



# Effects of Banxiaxiexin decoction on the intestinal microorganisms and enzyme activities in mice with spleen-deficiency constipation

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## Abstract

The present study was carried out to investigate the impact of Banxiaxiexin Decoction on the intestinal microorganisms and enzyme activities in mice with spleen-deficiency constipation. 24 experimental mice were randomly divided into 4 groups including the normal group, the model group with spleen-deficiency constipation, Banxiaxiexin Decoction treated group and Zhizhu Decoction treated group. Both the normal group and the model group with spleen-deficiency constipation were treated with sterile water. The other two groups were treated with traditional Chinese medicine respectively. The drugs were orally administered. After 6 days of treatment with traditional Chinese medicine, the intestinal microbes and enzyme activities of intestinal contents were measured. The results showed that the total number of colibacillus significantly increased from the Zhizhu Decoction treated group ( $p < 0.01$ ) compared to the normal group and the model group. Compared to the Zhizhu Decoction treated group, the total number of colibacillus obviously reduced in Banxiaxiexin Decoction treated group ( $p < 0.05$ ). Compared to the normal group, the amounts of bifidobacteria in the two treated groups significantly increased ( $p < 0.05$ ). Compared to the normal group, the activities of protease and amylase from Banxiaxiexin Decoction treated group obviously reduced ( $p < 0.01$ ) and the activities of xylanase and cellulase significantly improved ( $p < 0.05$  or  $p < 0.01$ ). Compared to the model group, protease activities from Banxiaxiexin Decoction treated group remarkably reduced ( $p < 0.01$ ) and the activities of xylanase and cellulase significantly increased ( $p < 0.01$ ). Compared to the Zhizhu Decoction treated group, the activities of protease and cellulase from the Banxiaxiexin Decoction treated group obviously reduced ( $p < 0.01$ ) yet amylase activities apparently improved ( $p < 0.01$ ). It is clearly evident from the results that Banxiaxiexin Decoction can regulate the intestinal microorganisms and enzyme activities to treat spleen-deficiency constipation.

**Keywords:** Spleen-deficiency constipation, Banxiaxiexin Decoction, Zhizhu Decoction, intestinal microorganism, intestinal enzyme activity

## Introduction

Constipation is a common gastrointestinal complaint clinically [1,2], affecting an estimated 27% of global population [3]. From the perspective of traditional Chinese medicine, constipation is relation to the dysfunction of large intestine. The disharmony between the spleen, stomach, liver and kidney is also concerned with constipation [4]. The intestine of a patient with constipation is a lack of insufficient fluid to lubricate the intestine and

stools, and the time of stools staying in the intestine is too long. The stools of a constipation patient are present with hard, dry, pellet-like and difficult to be passed. Constipation significantly affects health-related quality of life. There has a huge ecosystem in human intestine. Intestinal microbes involve in the pathogenesis and progression in various diseases [5]. Colonized germfree mice with *Bacteroides thetaiotaomicron* revealed that this commensal bacterium modulated expression of some vital genes

involved in several important intestinal functions [6]. These findings suggested that intestinal microbes were indispensable on the healthy development of the intestinal tract. Modern studies indicated that the imbalance of intestinal ecology in a patient with constipation was related to the increase of the number of harmful bacteria in intestine [7], and the number of *Clostridium* spp which belongs to pathogenic bacteria was significantly increased yet the number of bifidobacterium which belongs to probiotics was significantly decreased. The changes of the microbes and the enzyme activities in intestine of mice with spleen-deficiency constipation were significant [8]. Microbes such as colibacillus, bifidobacteria and lactobacilli were all proliferated. The activities of amylase and protease reduced but the activities of xylanase and cellulase raised. It is important to regulate the balance of intestinal microflora in the treatment of constipation.

Banxiaixixin decoction (BXD), one of a traditional Chinese medicine rescription, was originally chronicled in a classical Chinese book Treatise on Febrile Diseases of Han Dynasty and composed of seven herbs: pinellia, coptis, scutellaria, ginseng, dried ginger, jujube and honey-fried licorice. It has been prescribed the function of adjusting the balance between cold and heat and elimination of the nodules. The results of modern pharmacology research suggested that BXD has effect on a variety of diseases. BXD has effect on promoting gastrointestinal peristalsis, preventing reflux, protecting gastric mucosa, invigorating immune system and improving body's ability to resist hypoxia. BXD was mainly used to treat illness of the digestive system in clinic, such as reflux esophagitis, acute and chronic gastritis, peptic ulcer, dyspepsia and constipation [9-12]. In the present study, we investigated the effect of BXD on treatment of spleen-deficiency constipation in mice with ZZD used as a positive control drug, and elucidated the therapeutic mechanism of BXD with particular focus on the relationship between the changes of intestinal microflora, the activities of enzymes and constipation.

## Materials and methods

### Experimental animals

24 Kunming mice were provided by Shanghai Experimental Animal Company of Laikesi (SYXK (xiang) 2013-0005). The weight of each mouse was about  $20 \pm 2$ g, 12 male and 12 female. Mouse food was provided by Experimental Animal Center of Hunan University of Chinese Medicine. All the mice were fed for 2 days to adapt to the condition. The mice were randomly divided into 4 groups: the normal group, the model group with spleen-deficiency constipation, BXD treated group and Zhizhu Decoction (ZZD) treated group. The normal group's mice were administered with sterile water (0.8 mL/mouse) for twice a day. The mice of the other groups were treated with senna water decoction (0.8 mL/mouse) by mouth, twice a day, for 7 days, then the diet of the mice was controlled by irregular eating for 8 days to prepare the model with spleen-deficiency constipation [8].

### Drug treatment

500 g senna (produced in Yunnan) were immersed in 5000 mL boiling water for 10 minutes, the filtrate is evaporated and condensed to 100% decoction ( $1 \text{ g} \cdot \text{mL}^{-1}$ ) in water bath of  $75^\circ\text{C}$ . BXD [13] was composed of pinellia of 12 g, scutellaria of 9 g, dried ginger of 9 g, ginseng of 9 g, honey-fried licorice of 9 g, coptis of 3g and four jujubes of about 9 g. ZZD [14] was composed of raw atractylodes macrocephala and citrus in proportion to 2:1. Each BXD and ZZD were made into water decoction of appropriate concentration that equivalent to  $1 \text{ g} \cdot \text{mL}^{-1}$  of crude drug concentration in the whole group. The decoctions were stored at  $4^\circ\text{C}$ . Both the model group with spleen-deficiency constipation and the normal group were treated with sterile water. The other two groups with constipation were treated with water decoction of Chinese medicine according to the clinical equivalent dosage of mice. Mice of the BXD group were treated with dose of  $0.05 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  BXD water decoction, and mice of the ZZD group were treated with dose of  $0.27 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  ZZD water decoction. All the drugs were orally administered to the mice. The treatment lasted for 6 days.

### Media

For bacteria: Beef extract-peptone medium; for colibacillus: EC medium; for *Lactobacillus* spp.: MRS medium; for *Bifidobacteria* spp.: BBL medium; for fungi: martin Bengal Red-Streptomycin Medium [15].

### Extraction of intestinal contents

All the mice were sacrificed on the morning of the seventh day after the drug treatment. Mouse intestinal contents were collected in a sterile environment and mixed for spare [8].

### Determination of microorganisms of intestinal contents

A certain amount of intestinal contents from the mice of each group were weighted and put into conical flask equipped with glass beads and sterile water. Put the conical flask on an oscillator for 30 min with 120 rpm shaking to release microorganisms from intestinal contents into sterile water sufficiently. The appropriate dilution was selected and inoculated into the corresponding medium with the coating method and mixed bacteria method at  $37^\circ\text{C}$ . Total numbers of bacteria and colibacillus were detected after being cultured for 24 hours, the numbers of lactobacillus and bifidobacteria were determined after being cultured for 48 hours, the numbers of fungi were determined after being cultured for 96 hours.

### Analyze enzyme activities of mice intestinal contents

A certain amount of intestinal contents from the mice of each group were weighted and put into centrifuge tube and diluted with proper sterile water. Put the centrifuge tube on an oscillator for 30 min with 120 rpm shaking to release the enzyme from intestinal contents completely. The enzyme extracts were centrifuged for 30 min at 4000 r/min following which the enzyme activities in the supernatant were analyzed.

The activities of cellulase and amylase and xylanase were determined by DNS colorimeter. The activity level of protease was determined with Folinphend method. Cellulase activity was defined as an unit of the cellulase activity by generating 1 mg reducing sugar of 1 g intestinal contents at 46°C for 30 min. Amylase activity was defined as an unit of the amylase activity by generating 1 mg reducing sugar of 1 g intestinal contents at 37°C for 60 min. Xylanase activity was defined as an unit of the xylanase activity by generating 1 mg reducing sugar of 1 g intestinal contents at 46°C for 60 min. Protease activity was defined as an unit of protease activity by generating 1 g amino acid of 1 g intestinal contents at 37°C for 40 min.

### Statistical analysis

Measurement data of every group was represented with mean-standard deviation ( $\bar{x} \pm s$ ). The data was analyzed with SPSS 19.0 statistical software.

### Results

#### The apparent character of the mice observed in general

Each group's mice were observed. The mice of the normal group were eyesight and vitality. The diet and the amount of water drinking for each mouse were normal. The hair on the back was thick and shiny. The feces was normal too. In order to prepare the animal models with spleen-deficiency constipation, the mice were administered senna water decoction by mouth. On the first day, the mice began to appear loose stools and perianal contamination, food intake and the activity reduced, the hair of the mice frizzled on the third day, accompany with tired lying, lethargy, weakness of limbs, arched back and marasmus. The mice appeared constipation symptoms on the ninth day including the amount of feces decreased. The particles of the feces got smaller, harder and drier. The center of the feces was drier and short of moisture. From the ninth day to the fifteenth day, the mice of the model group and the BXD group and the ZZZ group lasted the symptoms of spleen-deficiency constipation. After treated with ZZZ and BXD for five days, the mice of the BXD group and the ZZZ group restored their vitality in eyes and vigorous, the mice hair on back became thick and shiny, the diet and water drinking of the mice returned to normal, the particles of the feces became soft covering with mucus on the surface, the center of the feces was rich in water. The phenomenon suggested that BXD and ZZZ had effect on spleen-deficiency constipation in mice.

#### Effect of BXD on main intestinal microbes in mice with spleen-deficiency constipation

A certain amount of mice intestinal contents were collected. The microbes in the intestinal contents were analyzed. The result showed that the total number of bacteria in the intestinal of the four groups had no clear distinction ( $p > 0.05$ ). We could see from **Table 1**, the amount of colibacillus in mice intestine treated with ZZZ were significantly higher than that in mice of

**Table 1. Effect of BXD decoction on main intestinal microbes in mice with spleen-deficiency constipation.**

Groups	Total bacteria ( $10^7$ CFU·g <sup>-1</sup> )	Colibacillus ( $10^7$ CFU·g <sup>-1</sup> )	Fungi ( $10^4$ CFU·g <sup>-1</sup> )
Normal group	12.00±1.00	14.67±1.53	0
Model group	12.67±1.52	11.67±4.04	0
ZZD group	13.00±1.00	30.00±3.61AB	0
BXD group	12.33±1.53	16.00±7.00C	0

Note: Compared to normal group: A: $p < 0.01$ ; compared to model group: B: $p < 0.01$ ; compared to ZZZ group: C: $p < 0.01$ .

the normal group and the model group ( $p < 0.01$ ). BXD group had lower amount of colibacillus than ZZZ group ( $p < 0.05$ ). The fungi from the four groups failed to be cultured. The results suggested that BXD could restore the amount of colibacillus.

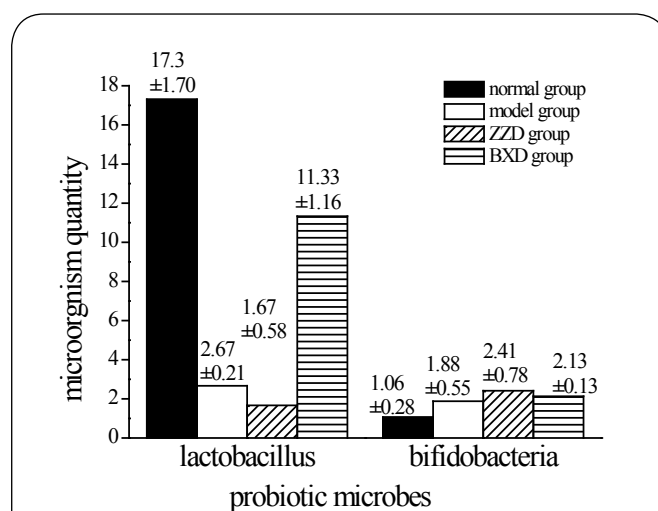
#### Effect of BXD on intestinal probiotic microbes in mice with spleen-deficiency constipation

Bifidobacteria and lactobacillus are beneficial bacteria in the gastrointestinal tract. Compared to the normal group, the amounts of bifidobacteria from BXD group and ZZZ group increased significantly ( $p < 0.05$ ) (**Figure 1**). The amounts of lactobacillus from four groups had no significant difference ( $p > 0.05$ ), and differences in the same group may be the mainly reason.

The research suggested that BXD had obvious regulation effect on restoring the intestine microorganism balance by adjusting the amounts of colibacillus and lactobacillus, at the same time BXD could promote the growth of bifidobacteria.

#### Effect of BXD on intestinal enzyme activity in mice with spleen-deficiency constipation

The amylase and protease are vital digestive enzymes in



**Figure 1. Effect of BXD decoction on intestinal probiotic microbes in mice with spleen-deficiency constipation.**

Note: In figure lactobacillus ( $10^7$  CFU·g<sup>-1</sup>), bifidobacteria ( $10^8$  CFU·g<sup>-1</sup>). Compared to normal group: a:  $p < 0.05$ .

**Table 2. Effect of BXD decoction on intestinal enzyme activities in mice with spleen-deficiency constipation.**

Groups	Protease (U·g <sup>-1</sup> )	Amylase(U·g <sup>-1</sup> )	Xylanase(U·g <sup>-1</sup> )	Cellulase (U·g <sup>-1</sup> )
Normal group	13.233±1.400	3.833±0.084	2.635±0.076	2.161±0.058
Model group	19.517±0.814A	0.815±0.062A	1.983±0.025A	2.216±0.127
ZZD group	19.383±0.425A	0.671±0.045Ab	2.983±0.077AB	1.663±0.098AB
BXD group	8.600±0.450ABC	0.865±0.057AC	2.862±0.089aB	2.572±0.110ABC

Note: compared to normal group: a:  $p < 0.05$ , A:  $p < 0.01$ ; compared to model group: b:  $p < 0.05$ , B:  $p < 0.01$ ; compared to ZZD group: C:  $p < 0.01$ .

intestine released by body. If the activities of amylase and protease declined, body's digestion and absorption function will be affected [16]. The activity of protease in mice treated with ZZD was evidently higher than that of the normal group ( $p < 0.01$ ) (Table 2). On the contrary, the protease activity in mice treated with BXD declined obviously ( $p < 0.01$ ) compared to the normal group. The activity of amylase from the model group, ZZD treated group and BXD treated group decreased significantly compared to the normal group ( $p < 0.01$ ). The activity of amylase from BXD treated group had little difference from the model group ( $p > 0.05$ ) but was higher than ZZD treated group. The data showed that BXD could enhance the amylase activity in mice ( $p < 0.01$ ). The activity of xylanase from BXD treated group and ZZD treated group was significantly higher than that from the normal group, and the xylanase activity from the model group decreased evidently ( $p < 0.01$ ). The activity of cellulase in mice treated with BXD was the highest among the four groups but the cellulase activity from ZZD treated group was the lowest. All these suggested that BXD had effect on regulating the activities of enzymes in intestine.

## Discussion

The mechanism of Chinese herbal medicine to achieve its medicinal function is mainly through the intestinal microbial hydrolysis and reduction of the active ingredients and absorption after conversion [17]. Bacteria in the gastrointestinal tract of mammals are the main contributors to the chemical reactions in the gastrointestinal tract [18,19]. *Bifidobacterium* is the most beneficial bacteria in intestine, lactic acid bacteria can produce lactic acid bacteria peptide to protect the intestinal mucosa from invasion of pathogenic microorganisms and reduce colonization of harmful bacteria, like colibacillus and so on [20]. The present study showed that BXD had effect on restoring the amount of colibacillus and lactobacillus and promoting the growth of bifidobacterium to adjust the intestinal micro-ecological balance.

The intestinal microbiota plays an important role in the metabolism and nutrition, the development and control of the immune system, the development of the brain, the synthesis of vitamins and the energy balance [21,22]. Amylase and protease are pivotal digestive enzymes in intestine mainly secreted by the body itself. Amylase can reduce chyme viscosity, prevent the breeding of harmful microorganisms

and promote the growth of beneficial microorganisms [23]. If the activity levels of amylase and protease declined, body's digestion and absorption function will certainly be affected. Cellulase and xylanase are secreted only by gastrointestinal microbes, not by the host itself, so the variety of the activities of cellulase and xylanase could reflect the change of microbes who secrete cellulase and xylase [24]. Our experimental results suggested that ZZD further decreased the activity of amylase and had very little effect on the activity of protease, enhanced the activity of xylanase and inhibited the activity of cellulase significantly. The analysis of enzyme activities in mice intestine showed that BXD reduced the activity of protease clearly, increased the activity of xylanase and further improved the activity of amylase, which indicated that BXD could reduce the digestive function of mice and enhance the activities of the enzymes secreted by intestinal microbes. On the basis of analysis above, we can draw a conclusion that BXD had effect on treatment of spleen-deficiency constipation by regulating the balance of intestinal microorganisms and enzyme activities.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

Authors' contributions	YG	LH	XZ	LS	HH	ZT
Research concept and design	✓	--	--	--	✓	✓
Collection and/or assembly of data	✓	✓	✓	--	✓	✓
Data analysis and interpretation	✓	--	✓	✓	✓	✓
Writing the article	✓	--	--	--	✓	✓
Critical revision of the article	✓	✓	✓	✓	✓	✓
Final approval of article	✓	✓	✓	✓	✓	✓
Statistical analysis	✓	✓	--	--	✓	✓

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