

Basic Study

Inhibitory effect of TongXie-YaoFang formula on colonic contraction in rats

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Abstract

AIM: To investigate the pharmacological effect of

TongXie-YaoFang (TXYF) formula and its underlying mechanisms.

METHODS: A neonatal maternal separation plus restraint stress (NMS + RS) model of diarrhea-predominant irritable bowel syndrome was developed by subjecting male Sprague-Dawley rats to daily maternal separation from postnatal days 2 to 21 plus restraint stress from days 50 to 59. Rats were randomly divided into two groups (NMS + RS and TXYF formula), and rats with no handling or separation were used as normal controls. Starting from postnatal day 60, rats were administered TXYF formula (9.84 g/100 g body weight) orally twice daily for 14 consecutive days, while the normal and NMS + RS groups were given distilled water. The distinctions of movement index (MI, area under the curve of contraction intensity/min, mg/min) and contraction frequency (CF, number of contractions/min, times/min) of isolated colonic longitudinal smooth muscle strips (CLSMs) in the three groups before and after treatment were observed with a Power Lab system. Different inhibitors were applied, and then 10^{-4} mol/L acetylcholine chloride (Ach) was added to CLSMs to induce muscle contraction.

RESULTS: Before treatment, the MI of CLSMs in the NMS + RS and TXYF formula groups was similar and both higher than that in the normal group (545.49 ± 73.66 mg/min vs 245.76 ± 34.44 mg/min and 551.09 ± 54.29 mg/min vs 245.76 ± 34.44 mg/min, $P < 0.01$, respectively). After treatment, the MI in the TXYF formula group was lower than that in the NMS + RS group (261.39 ± 38.59 mg/min vs 533.9 ± 61.63 mg/min, $P < 0.01$). In the same way, the CF of CLSMs in the NMS + RS and TXYF formula groups was similar and both higher than that in the normal group (3.42 ± 0.25 times/min and 3.31 ± 0.21 vs 1.1 ± 0.17 times/min, $P < 0.01$) before treatment. After treatment, the CF in the TXYF formula group was lower than that in the NMS + RS group (1.42 ± 0.87 times/min vs 3.11 ± 0.82 times/min, $P < 0.01$) and similar to that in the normal group (1.42

± 0.87 times/min *vs* 1.09 ± 0.13 times/min). When 8-(*N,N*-diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride and 4-aminopyridine were added to the bath and equilibrated for 30 min, respectively, and 10^{-4} mol/L Ach was added to CLSMs to induce muscle contraction, MI of the CLSMs in the TXYF formula group was lower than that in the normal group (666 ± 36.32 mg/min *vs* 747.77 ± 49.47 mg/min, and 686.53 ± 39.17 mg/min *vs* 750.45 ± 29.39 mg/min; $P < 0.01$, respectively). The MI of CLSMs in the TXYF formula group was lower than that in the normal group after treatment with nifedipine (689.48 ± 30.84 mg/min *vs* 741.65 ± 32.41 mg/min; $P < 0.05$).

CONCLUSION: TXYF formula inhibits colon contraction in rats. This may be related to activation of specific potassium channels and inhibition of extracellular calcium internal flow.

Key words: Irritable bowel syndrome; Neonatal maternal separation; Herbal medicine; Colonic longitudinal smooth muscle; Potassium channel

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Core tip: Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic gastrointestinal disease that seriously affects quality of life. Colon dysmotility is recognized as one of the pathophysiological factors. TongXie-YaoFang formula can effectively relieve diarrhea and lower visceral hypersensitivity in D-IBS rats, mainly *via* inhibiting the amplitude and frequency of colonic longitudinal smooth muscle contraction. Its action of mechanism may be related to the activation of specific potassium channels, inhibition of extracellular calcium internal flow, and reduction of intracellular free Ca^{2+} concentration.

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INTRODUCTION

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. The diagnosis of D-IBS is based on symptom assessment and the Rome III diagnostic criteria^[1]. The pathogenesis of D-IBS has not been fully clarified and colon dysmotility is recognized as one of the pathophysiological factors^[2]. The usual treatment of the disease in Western medicine involves symptomatic therapy, which is unsatisfactory for patients, while simultaneously increasing the use of healthcare resources^[3,4]. Traditional Chinese medicine (TCM)

can significantly improve symptoms and quality of life, therefore, increasing numbers of patients have begun to seek treatment with TCM^[5,6]. Many clinical studies have shown that TongXie-YaoFang (TXYF) formula can significantly improve D-IBS clinical symptoms and enhance quality of life^[7,8]. However, the specific mechanism of action has not been completely elaborated. The purpose of this study was to observe the effect of TXYF formula on contraction of colon smooth muscle in D-IBS rats, and discuss its mechanism of action.

MATERIALS AND METHODS

Neonatal maternal separation plus restraint stress

Neonatal Sprague-Dawley rats were obtained on postnatal day 1 from Vital River Laboratories Animal Technology Co. Ltd. (Beijing, China), and kept at Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine. The pups were randomly assigned to one of the following two rearing conditions: (1) neonatal maternal separation plus restraint stress (NMS + RS); or (2) no handling or separation (normal).

The NMS + RS litters were removed from their cages and separated from their dams for 3 h each day, whereas the normal pups remained in their home cages during the period of postnatal days 2-21^[9,10]. During the 3-h period of separation, the pups were removed from the nest to stand-alone compartments, where the temperature was maintained at 23 ± 0.5 °C in a thermally regulated facility. The litters were returned to their home cages immediately after separation^[11]. All the rats were reared on a 12:12 h light-dark cycle (lights on at 08:00 h) with access to food and water *ad libitum*. On day 22, the sexes of the pups, including those in the normal group, were distinguishable, so the females were removed and the males retained^[12].

On days 50-59, the NMS + RS rats were placed in transparent plastic restraint cylinders (4 cm × 4 cm × 18 cm), in which they could move forward and backward but could not turn around^[13]. The rats remained in the restraint cylinders for 3 h, with access to food and water *ad libitum*, in the morning and afternoon of each day. The NMS + RS rats were divided into two groups (NMS + RS and TXYF formula).

All animal care and experimental procedures were conducted according to the institutional ethical guidelines and conformed to the requirements of the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine and the Animal Ethics Committee of Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine.

Herb materials and drug administration

TXYF formula is composed of the following traditional Chinese herbal medicines: Bai zhu (*Atractylodes macrocephala* Koidz - *Acta Horti Gothoburgensis* 1938;

12: 310), 93.75 g; Shao yao (*Paeonia lactiflora* Pall - *Reise Russ Reich* 1776; 3: 286), 62.5 g; Chen pi (*Citrus reticulata* Blanco - *Fl Filip* 1837; 610), 46.875 g; and Fang feng [*Saposhnikovia divaricata* (Turcz) Schischk - *Fl URSS* 1951; 17: 359], 31.25 g. It was manufactured by Preparation Room for TCM of Beijing Chinese Medicine Hospital. All raw materials in the formula were examined according to the quality control criteria in the Chinese Pharmacopeia^[14].

From postnatal day 60, the rats in the TXYF formula group were treated daily with orally administered TXYF formula (9.84 g/100 g body weight). The normal and NMS + RS groups were given distilled water. The delivery volume in each group was always 2 mL/100 g/d, for 14 consecutive days.

Tissue preparation and experiments

Rats were anesthetized abdominally with 7% chlorohydrate (35 mg/100 g body weight). The distal colon (6-7 cm from the anus) was quickly taken and incised longitudinally along the mesenteric border. Two longitudinal smooth muscle strips (0.8 cm × 0.2 cm) were obtained^[15]. Specimens of intestinal muscle were ligatured with a medical thread at both ends. One end of the specimen was fixed to the bottom of the bath with the other connected to a physiological recorder through the tonotransducer. Specimens of the initial load was 1 g (2 mV) to maintain basic tension. Krebs solution (15 mL) was injected into the four baths at a temperature of 37 °C with consecutively circulating 95% oxygen and 5% CO₂. The tissues were allowed to equilibrate for 60 min with fresh Krebs solution at 15-min intervals before the experiment^[16].

Reagents

Krebs solution had the following composition: 117 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L MgCl₂, 24.8 mmol/L NaHCO₃, 1.2 mmol/L KH₂PO₄, 2.56 mmol/L CaCl₂ and 11.1 mmol/L glucose. 8-(*N,N*-diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8), 4-aminopyridine (4-AP), nifedipine, apamin, *N*-ethylmaleimide (NEM), methylene blue, and *N*^G-nitro-*L*-arginine methyl ester (L-NAME) were used. All of the reagents were purchased from Sigma-Aldrich (St Louis, MO, United States).

Apparatus

ML110 Powerlab amplifier, ML740 four-channel recorder, MLT02021D tonotransducer, and Power Lab/4sp analysis system for physiological data were all purchased from AD Instruments Shanghai Trading Co., Ltd (Pudong New Area, Shanghai, China).

Statistical analysis

All experimental data are expressed as mean ± SE. The changes before and after treatment were analyzed using one-way analysis of variance. The differences between groups were analyzed by comparing the

values for each group using a paired *t* test. *P* < 0.05 was considered statistically significant.

RESULTS

TXYF formula inhibits movement index and contraction frequency of colonic longitudinal smooth muscle strips in rats with D-IBS

Under baseline conditions, the movement index (MI) of colonic longitudinal smooth muscle strips (CLSMs) in the NMS + RS and TXYF formula groups was similar and higher than that in the normal group (545.49 ± 73.66 mg/min and 551.09 ± 54.29 mg/min vs 245.76 ± 34.44 mg/min, *n* = 8, *P* < 0.01; Figure 1A). After treatment, MI in the TXYF formula group was lower than that in the NMS + RS group (261.39 ± 38.59 mg/min vs 533.9 ± 61.63 mg/min, *n* = 8, *P* < 0.01; Figure 1A) and similar to that in the normal group (261.39 ± 38.59 mg/min vs 244.78 ± 32.15 mg/min, *n* = 8, *P* = 0.365; Figure 1A).

The same result was seen for contraction frequency (CF) of CLSMs. Before treatment, CF in the NMS + RS and TXYF formula groups was similar and higher than that in the normal group (3.42 ± 0.25 times/min and 3.31 ± 0.21 times/min vs 1.1 ± 0.17 times/min, *n* = 8, *P* < 0.01; Figure 1B). After treatment, CF in the TXYF formula group was lower than that in the NMS + RS group (1.42 ± 0.87 times/min vs 3.11 ± 0.82 times/min, *n* = 8, *P* < 0.01; Figure 1B). After treatment, the CF in the TXYF formula group was lower than that in the NMS + RS group and close to that in the normal group.

Significant differences in MI of CLSMs after treatment with TMB-8, 4-AP and nifedipine between the TXYF formula and normal groups

When TMB-8 was added to the bath and equilibrated for 30 min, and 10⁻⁴ mol/L Ach was added to CLSMs to induce muscle contraction, the MI of rat CLSMs in the TXYF formula group was lower than that in the normal group (666 ± 36.32 mg/min vs 747.77 ± 49.47 mg/min, *n* = 6, *P* < 0.05; Figure 2A and B). There were significant differences in MI of CLSMs between the TXYF formula and normal groups after treatment with 4-AP and nifedipine (686.53 ± 39.17 mg/min vs 750.45 ± 29.39 mg/min, and 689.48 ± 30.84 mg/min vs 741.65 ± 32.41 mg/min, *n* = 6, *P* < 0.05; Figure 2A).

No significant differences in MI of CLSMs between TXYF formula and normal groups after treatment with apamin, NEM, methylene blue and L-NAME

After treatment with apamin and NEM, MI of CLSMs in the TXYF formula and normal groups was similar (736.15 ± 32.41 mg/min vs 766.27 ± 32.41 mg/min, and 786.47 ± 32.11 mg/min vs 775.33 ± 35.93 mg/min, *n* = 6, *P* > 0.05, respectively; Figure 3). The MI of CLSMs in the TXYF formula and normal groups

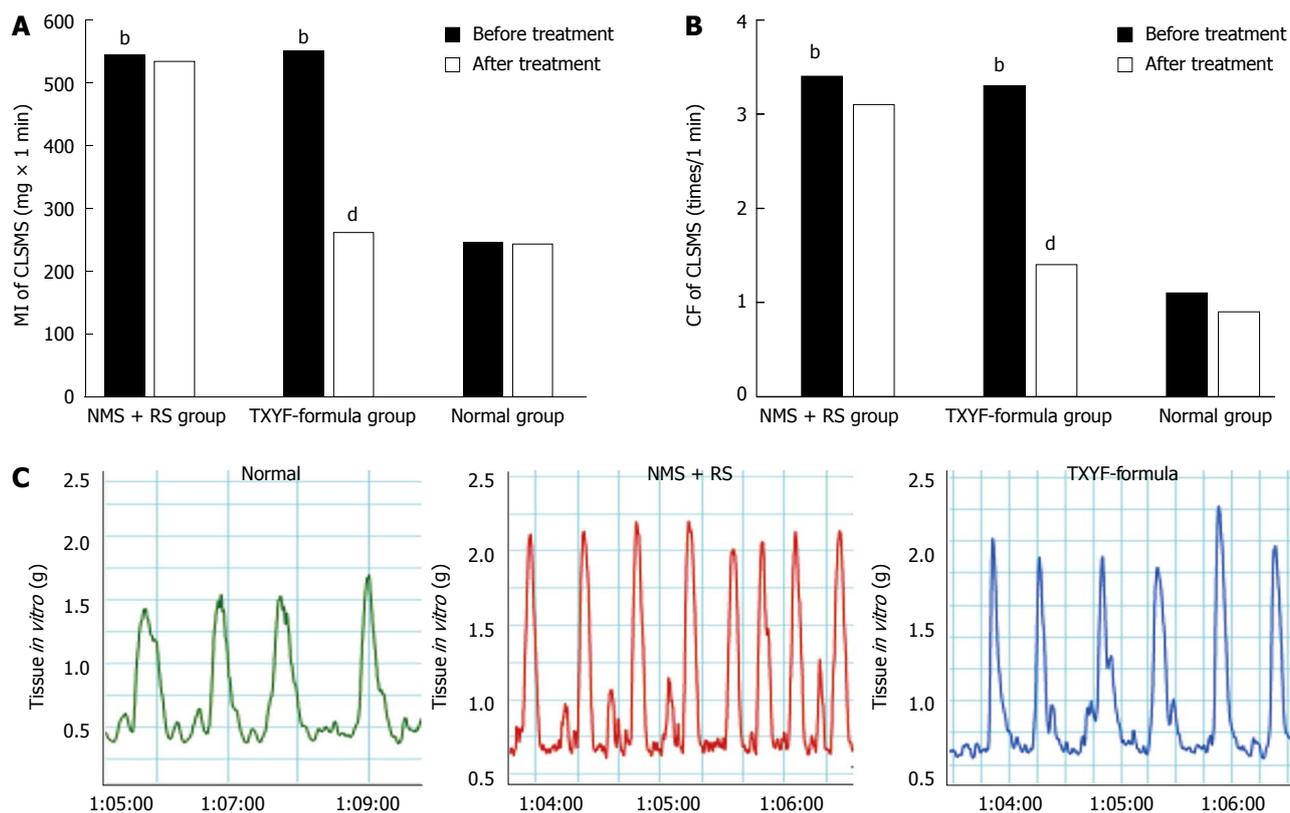


Figure 1 Effects of TongXie-YaoFang formula on movement index and contraction frequency of colonic longitudinal smooth muscle strips in rats. A: TongXie-YaoFang (TXYF) formula significantly reduced the movement index (MI) of colonic longitudinal smooth muscle strips (CLSMS) in neonatal maternal separation plus restraint stress (NMS + RS) rats; B: TXYF formula significantly reduced contraction frequency (CF) of CLSMS in NMS + RS rats; C: CF in the three groups after treatment. ^b*P* < 0.01 vs the normal group; ^d*P* < 0.01 vs the NMS + RS group.

was similar after treatment with methylene blue and L-NAME (744.48 ± 37.75 mg/min vs 754.27 ± 27.69 mg/min, and 755.43 ± 36.15 mg/min vs 760.7 ± 33.08 mg/min, *n* = 6, *P* > 0.05, respectively; Figure 3).

DISCUSSION

Diarrhea, abdominal pain and discomfort are the primary clinical symptoms of D-IBS, whose etiology and pathogenesis are complex. Its pathophysiological changes mainly include abnormal gastrointestinal dynamic disorder and visceral sensory sensitivity^[17,18]. In TCM, D-IBS belongs to diarrhea or abdominal pain, according to its clinical manifestation. Its main pathogenesis is stagnation of liver qi and spleen deficiency, disharmony between liver and spleen, and spleen deficiency wet-sheng^[19].

The role of Bai zhu, in the TXYF formula, is Jian-Pi Hua-Shi (invigorating spleen and eliminating dampness), while Shao yao is Yang-Yin Rou-Gan (nourishing yin and soothing liver). Modern pharmacological studies have shown that they had an analgesic effect on visceral pain and an inhibitory effect on bowel movement^[20,21]. The role of Chen pi, in the TXYF formula, is Li-Qi Xing-Pi (regulating qi-flowing and amusing spleen), and Fang feng is Qu-Shi Zhi-Tong (dispelling dampness and relieving pain). A previous pharmacological study has shown that Fang feng increases intestinal pressure

threshold in rats so as to exert its analgesic effect^[22]. In this study, we used the method of NMS combined with RS to establish an animal mode of D-IBS, with the main simultaneous symptoms of diarrhea and high visceral sensitivity. This recapitulates the clinical symptoms of patients with D-IBS.

Modern pharmacological studies have shown that TXYF formula has an inhibitory effect on bowel movement and reduces intestinal peristalsis^[23,24]. One study has demonstrated that NMS upregulates L-type Ca²⁺ channel expression in the colon, which contributes to colonic motility disorder^[25]. Yuan *et al.*^[26] have shown that TCYF inhibits the contraction of isolated rat colonic smooth muscle strips, mainly by preventing the influx of extracellular Ca²⁺.

In the present study, the contraction amplitude of CLSMS excited by Ach was lower in the TXYF formula group than in the normal group after incubation with nifedipine for 20 min. This indicated that the diastolic effect of TXYF-formula on CLSMS is probably achieved by inhibiting voltage dependent calcium channel and reducing intracellular Ca²⁺ concentration. After treatment with TMB-8 and 4-AP for 30 min, contraction amplitude of CLSMS induced by Ach in the TXYF formula group was lower than that in the normal group. This demonstrated that the inhibitory effects of TXYF formula on CLSM contraction might be achieved by means of: (1) inhibition of calcium release in

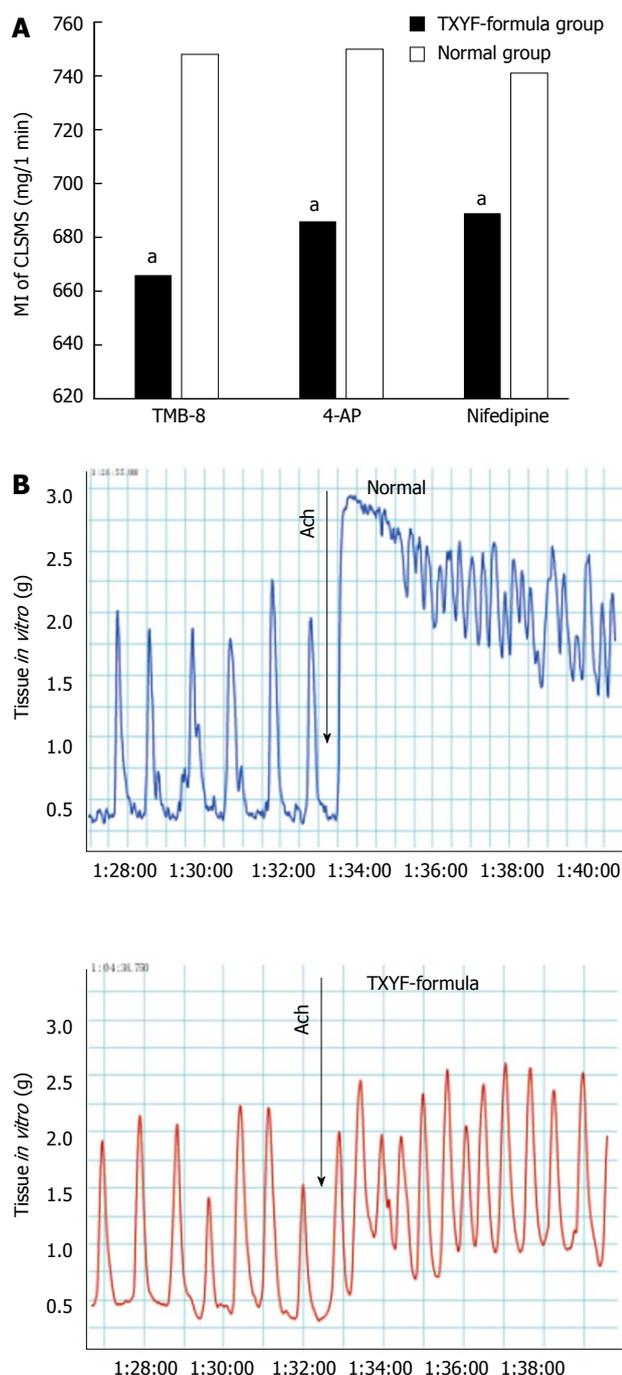


Figure 2 Effects of TMB-8, 4-aminopyridine and nifedipine on movement index of colonic longitudinal smooth muscle strips in rats. A: After treatment with 8-(*N,N*-diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8), 4-aminopyridine (4-AP) and nifedipine, the movement index (MI) of colonic longitudinal smooth muscle strips (CLSMS) in the TongXie-YaoFang (TXYF) formula group was lower than that in the normal group ($n = 8$, $P < 0.05$); B: After treatment with TMB-8, MI of rat CLSMS in the TXYF formula group was lower than that in the normal group. $^aP < 0.05$ vs normal group.

intracellular sarcoplasmic reticulum, which results in a decrease of intracellular calcium concentration; and (2) activation of specific potassium channels.

In conclusion, TXYF formula inhibited the contraction of isolated CLSMS in rats with D-IBS. Its mechanism may be related to the activation of specific potassium channels, inhibition of extracellular calcium internal flow,

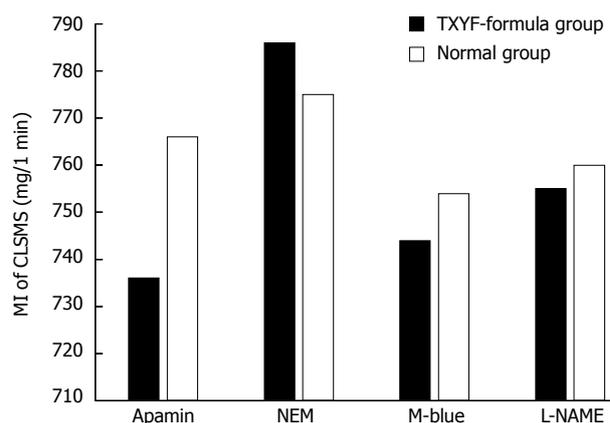


Figure 3 Effects of apamin, nifedipine, apamin, *N*-ethylmaleimide, methylene blue, methylene blue and *N*⁶-nitro-*L*-arginine methyl ester on movement index of colonic longitudinal smooth muscle strips in rats. NEM: Nifedipine, apamin, *N*-ethylmaleimide, Methylene blue; L-NAME: *N*⁶-nitro-*L*-arginine methyl ester; MI: Movement index; CLSMS: Colonic longitudinal smooth muscle strips.

and reduction of intracellular free Ca²⁺ concentration.

COMMENTS

Background

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. It seriously affects quality of life. The pathogenesis of D-IBS has not been fully clarified, although colon dysmotility is recognized as one of the pathophysiological factors. The usual treatment in Western medicine involves symptomatic therapy, which is unsatisfactory for patients, while simultaneously increasing the use of healthcare resources. Traditional Chinese medicine (TCM) can significantly improve symptoms and quality of life, so increasing numbers of patients have begun to seek treatment with TCM. Many clinical studies have shown that TongXie-YaoFang (TXYF) formula can significantly improve D-IBS clinical symptoms and enhance their quality of life.

Research frontiers

TXYF formula is a classic Chinese herbal formula for clinical treatment of D-IBS. In the area of treatment of D-IBS with TXYF formula, the research hotspot is its effect on colon movement, visceral sensitivity, and correlational ion channels.

Innovations and breakthroughs

Previous correlational studies have shown that TXYF formula can affect bowel movement mostly through the observation of intestinal fecal propulsion in D-IBS rats using the method of short-term building. There have been few studies on contraction amplitude and frequency of colon tissues *in vitro*. The current view is that D-IBS is a chronic disease and related to early adverse life events. In the present study, the movement index (area under the curve of contraction intensity/min, mg/min) and contraction frequency (the number of contraction/min, times/min) of isolated colonic longitudinal smooth muscle strips were observed with a Power Lab system.

Applications

The study results suggest that TXYF formula can inhibit the contraction of rat colon, including the amplitude and frequency. This may be related to the activation of specific potassium channels and inhibition of extracellular calcium internal flow.

Terminology

D-IBS is a chronic functional gastrointestinal disease and related to early adverse life events. It seriously affects quality of life. TXYF formula can inhibit contraction amplitude and frequency of colon tissues *in vitro*. This may be related to the activation of specific potassium channels and inhibition of extracellular calcium internal flow.

Peer-review

TXYF formula, a Chinese herbal formula, can effectively relieve diarrhea and lower visceral hypersensitivity of D-IBS rats mainly *via* inhibiting the contraction of the colonic longitudinal smooth muscle. This research shows that the

mechanism may be related to the activation of specific potassium channels, inhibition of extracellular calcium internal flow and reduction of intracellular free Ca^{2+} concentration.

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