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Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com**Original article****Effects of Sijunzi Dripping Pill on Gastrointestinal Motility of Mice**

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ABSTRACT

Objective To study the effects of Sijunzi Dripping Pill (SDP) on gastrointestinal motility of mice. **Methods** The diarrhea and swimming model of mice was made by *Rhei Radix* et *Rhizoma*-induced spleen deficiency. The intestinal transit, gastric emptying test, serum motilin (MTL), vasoactive intestinal peptide (VIP), and substance P (SP) were chosen to observe the effects of high-, mid-, and low-dose SDP on stomach movements, and the water extractive of Sijunzi Decoction was used as positive control. **Results** Compared with the control group, the gastric emptying rate in the gastrointestinal motility group was significantly decreased, the intestinal propulsion rate was obviously increased, the levels of MTL, prostaglandin E₂ (PGE₂), and SP were increased ($P < 0.05$), while the level of VIP was decreased ($P < 0.05$). Compared with the model group, SDP could decrease the intestinal transit rate, whereas increase the gastric emptying rate and the level of MTL ($P < 0.05$); The high-dose SDP could decrease the level of PGE₂ ($P < 0.05$) and the low-dose SDP could decrease the level of VIP ($P < 0.05$); Each group had no significant effect on SP. **Conclusion** SDP has the good effect on increasing the gastrointestinal motility of mice, and its function may partly relate to the regulation of the levels of MTL and VIP as well as PGE₂.

Key words

amount of gastric emptying; gastrointestinal motility; serum motilin; Sijunzi Dripping Pill

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1. Introduction

Sijunzi Decoction (SD) comes from a traditional Chinese medicine book called “*Taiping Benevolent Dispensary Bureau*”, which is the basic recipe of tonifying *qi* and repairing the weakness of spleen. In modern recipe, *Codonopsis Radix* replaces *Ginseng Radix*, and in this study SD consists of *Codonopsis Radix*, *Poria*, roasted *Atractylodes Macrocephalae Rhizoma*, and *Glycyrrhizae Radix* et *Rhizoma Praeparata cum Melle*. The polysaccharids from SD have

various functions and activities, such as aiding digestion, adjusting stomach movements, and affecting gastrointestinal hormone (Ye and Chen, 2005). Existing formulations made from above Chinese materia medica are water pill, mixture, and granules. Those formulations are produced by traditional methods which induce a lot of problems, such as complicated producing process, low absorbance, intake inconvenience, low efficiency, etc. Sijunzi Dripping Pill (SDP) mainly consisted of polysaccharids and lipid-soluble components in SD. This research focused on stomach movement function,

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examined the effects of SDP on intestinal propulsion, gastric emptying, serum motilin (MTL), vasoactive intestinal peptide (VIP), and substance P (SP) in mice with spleen deficiency.

2. Materials and methods

2.1 Experimental animals

Kunming mice (18–20 g), male and female in half, supplied by Experiment Center of Shanxi Medical University (SCXK-201101) were used in this study.

2.2 Drugs and reagents

Sijunzi Dripping Pill was supplied by Department of Pharmacology, Shanxi University of Traditional Chinese Medicine, and mainly contained 20% of polysaccharids and 0.02% of lipid-soluble components. *Rhei Radix et Rhizoma* (700 g, Jingwan Chinese Medicine Co., China, No. 110901) was immersed for 1 h in 2100 mL water, decocted for 15 min at 97 °C, filtered with a double layer gauze, then added with two fold amount of water to decoct the residues for 15 min, filtered and combined the decoction. The sample was concentrated to 1.5 g/mL, sealed, and kept in cool condition.

Codonopsis Radix (No. 100417101), *Poria* (No. 100301111), roasted *Atractylodis Macrocephalae Rhizoma* (No. 100428101), and *Glycyrrhizae Radix Rhizoma Praeparata cum Melle* (No. 105668192) were all purchased from Qiao Chinese Herbal Medicine Co., Ltd., China, at a ratio of 2:2:2:1, added with 1400 mL water to immerse 175 g medicine for 1 h, decocted for 2 h at 97 °C, and filtered with a double layer gauze after decocting. The sample was decocted for three times, the decoction was combined and concentrated to 1.75 g/mL, sealed, and kept in a cool condition. This decoction obtained was SD.

Polysaccharide sample (1 mg) was precisely weighed, together with dry KBr powder (200 mg) of chromatographic grade, finely grounded in agate mortar and mixed evenly, then shaped into tablet. MB104 FTIR (ABB Co., Canada) was used to characterize polysaccharide in SDP.

Xylitol (Shandong Futian Medicine Co., Ltd., No. 11031908), 1% methyl orange (Tianjin Guangfu Institute of Fine Chemicals), 5% sodium bicarbonate (Tianjin Shentai Chemicals Co., Ltd., No. 110801), and sodium chloride (Tianjin Kemimou Chemicals Co., Ltd., No. 20100427) were used. All reagents were of analytical grade. Motilin (MTL), vasoactive intestinal peptide (VIP), prostaglandin E₂ (PGE₂), and substance P (SP) were detected by ELISA kit (No. 201110, made by American R&D Co., imported, dispensed, and provided by Shanghai Chuangsai Co.).

2.3 Main instruments

721 Visible Spectrophotometer (Shanghai Qinghua Technology Co., Ltd.), FSH-II High-speed Electric Homogenizer (Jintan Danyangmen Quartz Glass Co.), LD5–10 Centrifuge (Beijing Medical Centrifuge Co.),

JA4003 Analytical Balance (Shanghai Liangping Instrument Co., Ltd.), Spectra Max 190 Microplate Reader (Molecular Co., USA).

2.4 Groups and models

The Kunming mice (18–22 g) were housed in a room at (22 ± 1) °C, with free access to water and standard mouse chow. The mice were randomly divided into six groups ($n = 8$, half male and half female), such as control, model (spleen deficiency group), SD (35 g/kg fresh medicine), high-, mid-, and low-dose (2, 1, and 0.5 g/kg) SDP groups. In the morning, the mice in the control group were fed with water, the mice in the model group were ig administered with xylitol (3.5 g/kg) and the rest mice were ig given SDP with the relative doses. In the afternoon, all the model mice were given water extract from *Rhei Radix et Rhizoma* (37.5 g/kg) to establish the spleen deficiency model (Xu et al, 2004) except the mice in the control group. The mice in the control group were still given water, and after administration all the mice were put into a box full of water with the depth of 30 cm at (25 ± 1) °C. After swimming for 10 min, the mice were taken out and dried. The experiment lasted for two weeks.

2.5 Experimental protocol and measurement of intestinal propulsion and gastric emptying

Before the experiment, the mice were fasting for 18–20 h, but were supplied with tap water until 10 min before the experiment. On the day of the experiment, the animals were ig given 1% methyl orange (10 mL/kg) and absorbed blood 20 min later, then sacrificed. The stomach and attached small intestine of mice were immediately exposed by laparotomy. After ligation of esophagogastric, gastroduodenal, and ileocaecal junctions, the stomach and small intestine were carefully removed and placed on a wooden board to observe the leading edge of the methyl orange in the intestine (Wu et al, 2002). The length of small intestine from pylorus to ileocecal and the length of methyl orange promoting were measured. The formula of calculating intestinal propulsion was as follows: intestinal propulsion rate = length of methyl orange / whole length of small intestine.

The stomach was cut out, immersed into 1% sodium bicarbonate solution for 30 min, and centrifuged at 2000 r/min for 10 min. The supernatant was measured by determining the absorbance (A), and expressed as a percentage of gastric emptying. The formula for calculating gastric emptying rate was as follows:

$$\text{gastric emptying rate} = (1 - A_n) / A_0$$

where A_n was the A value of stomach, A_0 was the A value of 1% sodium bicarbonate solution.

2.6 Experimental protocol and measurement of serum MTL, small intestine homogenate, VIP, PGE₂, and SP

The blood samples were collected and centrifuged at 3500 r/min for 10 min, and ELISA kit was used to measure

serum MTL. The intestine was weighed, made into 10% homogenate, and centrifuged at 2500 r/min for 10 min at 4 °C. The supernatant was used to test the concentration of VIP, PGE₂, and SP by the ELISA protocol.

3. Results

3.1 Characteristics of polysaccharides in SDP

Using glucose as standard, and phenol-sulfuric acid method for the determination of the polysaccharides in SDP, the extract content of total polysaccharides was 68%.

The resolution used was 4 cm⁻¹, the frequency of spectra ranged from 4000 to 400 cm⁻¹, and the spectra were obtained by averaging 32 scans. FTIR spectra of polysaccharides in SDP are shown in Figure 1. The wave numbers of characteristic absorption peaks were analyzed by software GRAMS/32AI.

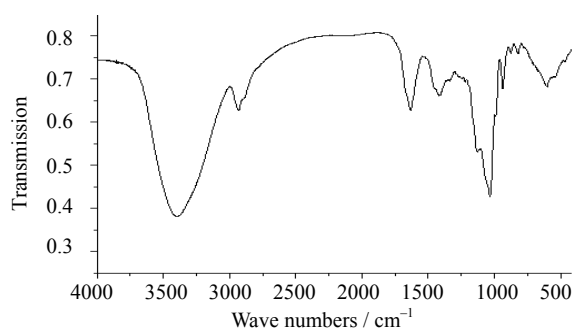


Figure 1 FTIR spectra of polysaccharides in SDP

The width absorption peak at the range of 3600–3200 cm⁻¹ represented the peak of O-H stretching vibration that polysaccharides existed intermolecular or extramolecular hydrogen bonds. The weak absorption peak near 2930 cm⁻¹ was caused by the CH stretching vibration of -CH₂- functional groups. The peaks near 1640 cm⁻¹ might be provided for the amide carbonyl stretching vibration. The peaks in 869 and 812 cm⁻¹ indicated that the polysaccharide had a chroman-type α-2 glycosidic bond, and a mannose glycosylation.

The samples of the polysaccharides solution at 0.08 g/L were precisely weighed, tested by the UV-vis spectrophotometry (Cary50, Varian) within the scanning range of 200–400 nm. The result is given in Figure 2. It can conclude that there is a strong polysaccharide absorption peak at 200 nm.

¹H-NMR spectra were measured at 600 MHz at 298 K, and 32 free induction decays (FIDs) were collected over a spectral width of 12345.679 Hz with a relaxation delay of 5 s. ¹H-NMR spectrum is the major approach to determine the configuration of glycosidic bonds. ¹H-NMR spectra showed that the proton signals within the range of δ 3.0–5.0, is a typical signal for polysaccharides (Figure 3).

3.2 Effects of SDP on intestinal propulsion and gastric emptying in mice

Compared with the control group, the rate of intestinal

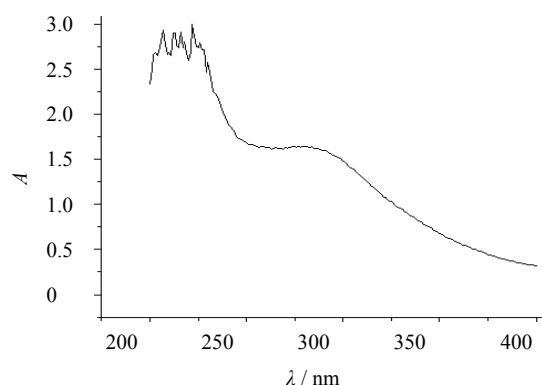


Figure 2 UV absorption spectrum of polysaccharides in SDP

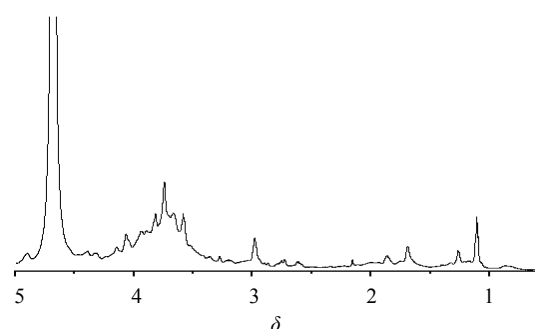


Figure 3 ¹H-NMR spectrum of polysaccharides in SDP (600 MHz)

propulsion was increased in the model group (Table 1), and compared with the model group, those of the other groups are decreased without significant differences.

It is shown in Table 1 that compared with the model group, the gastric emptying rates were significantly increased in SDP groups ($P < 0.05, 0.01$), and increasing trend without significant changes was shown in SD group.

Table 1 Effects of SDP on intestinal propulsion and gastric emptying in mice ($\bar{x} \pm s, n = 8$)

Groups	Doses / (g·kg ⁻¹)	Intestinal propulsion rates / %	Gastric emptying rates / %
control	—	59.17 ± 4.90	88.34 ± 7.05
model		61.91 ± 15.60	65.76 ± 7.80 ^{△△}
SD	35	41.03 ± 9.35	70.19 ± 6.61 ^{△△}
SDP	2	53.60 ± 10.96	81.07 ± 6.31 ^{*▲▲}
	1	53.09 ± 13.43	85.20 ± 7.64 ^{**▲▲}
	0.5	54.64 ± 10.26	82.25 ± 5.30 ^{*▲▲}

[△] $P < 0.05$ ^{△△} $P < 0.01$ vs control group; ^{*} $P < 0.05$ ^{**} $P < 0.01$ vs model group; [▲] $P < 0.05$ ^{▲▲} $P < 0.01$ vs SD group, same as below

3.3 Effects of SDP on MTL in mice

Table 2 shows that the MTL in SD, high-, mid-, and low-dose SDP groups had significant difference ($P < 0.01, 0.05$).

3.4 Effects of SDP on VIP, PGE₂, and SP in mice

Table 3 indicates that compared with the model group,

Table 2 Effects of SDP on MTL in mice ($\bar{x} \pm s$, $n = 8$)

Groups	Doses / (g·kg ⁻¹)	MTL / (ng·L ⁻¹)
control	–	10.85 ± 1.65
model	–	14.36 ± 1.45 ^{△△}
SD	35	22.87 ± 3.14 ^{△*}
SDP	2	10.92 ± 2.33 ^{*▲▲}
	1	12.09 ± 1.56 ^{*▲}
	0.5	14.10 ± 2.43 ^{△▲}

the levels of VIP in SD and low-dose SDP group were significantly decreased ($P < 0.05$, 0.01), and a decreasing trend was shown in high-dose SDP group. The levels of PGE₂ in SD, high- and low-dose SDP groups had significant difference ($P < 0.05$). The decreasing trend without significant difference was shown in mid-dose SDP group. Compared with the model group, the levels of SP in SD and SDP each dose group had no obviously different.

Table 3 Effects of SDP on VIP, PGE₂, and SP in mice ($\bar{x} \pm s$, $n = 8$)

Groups	Doses / (g·kg ⁻¹)	VIP / (ng·L ⁻¹)	PGE ₂ / (pg·L ⁻¹)	SP / (ng·L ⁻¹)
control	–	6.71 ± 4.98	25.82 ± 9.25	22.30 ± 5.32
model	–	14.60 ± 0.32 [△]	48.62 ± 3.691 ^{△△}	33.23 ± 3.06 ^{△△}
SD	35	10.95 ± 1.83 [*]	34.16 ± 5.75 [*]	31.06 ± 4.56 [△]
SDP	2	11.29 ± 2.68	35.08 ± 5.24 [*]	33.75 ± 3.92 ^{△△}
	1	11.27 ± 2.35	44.03 ± 5.80	30.16 ± 2.16 [△]
	0.5	9.24 ± 1.83 ^{**}	34.68 ± 6.51 [*]	31.30 ± 5.98

4. Discussion

SD, the basic recipe of activating *qi* and strengthening spleen, is deeply researched in gastrointestinal pharmacology. Researches have proved that total polysaccharides from SD had the effects on immune regulation and treatment of gastrointestinal diseases. Polysaccharides compounds, as new drugs, became more and more useful in clinic because they had less toxic side effects and more safety and effectiveness (Zhu and Luo, 2007; Zhang et al, 2010). The aim of our study is to explore if the total polysaccharides from SD could replace the SD to treat patients with spleen deficiency syndrome.

Administration of water extract from *Rhei Radix* et *Rhizoma* as well as swimming is one of the most commonly used methods for spleen deficiency model, with simple preparation and high modulus characteristics. In the present study, after administration of water extract from *Rhei Radix* et *Rhizoma* for 14 d, the mice had some symptoms such as towering hair, extrados, eating less, and so on, which showed that the spleen deficiency model had been established successfully (Jia et al, 2006). Gastrointestinal motility is an important physiological functions of the digestive system, and gastrointestinal hormones in the regulation of gastrointestinal motility play an important role, which regulate the secretion, movement, absorption, blood flow, and cell nutrition of the digestive tract (Hu et al, 2011; Yang et al, 2010). The disorder of gastrointestinal movement is a usual symptom of spleen deficiency. In this study, the rates of intestinal propulsion and gastric emptying were used to observe the amount of drugs on gastrointestinal digestion and absorption. In order to find the mechanism, MTL, VIP, PGE₂, and SP were selected to observe the drug effects.

MTL is a regulatory polypeptide of 22 amino acid residues and originates in MTL cells scattered in the gastrointestinal tracts of pigs, rats, cows, and cats, and in the central nervous system of rabbits. MTL could induce phase III contractions in the stomach. Phase III activity has an

important role in gastric emptying and digesting, and hence prevents the bacterial overgrowth in the upper gut, to increase the migrating myoelectric complex component of gastrointestinal motility, stimulate the production of pepsin, and improve peristalsis in the small intestine. A high level of MTL secreting into blood could stimulate the contraction of fundus and antrum, and accelerate the gastric emptying. It then contracts the gallbladder and increases the squeeze pressure of the lower esophageal sphincter (Iton, 1997). In our experiment, compared with the model group, SDP could decrease the rate of intestine transit, whereas increase the rate of gastric emptying, and SDP had some positive effects to regulate the disorder of spleen deficiency syndrome.

PGE₂ has a strong contraction on intestinal smooth muscle, and it accelerates the bowel movement, promotes intestinal movement, inhibits the absorption of water and electrolytes in the intestine, and stimulates the intestinal fluid secretion.

SP is found mostly in peripheral afferent nerve fibers and in the gastrointestinal system. It is thought to be the primary neurotransmitter for nociceptive information. And SP is one of the strongest excitatory peptide neurotransmitters in regulation of intestine, it can inhibit the secretion of the gastrointestinal tract, stimulate intestinal movement, and inhibit the gastric emptying (Zheng et al, 2008; Mineko and Akio, 2000). VIP plays an important role in the mediation of non-noradrenergic and non-cholinergic (NANC) relaxation of smooth muscles and in regulation of gastrointestinal motility. Strength stimulation of the rat colonic segment induced the descending relaxation and ascending contraction, the former being mediated by VIP-containing motor neurons and the latter by acetylcholine and SP-containing motor neurons (Mineko and Akio, 2000). VIP decreasing may result in the enteric nervous system transmission barriers. SP and VIP play the important roles in the regulation of intestinal movement and coordination role. The motility is increased because of these works together (Li and Ru, 2009).

The experimental results of the model group showed that

the levels of MTL, PEG₂, and SP were significantly increased. Using the water extract from *Rhei Radix et Rhizoma* to make model could cause the increase of MTL and PGE₂ levels, and this change was leading to the emergence of diarrhea. The inhibitory factor VIP did not reduce but increase, and this phenomenon was the regulatory role of the body reflex.

The experimental results showed that the SDP to some extents reduced the level of intestinal propulsion in mouse spleen and gastric emptying, MTL, and VIP, PGE₂ levels, and had no effect on SP. Compared to the traditional SD, SDP had more efficiency, and its mechanism may be related to the regulation of MTL, VIP, and the level of PGE₂.

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References

- Hu XJ, Huang SP, Deng SG, 2011. The decoction of invigorating spleen and regulating *qi* on functional dyspepsia rat gastrointestinal motility and motilin, gastrin effects. *China J Pharmacol Exp Ther* 17: 214-217.
- Iton Z, 1997. Motilin and clinical application. *Peptides* 18: 593-608.
- Jia HW, Zhao N, Zhang WD, Shan ZY, Zhang Z, Chen YP, Xiang LH, Wei L, Lin MC, Lu AP, 2006. Effects of Lizhong Wan and Sijunzi Tang on active movement of rats with spleen deficiency induced by reserpine. *Chin J Inf Tradit Chin Med* 13: 34-35.
- Li L, Ru LQ, 2009. Distribution and relationship of substance P, vasoactive intestinal polypeptide and cholinergic neurons in the intestinal tract of rat. *Chin J Neuroanat* 25: 663-666.
- Mineko F, Akio I, 2000. Peptidergic regulation of gastrointestinal motility in rodents. *Peptides* 21: 1565-1582.
- Wu CL, Hung CR, Chang FY, Lin LC, Pau KF, Wang PS, 2002. Effects of evodiamine on gastrointestinal motility in male rats. *Eur J Pharmacol* 457: 169-176.
- Xu S, Yan Q, Cheng XF, Zhu JH, Xu JL, 2004. The effect of Weileyin on MOT and GAS of spleen insufficiency rat. *J Chin Med* 19: 20-22.
- Yang LJ, Liu YL, Jin ZG, 2010. Acupuncture combined with medicine on motilin and gastrin secretion in rat model of spleen deficiency syndrome. *China J Basic Med Tradit Chin Med* 16: 1156-1157.
- Ye FQ, Chen YW, 2005. The four gentlemen decoction on the gastrointestinal effects of pharmacological research. *Lishizhen Med Mater Med Res* 16: 73-74.
- Zhang DP, Zhou L, Zhang ZM, Zhu XY, Cheng F, Wang Y, Xu JQ, Liu FH, 2010. Four polysaccharide on immune suppression of murine intestinal sIgA rats and its mechanism. *Tradit Chin Drug Res Clin Pharmacol* 21: 11-14.
- Zheng XB, Ye QL, Dai SX, Zou Y, Wu D, 2008. Targeted regulation of Zhishu Decoction on the genes expression of substance P and calcitonin gene-related peptide in mice with spleen-deficiency constipation. *Chin J Integr Trad West Med Dig* 16: 155-158.
- Zhu XX, Luo XG, 2007. The extraction and purification of polysaccharides. *Food Res Dev* 28: 186-188.