lowest, compared with the control group  $P < 0.01$ . After treating of Tiaoshen Jianwei Decoction, compared with model group were significantly increased ( $P < 0.01$ ), there is not significant difference compared with the control group and mosapride group ( $P > 0.05$ ). This describes that each treatment group were obviously improved and even to cured, The curative effect of Tiaoshen Jianwei Decoction group, is similar to Mosapride group, each index has recovered to the normal level (it is shown as Table 2).

**Table 2** The expression of SP in rat gastrointestinal of rats (n=10,  $\bar{x} \pm s$ , ng/ml)

Grouping	SP
control group	398.42 ±43.56
model group	266.13 ±37.34 *
Mosapride group	367.05 ±51.69 <sup>Δ</sup>
Tiaoshen Jianwei Decoction group	383.12 ±64.86 <sup>Δ</sup> #

**Note:** Compared with control group \*  $P < 0.05$ , compared with model group <sup>Δ</sup> $P < 0.05$ , compared with Mosapride group #  $P > 0.05$ .

## 4. Discussion

FD, belong to the "stomach stagnation, stomach pain, anorexia, stomach, noisy and other areas of traditional Chinese medicine, caused by the factors of emotional disorders, overstrain of improper diet, resistance by heat and wet, congenital deficiency damaged the spleen and stomach. Its pathogenesis is spleen deficiency and qi stagnation, abnormal movements, also related to liver failure catharsis. Disease is mainly located in stomach and related to the two dirty of liver and spleen, so the therapeutic principles should be soothing liver and invigorating spleen, and reconcile the stomach and make the zhongqi smooth, so as to the function of sending up the clear of spleen and descending the turbid of stomach returned to normal, essence of water and grain to normal operation and gastrointestinal motor function to coordination. Tiaoshen Jianwei Decoction, is prescription of clinical experience of Professor Li Junyan, is composed of Chaihu Shugan San add Poria cocos, polygala root, bark of silktree, woody, has the functions of Soothing the liver, calming the mind, strengthening the spleen and reconciling stomach. Modern research shows that the peony has the effect of calming and relaxing smooth muscle; bupleurum mainly contain saponin and volatile oil has sedative, analgesic, anti-inflammatory and cholagogic effect; Tangerine peel mainly contain volatile oil has relax smooth muscle, anti-inflammatory, anti ulcer effect, nutgrass galingale rhizome contain volatile oil and sugar can raise the pain threshold of mice has the effect of relieving pain, strengthening the stomach and promoting digestion [5]. Volatile oil of Fructus aurantii, has the function of first

exciting and then inhibitory effect on smooth muscle of isolated intestinal of rat, has obvious relaxation effect on spasmodic contraction induced by CaCl<sub>2</sub>, Ach and histamine phosphate, and can reduce gastric acid, Combating from forming pyloric ligature ulcer in rat, flavonoid glycosides of Fructus aurantii has an inhibitory effect on the intestinal smooth muscle [6]. Perfume mentioned fluid of wooden contains volatile oil and total alkaloids have exciting effects on the isolated small intestine of mouse. All of Poria, Radix Polygalae, Albizzia bark have a sedative effect.

According to the Rome III criteria [7], FD is divided into to two types that are postprandial distress syndrome (PDS) that take dynamic obstacles as the main syndrome and epigastric pain syndrome (EPS) that take organ neuroticism as the main syndrome. Traditional Chinese medicine believed that heart manages mind, wicth is similar to brain function in Western medicine. Modern medicine thought that the incidence is closely related to brain-gut peptides and gastrointestinal hormone is an important factor affecting the gastrointestinal motility on patients with FD. SP, is a brain-gut peptide that scholars studied more in recent years, exists mainly in the human central nervous system, spinal dorsal root and central nervous system of gastrointestinal tract, a small part in the intestinal enterochromaffin cells. It have both the transmission of pain information and analgesic effect, can promote gastrointestinal motility, stimulate secretion of gastrointestinal hormone [8]. Numerous studies have shown that because SP can promote the gastric emptying, small intestinal movement, and associated with increased sensitivity of gastrointestinal nerve, so the level of SP was decreased on PDS type of FD, and EPS type was increased. The experiment found that Tiaoshen Jianwei Decoction as the Mosapride can achieve the same therapeutic effect, but also overcome the side effects of Western medicine, its comprehensive treatment is better than mosapride. Tiaoshen Jianwei Decoction can regulate the content of SP in astrointestinal regulating, make the gastrointestinal motility recovery, thus plays the role of the treating of FD. In addition, it is of the functions of soothing the liver, calming the mind, strengthening the spleen and reconciling stomach so that regulating both body and physical on patients, is worth the clinical promotion.

## References

- [1] Zhang QH, Zhou FS. An analysis of "the correlation of heart and stomach". Journal of Traditional Chinese Medicine of Yunnan University, 24 (2): 2001 39.
- [2] Wu HB, Xu D, Bo WJ. Effect of Xiaoshi oral liquid on gastric emptying of functional dyspepsia rat. Lishizhen medicine and Chinese medicine, 24 (12): 2013 2855-2856.
- [3] Guo HJ, Lin J, Li GC. Study of animal model onFunctional dyspepsia. Xiaohua Journal of Integrated Traditional Chinese and Western Medicine of Chinese, 9 (3): 2001 141.
- [4] Yang F, Hu Y. The basis and technique of experimental zoology. Publishing House of Fudan University, 9: 2010 405.
- [5] Huang PS. Modified Chaihu Shugan powder combined with antidepressant drug treated 68 cases of depression. Shaanxi Journal of traditional Chinese medicine, 33 (6): 2012 666-667.
- [6] Xu H, Chen HF, Jie L. The research overview of chemical composition of Fructus aurantii and Fructus aurantii Immaturus and gastrointestinal motility. Journal of Jiangxi traditional Chinese Medicine University, 21 (1): 2009 43-42.
- [7] Bolino MC, Furia M, Facio L, Delli Quadri I, Lien Y, Espinosa F, Vera F, Corti R, Vázquez H, Iantorno G. Functional dyspepsia and the satiety test: its usefulness in clinical practice. Rev Gastroenterol Mex, 78 (3): 2013 127-134.
- [8] Yu T, Zhao LN. The brain-gut peptide and functional dyspepsia. Modern Digestion & Intervention, 17 (4): 2012 241-244.