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Original Article

Post-marketing Re-evaluation of Tongxiening Granules (痛泻宁颗粒) in Treatment of Diarrhea-Predominant Irritable Bowel Syndrome: A Multi-center, Randomized, Double-Blind, Double-Dummy and Positive Control Trial

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ABSTRACT **Objective:** To evaluate the efficacy and safety of Tongxiening Granules (痛泻宁颗粒, TXNG) in the treatment of irritable bowel syndrome with predominant diarrhea (IBS-D). **Methods:** A randomized, double-blind, double-dummy, and positive parallel controlled clinical trial was conducted from October 2014 to March 2016. Totally 342 patients from 13 clinical centers were enrolled and randomly assigned (at the ratio of 1:1) to a treatment group (171 cases) and a control group (171 cases) by a random coding table. The patients in the treatment group were administered orally with TXNG (5 g per time) combined with pinaverium bromide Tablet simulator (50 mg per time), 3 times per day. The patients in the control group were given TXNG simulator (5 g per time) combined with pinaverium bromide Tablets (50 mg per time), 3 times per day. The treatment course lasted for 6 weeks. The improvement of Irritable Bowel Syndrome Symptom Severity Score (IBS-SSS) was used to evaluate the primary outcome. Secondary outcomes included adequate relief (AR) rate, Irritable Bowel Syndrome-Quality of Life Questionnaire (IBS-QOL), Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), and the recurrence rate at follow-ups. Safety indices including the adverse events (AEs) and related laboratory tests were evaluated. **Results:** Primary outcome: IBS-SSS at baseline, weeks 2, 4, 6 showed no statistical significance in both full analysis set (FAS) and per protocol set (PPS, $P>0.05$). After 6 weeks of treatment, the total effective rate of IBS-SSS scores in the treatment group (147/171, 86.0%) was higher than the control group (143/171, 83.6%) by FAS ($P>0.05$). In regard to secondary outcomes, after 6-week treatment, there was no significant difference in AR rate, total score of IBS-QOL, improvement of HAMD and HAMA total scores between the two groups ($P>0.05$). The recurrence rate at 8-week follow-up was 12.35% (10/18) in treatment group and 15.79% (12/76) in control group, respectivery ($P>0.05$). A total of 21 AEs occurred in 15 cases, of which 11 occurred in 8 cases in the treatment group and 10 AEs in 7 cases in the control group. The incidence of AEs had no statistical significance between the two goups ($P>0.05$). **Conclusion:** Tongxiening Granules could relieve the symptoms of patients with IBS-D and the treatment effect was comparable to pinaverium bromide. (No. ChiCTR-IPR-15006415)

KEYWORDS Tongxiening Granules, irritable bowel syndrome with predominant diarrhea, post-marketing evaluation, randomized controlled trial

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Irritable bowel syndrome (IBS) is a common disease in digestive system. According to a prevalence research, the incidence of IBS in Guangzhou, China is about 5.6%,[1] of which IBS with predominant diarrhea (IBS-D) is the most common type. IBS-D is characterized by abdominal pain, diarrhea and abdominal discomfort. The pathogenesis may be related to factors such as brain-intestine axis disorders, visceral hypersensitivity, intestinal motility abnormalities, and intestinal flora alternation. According to Chinese medicine (CM) theory, the occurrence of IBS is related to emotional disorders, over-conscientiousness, and eating disorders. Gan (Liver) depression, Pi (Spleen) deficiency and overabundance of dampness is the primary pathogenesis.[2] In CM clinic, the treatment methods mainly focus on promoting qi flow of Gan, improving emotional depression, strengthening Pi, eliminating dampness and alleviating the pain and other symptoms. Our previous work has found that Chang'an Ⅰ Recipe (肠安Ⅰ号方), a Chinese medicinal formula acted as similar pathogenesis and showed a better clinical effect over placebos in patients with IBS-D.[3]

By the means of softening Gan, strengthening Pi, regulating qi and removing dampness, Chinese patent medicine Tongxiening Granules (痛泻宁颗粒, TXNG), including *Radix Paeoniae alba*, *Atractylodes Macrocephala*, *Pericarpium Citri Reticulatae Viride* and *Allium Macrostemon*, has been applied in patients with IBS-D who suffered from abdominal pain, diarrhea, abdominal distension and other symptoms induced by the pattern of Gan-qi attacking Pi.[4] Phase Ⅱ and Ⅲ clinical trials results have indicated that the symptoms of abdominal pain and diarrhea could be significantly relieved by TXNG in patients with IBS-D.[2,4] To gain a more specific effectiveness and safety data of TXNG, a randomized, double-blind, double-dummy, positive control, multi-center clinical trial was conducted.

METHODS

Diagnostic Criteria

IBS diagnosis and classification was referenced to Rome Ⅲ criterion.[5] IBS-D was diagnosed as loose (mushy) or watery stools more than or equal to 25% and hard or lumpy stool less than 25% of bowel movements.

Inclusion and Exclusion Criteria

Patients were included when they met the following criteria (1) aged between 18 and 65 year-old; (2) met the diagnostic criteria for IBS-D;[5] (3) the baseline IBS Symptom Severity Score (IBS-SSS) >75 points;[6] (4) the informed consent forms were signed. The exclusion criteria are listed below: diarrhea caused by infection, inflammatory bowel disease, malabsorption syndrome, hyperthyroidism, systemic diseases, intoxication, parasitic infection, malignant tumors, etc.; patients with organic diseases of the digestive system (chronic pancreatitis, etc.), or systemic diseases (hyperthyroidism, diabetes, chronic renal insufficiency, mental or nerve systems diseases, etc.) that affect the digestive motility; those who are taking or need to keep taking drugs that may affect the gastrointestinal function, e.g., anticholinergic drug, calcium channel blockers, 5-hydroxytryptamine ($5\text{-}HT_3$) receptor antagonists, antidiarrheal agents, antacids, prokinetic agents, antidepressants, antianxiety agent and intestinal flora regulators; patients with a history of abdominal surgery (e.g., cholecystectomy, etc.); patients who have taken other Chinese or Western medicines for treating IBS within 2 weeks; pregnancy or lactation; severe systemic diseases, such as heart, brain, liver, kidney, endocrine or blood diseases, among whom the blood ALT and AST > 1.5 times of the upper limit of normal value (ULNV), Cr > 1.2 times of the ULNV; allergic constitution or allergic to test drug components; disabled patients in law (blind, deaf, dumb, mental retardation, mental disorders, etc.); those who participate in other clinical trials in the last 3 months.

Withdrawal Criteria

The researcher decided to withdraw patients for following reasons: allergic reactions or serious adverse events (AEs) occurred, and the trial should be stopped by the judgment of the investigators; patients in worsening condition which should quit for other treatment; subjects with poor compliance (compliance < 80% or > 120%), or change for another medication or take forbidden Chinese and Western drugs; patients with unintended unblinding. The subject could withdraw from the trial for personal reasons, or just be unwilling or impossible to continue the clinical trial, and requests for another medication; or be unavailable and do not accept clinical intervention.

Suspension Criteria

Severe AEs occurred during the clinical trial;

serious mistake in clinical trial scheme or serious deviation in implementation that made it impossible to evaluate drug efficacy; poor therapeutic efficacy; the sponsor requested to discontinue the trial; and the administrative department canceled the trial.

Rejection Criteria

Serious violation of inclusion or exclusion criteria; those who took no drug in the trial; lack of test record; took prohibited drugs that made it impossible for statistical analysis, etc. The final rejection decision was determined by the database blind verification committee.

Patients

This trial was performed from October 2014 to March 2016. Subjects were included through a combination of outpatient clinic and open recruitment through advertisement from 13 clinical centers including Xiyuan Hospital, China Academy of Chinese Medical Sciences; Beijing Hospital of Traditional Chinese Medicine, Capital Medical University; Union Hospital, Tongji Medical College, Huazhong University of Science and Technology; Shengjing Hospital of China Medical University; West China Hospital, Sichuan University; Affiliated Hospital of Chengdu University of Traditional Chinese Medicine; Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine; First People's Hospital of Yichang; Second Affiliated Hospital of Shaanxi College of Traditional Chinese Medicine; Chongqing Traditional Chinese Medicine Hospital; Harbin Hospital of Traditional Chinese Medicine; Yuncheng Central Hospital of Shanxi Province and Second Affiliated Hospital of Shandong Traditional Chinese Medicine University. This clinical trial followed the Declaration of Helsinki and China's relevant clinical research regulations. It was approved by the Ethics Committee of Xiyuan Hospital of China Academy of Chinese Medical Sciences (Approval No. 2013XL067-4) and agreed by the ethic committees of all participating organizations, and all patients had signed written informed consent. The patients recruited in each clinical center are listed in Appendix 1.

Sample Size

Considering that this study was a small sample exploratory clinical trial for the post-marketing evaluation and referring to the phase Ⅱ clinical trial design, 150 cases were initially identified for the treatment group. According to the assigned ratio of 1:1 for treatment and control groups, and taking into account of 20% of dropout, 180 patients were accepted for each group, with a total of 360 patients in all centers.

Trial Design Overview

A randomized, double-blind, double-dummy, positive control, multicenter clinical trial design was applied. The research centers were stratified, and the treatment and control groups were allocated at the ratio of 1:1. Professional statisticians used the SAS software to generate random numbers for allocating codes to research center, trial cases, treatment groups and generating random coding tables for trial cases.

Blinding and Reservation

The biostatisticians conducted the blinding design. The sponsor appointed the unrelated personnel of clinical trial to distribute the medicine (test drug and control drug) according to the Test Drug Packaging Form. The abovementioned blinding scheme, together with initial random number, block length, etc., have been duplicated, sealed and reserved by the sponsor and principle investigator. All the drug coding processes should be recorded.

Blinding Method

The study adopted a double-blind design. Patients who met the inclusion criteria were assigned with the drug numbers sequentially according to the order of screening, and the drug administrator or researcher distributed the drug based on the drug number. Test drug, positive control drug, and the simulation agent were all in the same package. Subject did not know which drug was used, and the remaining drugs were returned to the researcher or drug administrator.

Emergency Unblinding

Each drug was equipped with a corresponding emergency letter. Only when serious AEs occur or the type of drug needs to be identified immediately, the primary investigator of each clinical center disclosed the letter for emergency unblinding. Once the letter was opened, the patient would be suspended from the trial and treated as a dropout case. The result should be informed to the monitor at the same time.

Unblinding Provisions

After all the research data were recorded and locked, staff who were responsible for the blinding scheme reservation, principle investigator, sponsor, and the statisticians together worked for the 2 phrases work of unblinding.

Interventions

Patients in the treatment group took TXNG (5 g per time, Chongqing Pharscin Pharmaceutical Co., Ltd., Lot No. 140402, 150101) and pinaverium bromide Tablet simulator (50 mg per times, Chongqing Pharscin Pharmaceutical Co., Ltd, Lot No.140401). Patients in the control group took TXNG simulator (5 g per time, Chongqing Pharscin Pharmaceutical Co., Ltd, Lot No. 140401) and pinaverium bromide Tablet (50 mg per time, Solvay Pharmaceuticals, Lot No. 629183, 634182). All drugs were orally administrated 3 times per day for 6 weeks. Other Chinese medicines, drug, and therapies related to IBS were prohibited during the observation period.

Primary Outcomes

IBS-SSS was calculated as the primary outcome. The degree and frequency of abdominal pain, degree of abdominal distention, defecation satisfaction, and qualiyt of life were analyzed. The total score was 500 points including 5 items with scale varying from 0 to 100 points. Patients were required to fill the form before test and at the time of 2, 4 and 6 weeks after treatment.

According to IBS-SSS,[6,7] there are 4 graded outcomes including remission (less than 75 points), mild (76–175 points), moderate (176–300 points), and severe (over 300 points), respectively. Remission was considered as cured, 2 grades improvement as markedly improved, 1 grade improvement as improved, no improvement or worsen condition as ineffective. Efficacy rate = (cured + markedly improved + improved)/ total cases × 100%.

Secondary Outcomes

Adequate Relief

In regard to the question "over the past week, have you had adequate relief of your IBS symptoms?", the subject was required to answer "yes" or "no". The data was observed and recorded at the time of enrollment, 2, 4 and 6 weeks after treatment, respectively.[8] Adequate relief (AR) rate = AR/overall enrollment × 100%.

IBS-Quality of Life Questionnaire Score

The questionnaire consisted of 34 items, each item included 5-point response scale. The questionnaire was filled in by the patients themselves at the time of enrollment, 2, 4 and 6 weeks after the treatment, respectively.[9] The total score of each item was calculated and analyzed.

Hamilton Anxiety Scale and Hamilton Depression Scale

The Hamilton Anxiety Scale (HAMA)[10] and Hamilton Depression Scale (HAMD)[11] were used for evaluating the psychosocial status of patients at the enrollment and 6 weeks after treatment at each group. The total score of each item was calculated and analyzed.

Recurrence Evaluation

Patients who had achieved the therapeutic goals were followed up for another 8 weeks. The recurrence rate was observed at 4 and 8 weeks after drug discontinuance. If cured patients whose IBS-SSS changed from remission to mild or a more severe state, they were considered as recurrence. The recurrence rate = recurrent patients/total follow-up patients × 100%.

Safety Evaluation

During the study period, the patient's vital signs, AEs, and safety findings were observed. The blood, urine, stool routine and occult blood, blood sedimentation rate, fasting blood-glucose, liver function, renal function, and electrocardiogram were examined for the safety evaluation.

Follow-Up

After 6-week treatment, the patients whose IBS-D symptoms were successfully relieved (IBS-SSS rates between 0–75 points) were followed up for next 8 weeks.

Statistical Analysis

SAS9.1.3 software was used for statistical analysis. Data analysis set included full analysis set (FAS) and per protocol set (PPS). FAS refers to cases which at least one medication was taken and at least one major index data was measured after the treatment; PPS refers to cases that have completed the treatment, follow-up visit and no serious violations of the protocol. Safety set (SS) included all cases that are randomized in this trial and at least one

medication had been taken.

For repeated measurement data, mixed effects model analysis was adopted, *t*-test, paired sample *t*-test, and nonparametric test were taken for other measurement data analysis. Enumeration data was analyzed by Chi-square test. FAS and PPS analysis were performed for the primary efficacy index and FAS analysis set for secondary efficacy indices. Employing a bilateral difference examination, *P* value less than 0.05 was considered statistically significant. For confounding factors that were difficult to control or uncontrolled before treatment, such as imbalances between groups before treatment, covariance analysis or logistic regression analysis were used.

RESULTS

Subject Enrollment and Baseline Data

Totally 342 patients were enrolled who all entered the FAS group including 171 patients in treatment and control groups, respectively. Totally 305 patients entered the PPS, with 155 in the treatment group, and 150 in the control group. The primary cause for dropout and rejection were loss to follow-up, violation of protocols and exceed visiting time limit (Figure 1). There was no statistical significance between the two groups in gender, age, course of disease, previous medication, and drug combination (*P*>0.05, Table 1). The differences of IBS-SSS, IBS-QOL, HAMD, and HAMA scores before treatment had no statistical significance between the two groups (*P*>0.05).

Primary Outcome

The primary outcome included IBS-SSS (FAS and PPS analysis). When the conclusions of the two are inconsistent, the FAS analysis results were mainly considered.

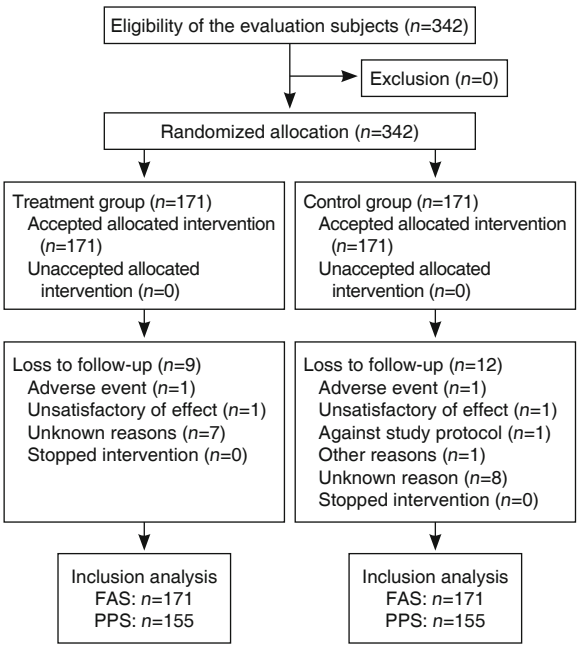

Figure 1. Flow Diagram of Clinical Trial of TXNG in Treatment of IBS-D Patients

Improvement of IBS-SSS

At the 2, 4 and 6 weeks after treatment, the mixed effects model analysis showed that compared with the pre-treatment in each group, the total IBS-SSS gradually decreased (*P*<0.05). The symptoms scores in both groups were significantly improved at a good time-effect manner. The IBS-SSS have no statistical significance between the two groups after treatment (*P*>0.05). The FAS result is consistent with PPS (Figure 2).

IBS-SSS Efficacy Rate

At 6 weeks after treatment, the cured, markedly improved, improved rates were 47.4% (81/171), 7.6%

Table 1. Baseline Data in Two Groups ($\bar{x} \pm s$, FAS)

Items	Treatment group (171 cases)	Control group (171 cases)	Statistics	*P* value
Age (Year)	43.97 ± 13.82	45.59 ± 12.81	–1.123[a]	0.2622
Gender (Cases, male/female)	85/86	93/78	0.750[b]	0.3865
Course of disease (Month)	24.22 ± 23.04	25.93 ± 35.27	0.206[c]	0.8366
Previous drugs (Case, Yes/No)	50/121	42/129	0.958[b]	0.3293
Drug combination (Cases, Yes/No)	9/162	5/166	1.192[b]	0.2750
IBS-SSS	240.49 ± 65.13	238.39 ± 63.59	0.302[a]	0.7625
IBS-QOL score	29.26 ± 20.10	28.99 ± 20.20	0.123[b]	0.9018
HAMD score	7.25 ± 4.64	7.04 ± 4.45	0.428[b]	0.6688
HAMA score	6.54 ± 3.92	6.49 ± 3.94	0.138[b]	0.8906

Notes: [a] *t* value; [b] Chi-square value; [c] *Z* value

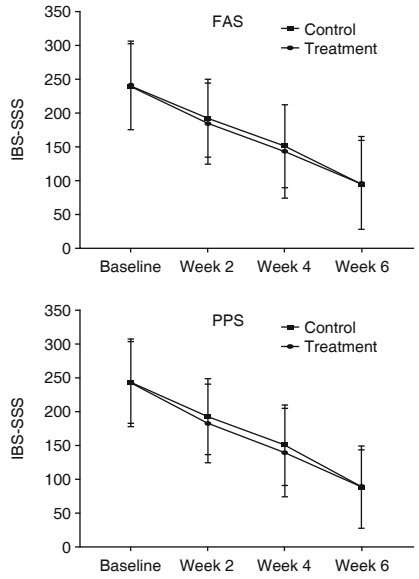

Figure 2. IBS-SSS in Both Groups at Pre-treatment and Post-treatment

(13/171), 31% (53/171) and the efficacy rate was 86.0% in the treatment group, respectively, whereas 44.4% (76/171), 9.4% (16/171), 29.8% (51/171) and the efficacy rate was 83.6% in the control group (all *P*>0.05). The efficacy of the TXNG was comparable to pinaverium bromide.

Secondary Outcomes

AR Rate

In FAS, the AR rate in treatment group was 58.93% (99/168), 65.48% (110/168) and 80.95% (136/168) respectively at the 2, 4 and 6 weeks after treatment, whereas 48.81% (82/168), 61.31% (103/168) and 78.57% (132/168) in the control group, respectively. There was no statistical significance between two groups in AR rate (*P*>0.05).

IBS-QOL, HAMD and HAMA Scores

After 6-week treatment, the difference of the total scores of IBS-QOL, HAMD and HAMA have no statistical significance between the two groups (*P*>0.05, Table 2).

Resurrence Rate in Follow-Up

Follow-ups were carried out in 4 and 8 weeks after the drug withdraw. The recurrence rate was 11.11% (9/81) and 14.47% (11/76) in the treatment and control groups in 4 weeks as well as 12.35% (10/81) and 15.79% (12/76) in 8 weeks, respectively. There was no statistical significance between the two groups in both time points (*P*>0.05).

Table 2. Comparison of IBS-QOL, HAMD, HAMA Scores between Two Groups ($\bar{x} \pm s$, FAS)

Group	Case	Time	IBS-QOL	HAMD	HAMA
Treatment	162	Baseline	29.26±20.10	7.25±4.64	6.54±3.92
	162	Week 6	12.44±10.13	4.31±3.42	3.78±3.16
Control	159	Baseline	28.99±20.20	7.04±4.45	6.49±3.94
	159	Week 6	12.53±9.62	4.03±2.91	3.53±2.54

Safety Evaluation

No severe AEs and reactions occurred during the trial. Adverse reactions occurred in 9 cases, among which 5 in the treatment group and 4 in the control group. The incidence rate was 2.92% and 2.34%, respectively (*P*>0.05). In the treatment group, 1 case presented with mild erythra, 1 case of slightly elevated AST, drugs was suspended for the above 2 cases and the symptoms vanished and elevated AST became normal at the end of clinical trial. One case presented with mild nausea and vomiting, the symptoms disappeared without special treatment. Two cases recovered from mild urinary erythrocyte abnormalities without special medical treatment. In the control group, there was 1 case of slightly elevated GGT and ALT, drugs was suspended and abnormities became normal. Three cases recovered from mild urine erythrocyte abnormalities without special medical treatment.

DISCUSSION

Tongxiening Granules is modified from the Tongxie Formula (痛泻要方) in the *Danxi's Experiential Therapy* written by the famous doctor ZHU Dan-xi in the Yuan Dynasty, and has been approved for by the China Food and Drug Administration in 2009. In this formula, *Radix Paeoniae alba* and *Pericarpium citri reticulatae viride were* used to soften the Gan and relieve pain; *Rhizoma Atractylodis Macrocephalae* was used to invigorate Pi qi and remove dampness to relieve diarrhea, *Allium Macrostemon* was used to relieve the diarrhea. Further animal studies showed that TXNG has significant anti-diarrheal effects on diarrhea caused by *folium sennae*, and exerts an obvious analgesic effect on acetic acid-induced abdominal pain, as well as significantly relieves the small intestine propulsion hyperfunction that caused by neostigmine. Besides, the drug can inhibit the pain reaction of rat caused by the thermal stimulus and has a significant spasmolytic effect on the isolated rabbit ileum *in vitro* (unpublished data).

Researches showed that the cause of IBS is

closely related to level of 5-HT, a substance that increases intestinal motility, decreases fluid absorption, and increases visceral sensitivity in patients with IBS-D, resulting abdominal pain, diarrhea, visceral discomfort and other symptoms.[12] TXNG was found to inhibit degranulation of peritoneal mast cells in sensitized rat, reduce the release of 5-HT, histamine and other transmitters, and reducing serum 5-HT and plasma substance P levels in IBS model rats. Several studies have shown that the main therapeutic goal of IBS including upregulation of calcitonin-gene-related peptide, down-regulated the excitability of dorsal horn neurons in order to increase the visceral pain threshold, as well as eliminating the intestinal allergies.[13-15] Two studies suggested that by up-regulating the expression of interleukin (IL)-10, an inhibitor of inflammatory cytokines in IBS-D can improve the patients' immune function by down-regulating the expression of pro-inflammatory cytokine IL-18.[16,17] Yang, et al[18] found TXNG can produce a significant relaxation effect on acetylcholine-induced intestinal smooth muscle spasm, which exerts antispasmodic and analgesic effects. Other *in vitro* results indicated that the granules had effects on promoting macrophage phagocytosis and inhibiting the growth of intestinal pathogens.[19]

Toxicity results showed that 2 months of continuous intragastric administration of TXNG extracts to SD rats in high-dose (100 times of the clinical daily doses for human), can lead to reduce on red blood cell count and hemoglobin and increase the serum ALT, AST and blood urea nitrogen (BUN) level. Given in medium- and low doses (50 times and 25 times of the clinical daily doses for human, respectively), the red blood cells count can also reduce and the BUN increased in medium-dose group. After 21 days of discontinuation, abnormal hematology and blood biochemical indices in high-, medium- and low-dose groups all recovered. Histological examinations of major organs were performed, no obvious histopathological changes were observed in the administered group compared with the control group. Furthermore, toxic reaction was observed during experiment which then eliminated after drug withdrawal (unpublished data).

Our present study results revealed that TXNG significantly improved the IBS-D symptoms by improving the degree of abdominal pain, bowel satisfaction and abdominal distension, which led to improvement on anxiety and depression status. The treatment effect was comparable to pinaverium bromide. One main concern is that there is a low recurrence rate after drug withdrawal and no severe adverse reactions and abnormal changes were found in both groups by lab tests. In general, the result of the study showed a favorable safety and effectiveness of TXNG in the treatment of patients with IBS-D.

Conflict of Interest

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article. This study was funded by Chongqing Pharscin Pharmaceutical Co., Ltd.

Author Contributions

Tang XD and Li ZH designed the study; Zhang SS, Hou XH, Chen S, Feng PM, Yang XN, Li HZ, Wu JQ, Xia PJ, Yang XJ, Zhou HJ, Wang HY and Ai YW carried out the trial; Li K performed the statistical analysis; Tang XD designed and wrote the manuscript.

Electronic Supplementary Material: Supplementary material (Appendix 1) is available in the online version of this article at http://dx.doi.org/10.1007/s11655-019-3030-x.

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