Formulation of traditional Chinese medicine and its application on intestinal flora of constipated rats

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Research

Keywords: Constipation, Chinese medicine, Gut microbiota, Lactobacillus, Probiotics

DOI: https://doi.org/10.21203/rs.3.rs-36144/v1

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Abstract

In this study, the self-extracted constipation treatment of traditional Chinese medicine extracts was applied to constipated rats. To explore the mechanism and role of the Chinese medicine for the treatment of constipation, the 16S rRNA sequencing and qRT-PCR technology were used to analyze the abundance and diversity of intestinal flora in rats. We found that the relative abundance of Firmicutes in the intestinal flora of rats with constipation was significantly higher than that in the control group, and the Bacteroides was significantly lower than that in the control group, while the relative abundance of Firmicutes was significantly changed after taking a certain dose of Chinese medicine, and reduced the relative abundance of Lachnospiraceae, and greatly increased the relative abundance of Lactobacillus, enhanced the symbiotic relationships of Lactobacillus with other intestinal flora. There was no significant difference in the total copies of intestinal bacteria between the constipated rats and the control group. The total copies of intestinal bacteria in the constipated rats decreased after taking the traditional Chinese medicine. Finally, this study results provides a theoretical basis for the treatment and understand the mechanism and effect of traditional Chinese medicine on rate constipation.

1. Introduction

Constipation is a common clinical gastrointestinal disease, mainly manifested as irregular defecation, difficulty in defecation, dry stool and abdominal discomfort. The total incidence rate of the disease is around 16% [1]. As the disease spectrum changes, Chronic constipation has gradually become a common problem affecting children's physical and mental health. It affects 0.7% − 29.6% of children in the world [2, 3]. According to a US survey, constipation plagues in healthy people, and constipation are also associated with colon cancer, heart, brain vessels, and the elderly dementia and so on [4]. Meanwhile, long term constipation can lead to intestinal wall damage, hemorrhoids and anal fissure, and the accumulation of enterotoxin can also easily lead to cytopathic changes, thus leading to colorectal cancer; about 22% of patients with constipation have hypertension complications [5]; elderly constipation patients with cardiovascular and cerebrovascular diseases can induce acute myocardial infarction, heart failure, intracranial hemorrhage and other serious diseases due to excessive abdominal pressure due to forced defecation. In addition, constipation has an important relationship with hepatic encephalopathy, breast disease, Alzheimer's disease, women's dysmenorrhea, urinary tract infection and other diseases [4].

The pathogenesis of chronic constipation mainly includes the reduction of colonic contraction, the dysfunction of pelvic floor and defecation, the abnormality of intestinal neurons and neurotransmitters, the decrease of interstitial cells and intestinal glial cells, and the abnormality of intestinal neurochemical signals [6]. In recent years, it has been found that the imbalance of gut microbiota plays an important role in the pathogenesis of chronic constipation. Intestinal microflora have an important functions in maintaining homeostasis and improving immunity [7]. However, if the feces of patients with constipation stay in the intestine for a long time, it may change the number and type of normal intestinal microflora, metabolic molecules of intestinal microflora, such as methane and short chain fatty acids. Between the
microflora and the host immune system plays an important role in the pathogenesis of chronic constipation [6, 8].

Traditional Chinese medicine has been used to treat diseases for a long time. Because many effective components of traditional Chinese medicine are produced with strong biological activity after metabolism of intestinal flora and playing a therapeutic role [9, 10]. Puerarin, iso-flavone and other ingredients contained in Huangta, Pueraria and Douchi are commonly found in many prescriptions and health products. In vitro studies have shown that puerarin and iso-flavone can be metabolized into more effective daidzein and maorui iso-flavone by bacteria in the intestinal tract, which has a strong therapeutic effect [11]. The intestinal flora is related to the physiology and pathology of the human body, and closely related to the medicine and food taken cut contact [12–14]. The composition of traditional Chinese medicine is complex, therefore, the addition to the ingredients with good therapeutic effect, and it also contains polysaccharides, microelements, proteins, vitamins and other nutrients. This makes the composition of traditional Chinese medicine in clinical treatment varied [15]. The intestinal flora in the body plays a vital role in the treatment of traditional Chinese medicine entering the body, and its corresponding effective components to maintain the balance of the number of intestinal flora in the body. Traditional Chinese medicines, such as rhubarb, thenardite, etc. contain irritating ingredients, which can inhibit the resident some bacteria in the gut. Xie et al. [16] found that Rhizoma Coptidis and berberine can significantly reduce the proportion of Firmicutes and Bacteroidetes in feces of HFD mice in the total number of bacteria.

In the current treatment of diseases, traditional Chinese medicine has the advantages of small adverse reactions, not easy to remain in the body, no dependence and so on, which has gradually been accepted by the world. For functional constipation, colitis, chronic diarrhea and other diseases, the use of traditional Chinese medicine has often achieve good results. Therefore, the demand for traditional Chinese medicine is increasing. Hence, the present study objective was mosapride used as a positive control, and diphenoxylate, a commonly used anti abdominal cathartic, combined with the qi stagnation and constipation rats caused by tail entrapment. In addition, self-developed traditional Chinese medicine decoction on constipation was used as the research object to explore the efficacy and the relationship between its efficacy and intestinal flora, to reveal its micro ecological mechanism of action. The development of the efficacy and function of traditional Chinese medicine provides certain theoretical basis to treatment of the disease.

2. Materials And Methods

2.1. Animals and ethics statement

The SPF Wistar rats, male, were used for experiment purposes with body weight 110 ± 10 g. All animal tests were conducted in accordance with the NIH Publication No. 80 – 23 and approved by Fujian University of traditional Chinese medicine.

2.2. Preparation of Chinese medicine
The traditional Chinese medicine "Li Qi Run Chang Fang" was prepared by the pharmacy of the second people's Hospital Affiliated to Fujian University of traditional Chinese medicine. Drug composition: Magnolia officinalis 100 g, Fructus aurantii 100 g, hemp seed 150 g, plum seed 100 g, Fructus Trichosanthis 150 g, fried semen Raphani 100 g, bupleurum 90 g, paeony 120 g, tangerine peel 90 g, and mirabilite 30 g. When cooking, add eight times of water to soak for 0.5 hours, then cook three times, the first, second and third time were 1.5 h, 1 h and 0.5 h, respectively, combine the supernatant and filter. The filtrate was concentrated to 500 ml under reduced pressure at 60 °C – 70 °C, and the concentration of the solution is 2.06 g·ml\(^{-1}\), which was sterilized and repacked, and stored in refrigerator at 4 °C.

2.3. Constipation FC rat model

This model was based on a FC rat model that made by intragastric administration of diphenoxylate, and combines the stagnation and constipation type caused by tail irritation: in addition to the blank control group, 5 rats in each model group are placed in the same cage, and the tail of the rats is entrapped with hemostatic forceps, the rats in the whole cage were enraged and kept tearing and beating with other rats. The rats were stimulated for 30 minutes every time, 4 times a day, and the interval between the two stimulation was 3 hours, a total of 14 days. Then, 10 mg/kg compound diphenoxylate was given to the stomach once a day, continuously for 7 days as a course of treatment. When the rats appeared irritable and fidgety, the first black stool excretion time was prolonged when they were fed with common diet, which was a successful model. Three rats were killed and their stomachs were taken to verify the success of the model.

2.4. Study design

The 10 rats in the blank control group were fed SPF standard diet until the end of the experiment. 50 FC rats models were made according to the above method. After successful modeling, rats were randomly divided into FC model group (n = 10), low dose group (n = 10, DL), medium dose group (n = 10, ZH), high dose group (n = 10, GA) and Mosapride group (n = 10, MO). According to the dosage conversion of clinical application, rats in the low, middle and high dose groups were 5.15 g·(kg·d)\(^{-1}\), 10.3 g·(kg·d)\(^{-1}\) and 20.6 g·(kg·d)\(^{-1}\), respectively, while the solution was diluted to 4 ml / rat with physiological saline according to the weight of rats. According to the clinical dosage conversion, mosapride group was given 2 mg· (kg·d)\(^{-1}\) gavage of 4 mL/animal each time. The blank group and FC model control group were given 4 mL normal saline every time and gavage for 2 weeks.

2.5. Bioinformatics and statistical analysis

Operational taxonomic unit (OTU) was generated by clustering clean tags. According to different similarity levels, OTUs of all sequences were divided, and the bio-information of OTUs above 97% similarity level was analyzed by Qiime software (version 1.8.0). The diversity analysis of single sample (alpha diversity) was reflect the richness and diversity of microbial community. Qiime software (version v1.8.0) was used to estimate the species richness and diversity of environmental community. R software was used to plot and analyze PCoA based on Bray Curtis with two-dimensional graph. Lefse analysis was used to detect species with significant difference in abundance between different groups by ANOVA test,
and linear discriminant analysis (LDA) was used to evaluate the influence of species with significant
difference in data dimensionality. Through spearman test, R software was used to analyze the correlation
between OTU and gate level annotation results in the first 20 absolute abundance samples.

2.6. DNA extraction and quantitative real time PCR (qRT-PCR) analysis

Used the DNA Stool kit to extract DNA from feces for the next test. qRT-PCR was performed to calculate
the total number of bacterial cells by measuring the 16S rDNA gene copy number with the primers 338F
5′-GTACTCCTACGGGAGGCAGCA-3′ and 806R 5′-GTGGACTACHVGGGTWTC. TAAT-3′. The PCR
amplification of 16S V3-V4 region was carried out with the above primers using the total bacterial DNA as
template. The reaction conditions were: pre denaturation at 94 °C for 5 min, 94 °C for 30 s, 55 °C for
30 s, and 72 °C for 30 s. After 30 cycles, 72 °C extended for 10 min, 4 °C insulation. After that, the
purified product was connected to T-vector pmd19 vector and transformed into E. coli DH-α 5 sensitive
cells. Plasmid DNA was extracted, and plasmid standards of different concentrations were used as
templates for qRT-PCR reaction. The cross coordinate of template copy number was used as the cross
coordinate and Ct value was used as the vertical coordinate to prepare the standard curve. SYBR green
was used as fluorescent dye to carry out real-time quantitative PCR reaction on fecal samples and
calculate copy number. The sequencing was carried out on MiseqPE300 platform. According to the
regulations of cut-adapt, the original reading was filtered by mass under specific filtering conditions to
obtain high-quality pure reading.

2.7. Statistical analysis

Data shown are the means ± SD. T-test (prism 6.0) was used to analyze the data differences between the
two groups. One way ANOVA (prism 6.0) was used to analyze the data difference more than two groups
and the difference was significant (p < 0.05).

3. Results

3.1. Overall structural changes in microbiota composition

Dilution curve is used to reflect the rationality of sequencing data and indirectly the species richness. As
shown in Figure S1-2, the curve gradually flattens, indicating that the amount of sequencing data is
sufficient. At the same time, we found that the good coverage of all experimental groups was greater than
99.0%, which indicated that the sequencing depth of this microbiome analysis was very deep (Fig. 1a).
For alpha diversity analysis, Chao and Shannon indexes were used to evaluate the richness and diversity
of flora. As shown Fig. 1B, the richness of the bacterial population (Chao1 indices) in the GA group was
significantly lower than that in the normal group (p < 0.01), and there was no significant difference in the
species richness of intestinal flora between KO, FC, MO and DI Group compared with normal rats. The
species richness of constipation model, mosapride group and low- medium- dose Chinese medicine
group are similar to that of normal healthy rats, and there were no significant difference. The observed
species indicates that the number of OTU actually observed with the increase in sequencing depth. As shown Fig. 1C, the number of OTU actually observed in the MO group was the largest, which is significantly higher than that in the FC (p < 0.01) and the DI group (p < 0.05). There was no significant difference in KO between the FC group and the control group, and the low-medium-high-dose Chinese medicine group showed a gradually decreasing trend (p < 0.05). From Fig. 1D, the Shannon index of MO and DI group was significantly higher than that of normal group (p < 0.05), which were indicates that the diversity of intestinal flora of mosapride and low-dose Chinese medicine group was more than that of healthy rats, while the diversity index of FC were not significantly different from normal group, which is clearly indicating that the intestinal diversity of constipation group was not significantly different from that of healthy rats. The intestinal diversity of GA group was significantly lower than that of normal control group (p < 0.01) and ZH group (p < 0.01), which were indicating that Chinese medicine at a high dose reduced the intestinal flora diversity.

In order to further study, the similarity or difference of the composition of the intestinal flora of the sample, cluster, Non-metric multidimensional scaling method (NMDS) and principal co-ordinates analysis (PCoA) were performed. Cluster analysis uses tree structure to describe and compare similarities between multiple samples. It can be clearly seen from Fig. 1E that the microbiological composition of the samples in the group is similar, and the samples of the same treatment group was gathered together, indicating that the differences between the groups was small and the sample repeatability good. It can be seen from NMDS and PCoA analysis (Fig. 1F-G) that the microbial composition of the normal control group was different from that of DI, ZH and GA groups, indicating that the intake of low, medium and high doses of traditional Chinese medicine changes the composition of the whole intestinal flora, and the degree of change is also different under different doses. In addition, it can be found that the differences between FC group and MO and DI groups are not obvious, and the composition of the flora tends to be the same, indicating that the intestinal flora composition of the constipation group, the mosapride group and the low-dose Chinese medicine group similar.

[Figure 1]

3.2. Classification based comparison of phylum and genus levels

From the phylum-level analysis (Fig. 2A), we could clearly found that 90% of the intestinal microorganisms in six groups were mainly composed of *Firmicutes* and *Bacteroides*. The abundances of *Firmicutes* in KO, FC, MO, DI, ZH and GA groups were 76.0%, 86.7%, 85.1%, 81.0%, 83.4% and 87.8%, respectively. The relative abundances of *Bacteroides* in KO, FC, MO, DI, ZH and GA groups were 15.7%, 7.3%, 9.0%, 12.0%, 8.2% and 6.0%, respectively. It can be seen from Fig. 2B that the relative abundance of *Firmicutes* in the constipation model group, mosapride group and high-dose traditional Chinese medicine group were significantly higher than that in the control group (p < 0.01), and there were no significant difference between the three groups. Meanwhile, the abundance of *Firmicutes* in the DI group was significantly lower than that in the middle dose ZH and constipation group (p < 0.05). As can be seen
from Fig. 2C, the relative abundance of *Bacteroidetes* in the constipation model group, the mosapride group and the different doses of the Chinese medicine group was significantly lower than the control group (p < 0.01), and there was no significant difference between the constipation model group and the mosapride group. At the same time, the relative abundance of *Bacteroidetes* in the DI group was significantly higher than that in the constipation group. At the same time, it showed a gradual decrease with the rising the dose of Chinese medicine (p < 0.05).

We could clearly found that the intestinal microorganisms in six groups were mainly composed of *Lachnospiraceae, Lactobacillus* and *Ruminococcaceae* (Fig. 2D). The abundances of *Lachnospiraceae* in KO, FC, MO, DI, ZH and GA groups were 19.5%, 16.9%, 10.8%, 11.7% and 8.8%, respectively. The abundances of *Lactobacillus* in KO, FC, MO, DI, ZH and GA groups were 12.3%, 8.3%, 6.1%, 6.3%, 9.4% and 23.1%, respectively. The abundances of *Ruminococcaceae* in KO, FC, MO, DI, ZH and GA groups were 8.3%, 11.4%, 12.9%, 11.0%, 12.5% and 11.7%. We found that there was no significant difference in the relative abundance of *Ruminococcaceae* among the six groups. The abundance of *Lachnospiraceae* decreased with the increasing of the dosage of Chinese medicine (p < 0.05), at the same time, the intake of Mosapride significantly reduced the relative abundance of *Lachnospiraceae* (p < 0.01) (Fig. 2E). The abundance of *Lactobacillus* increased with the increasing of the dosage of Chinese medicine, and the high-dose Chinese medicine group was significantly higher than the low-dose group, the constipation group and the normal control group (p < 0.01) (Fig. 2F). The results indicated that the abundance of *Lactobacillus* in the constipation group was significantly lower than KO (p < 0.05). *Lactobacillus* has a significant role in promoting intestinal peristalsis, so we speculate that the role of traditional Chinese medicine in the treatment of constipation may be related to promoting the proliferation of probiotics.

[Figure 2]

### 3.3. The differences in the dominant members of the microbiota

In order to verify and further determine the LEfSe was used to identify the specific phylotypes responding to FC and DI, ZH, GA groups. As shown Figs. 3A and B, the main differential microbial species between the constipation simulation group and other groups were *Camobacteriaceae* and *Clostridiales*, the main differential microbial species between the DI group and other groups were *Porphyromonadaceae* and *Lachnospiraceae*, the main differential microbial species between the ZH group and other groups were *Methylobacteriaceae, Christensenellaceae* and *Erysipelotrichaceae*, the main differential microbial species between the GA group and other groups were *Lactobacillaceae, Bacilli* and *Peptostreptococcaceae*, the main differential microbial species between the MO group and other groups were *Bifidobacteriaceae, Actinomycetaceae, Deferribacteraceae, Aerococcaceae, Clostridiaceae, Peptococcaceae* and *Ruminococcaceae*.

[Figure 3]

### 3.4. Network relationship and functional prediction analysis
Faust et al. [17] proposed a network inference analysis based on the relationship between microbial members. The fundamental purpose of this analysis was to examine the interaction patterns between different microbial community members in a sample. The numbers of the nodes and links were counted through statistical network symbiosis, it can be seen from Fig. 4A that the dominant symbiotic dominant flora in the KO group is *Firmicutes*, *Spirochaetae* and *Proteobacteria*. At the same time, the genus level of *Firmicutes*, the dominant symbiotic flora includes *Turibacharacter*, *Roseburia*, *Eubacterium*, *Ruminocaeae*, *Ruminostridium*, *Ruminococcus*, *Allobaculum*, *Lactobacillus*, *Spirochaetae*, *Treponema*, *Proteobacteria* and *Desulfovibrio*. In addition, 7 symbiotic relationships between *Lactobacillus* and other bacteria, 5 positive and 2 negative ones. From Fig. 4B that the dominant symbiotic dominant flora in the FC group is *Firmicutes* and *Spirochaetae*. At the genus level of *Firmicutes*, the dominant symbiotic flora includes *Turicibacter*, *Eubacterium*, *Ruminocaeae*, *Ruminostridium*, *Ruminococcus*, *Allobaculum*, *Christensenellaceae*, *Lactobacillus*, *Spirochaetae* and *Treponema*. There are 7 symbiotic relationships between *Lactobacillus* and other bacteria, 2 positive and 5 negative ones. The above shows that in constipation group FC was significantly reduce the symbiotic relationship between *Proteobacteria* and other intestinal bacteria, increase symbiosis of *Firmicutes* and other flora, but it does not change and affect the symbiotic flora of *Spirochaetae*. Compared with control group KO, the negative symbiotic relationship of Lactobacillus was increased in the constipation group. From Fig. 4C that the dominant symbiotic flora in the GA group was *Firmicutes* and *Spirochaetae*. At the genus level of *Firmicutes*, the dominant symbiotic flora includes *Lactobacillus*, *Turicibacter*, *Eubacterium*, *Ruminocaeae*, *Ruminostridium*, *Ruminococcus*, *Allobaculum*, *Christensenellaceae*, *Lachnospiraceae*, *Spirochaetae* and *Treponema*. There are 24 symbiotic relationships between *Lactobacillus* and other bacteria, 14 positive ones and 13 negative ones. In this study, we found that a certain dose of Chinese medicine significantly increased the symbiotic relationship between *Lactobacillus* and other intestinal flora, making *Lactobacillus* from the edge of the symbiotic relationship to the dominant dominant flora.

Changes in the symbiotic relationship of the gut microbiota often also indicate functional changes; thus, functional prediction analysis was also performed. From Fig. 4D, it can be seen that the distribution of functional genes in these six groups is mainly concentrated in glycan biosynthesis and betabolism, followed by Lipid metabolism, biosynthesis of other secondary metabolites and transport and catabolism. The less distributed ones were neurodegenerative diseases, and the other ones, such as Digest System, account for less than 1%. At the same time, the Fig. 4E also indicted that the expression of glycosaminoglycan degradation, apoptosis, G protein-coupled receptors, stilbenoid, diarylheptanoid and gingerol biosynthesis, protein digestion and absorption, glycosphingolipid biosynthesis - ganglio series and bil secretion were significantly up-regulated by Chinese medicine compared with FC group, significant down-regulation of glycosphingolipid biosynthesis - globo series, amyotrophic lateral sclerosis (ALS), amoebiasis, prion diseases, alpha-linolenic acid metabolism, chlorocyclohexane and chlorobenzene degradation and steroid hormone biosynthesis.

[Figure 4]

3.5. qRT-PCR analysis
The total copy number range of 16S rDNA gene of bacteria was from $8.4 \times 10^7$ to $8.2 \times 10^8$, $2.1 \times 10^8$ to $1.1 \times 10^{10}$, $1.4 \times 10^{10}$ to $9.0 \times 10^{13}$, $4.1 \times 10^{10}$ to $5.9 \times 10^{12}$, $3.2 \times 10^6$ to $1.4 \times 10^9$, $2.5 \times 10^6$ to $1.3 \times 10^8$ copies per gram of tissue content in the KO, FC, MO, DI, ZH and GA samples, respectively. We found that the total copy number of bacteria in the constipation group was not significantly different from that in the control group, and that in the mosapride group and the low-dose traditional Chinese medicine group was significantly higher than that KO ($p < 0.05$). The difference between the medium dose traditional Chinese medicine group and the control group was not significant, while the copy number of bacteria in the high-dose group was significantly lower than that KO (Table 1).

### Table 1

| Total bacteria (copy number/g) | FC          | MO          | DI          | ZH           | GA           | KO           |
|-------------------------------|-------------|-------------|-------------|---------------|--------------|--------------|
| 1                             | 148213100   | 144243900   | 1.30734E+11 | 877964800     | 2532737      | 821453400    |
| 2                             | 530056400   | 662550200   | 5.94403E+12 | 137596100     | 45190660     | 84881970     |
| 3                             | 476350900   | 141332000   | 4.09941E+12 | 65151450      | 69905180     | 84399230     |
| 4                             | 323872900   | 1.35541E+12 | 412783900   | 16213720      | 144515900    | 311785000    |
| 5                             | 216104600   | 2.33782E+11 | 522876000   | 17307860      | 84531920     | 178919400    |
| 6                             | 241080800   | 9.06385E+13 | 548494400   | 10236970      | 107940800    | 168030700    |
| 7                             | 459315300   | 651434800   | 5.61591E+11 | 3292147       | 120057200    | 820202500    |
| 8                             | 114310600   | 634031000   | 1.96913E+11 | 5663928       | 133493100    | 490321300    |

FC: constipation model group, MO: mosapride group was given 2mg·(kg·d)$^{-1}$ gavage, DI: low dose group with 5.15g·(kg·d)$^{-1}$, ZH: medium dose group with 10.3G·(kg·d)$^{-1}$, GA: high dose group with 20.6g·(kg·d)$^{-1}$ doses, KO: control group.

[Table 1]

### 4. Discussion

Gut microbes were closely related to human health, which help regulate the metabolism of the host and the development of the immune system [18]. Numerous studies have shown that intestinal microbes
significantly associated with many diseases of the human body, such as intestinal inflammation, obesity, diabetes, and tumors [19, 20]. At the same time, it was found that constipation was closely related to intestinal flora. On the one hand, constipation reduces the number of beneficial bacteria in the intestine and increases the number of pathogenic bacteria or conditioned pathogens; when the structure of the flora is disordered, conditioned pathogens and pathogens can cause the displacement of intestinal bacteria, and then lead to the release of a large number of inflammatory factors, which can further aggravate the symptoms of constipation. Therefore, in the process of constipation, intestinal flora disorder was often accompanied by some inflammatory reactions [13].

It has been found that there are differences in flora composition and diversity between obese children with constipation and non-constipation. In children with constipation, the abundance of Prevotella was significantly reduced, Firmicutes is increased, and the diversity of flora is reduced [21]. Khalif et al. [22] studied 57 adult patients with functional constipation, and found that the content of Bifidobacteria and Lactobacillus decreased significantly, but the potential pathogenic bacteria or fungi increased. In recent years, 16S rRNA high-throughput sequencing technology has been used to find that the fecal flora diversity of children with chronic constipation is different from that of healthy children. The Firmicutes / Bacteroides value of children with chronic constipation is higher than that of healthy children, the number of Prevotella is significantly reduced, and the number of Bifidobacteria and Fusobacteria is not significantly reduced [23]. This result is consistent with that of our study, where we found that the relative abundance of Firmicutes in the intestine of constipation model rats was significantly higher than normal group, and the relative abundance of Bacteroides decreased. Zhu et al. [23] studied the fecal microbial composition of 8 children with constipation and 14 healthy children. From the level of phylum, the proportion of Bacteroides in healthy people 59.18%, which were the first dominant group, Firmicutes accounting for 34.98% of the total, accounting for 93.56% of the total. It is the main part of the intestinal flora. The proportion of constipation population was significantly different from that of healthy people, with the proportion of Firmicutes accounting for 57.98%, being the first dominant flora, with the proportion of Bacteroides accounting for 33.69%.

After observation of different doses of traditional Chinese medicine to rats for a period of time, through 16S rRNA sequencing results, we found that different doses of traditional Chinese medicine significantly reduced the relative abundance of Lachnospiraceae, and greatly increased the relative abundance of Lactobacillus. As a probiotic, Lactobacillus can promote intestinal peristalsis, it can promote intestinal peristalsis. Therefore, we speculated whether the reason why this traditional Chinese medicine plays a role in reducing constipation was related to promoting the relative abundance of Lactobacillus. According to relevant literature research, we found that the depth of colonic crypt in constipated rats decreased, there were a lot of inflammatory reactions, goblet cells were lost, and intestinal crypt had the function of protecting intestinal stem cells and preventing intestinal cells from being damaged. At the same time, the goblet cells secrete mucin, which were forms the mucus barrier, provides a place for the symbiotic bacteria in the host, prevents the colonization of pathogenic microorganisms, and the mucus secreted can lubricate the intestinal tract and promote food evacuation. Therefore, the loss of goblet cells in the colon of constipated rats is closely related to the occurrence of constipation. At the same time, some
studies have found that probiotics such as *Lactobacillus* can enhance intestinal epithelial barrier function and promote intestinal growth in mice with ulcerative colitis, thereby reducing the severity of colon damage [24], so we speculate that the reduction of constipation may be closely related to the significant increase of *Lactobacillus* in the feces, which can protect the colon tissue.

At the same time, *Lactobacillus* can promote intestinal peristalsis, metabolize short chain fatty acids and other beneficial products, and promote intestinal health. By GC/ MS and targeted metabolites, *Lactobacillus casei* strains were found to significantly improve defecation frequency, and 14 kinds of nonvolatile fecal metabolites, as possible constipation related metabolites, were regulated by *Lactobacillus casei* [8]. In the next step of this study, we would like to build the constipation model of germ-free mice, transplant *Lactobacillus* into aseptic mice to explore the specific mechanism of improving constipation, and combine 16S rRNA sequencing technology with metabolome to explore the mechanism.

### 4. Conclusion

In general, we found that *Firmicutes* in the intestinal flora of rats in constipation group was significantly higher than that in the control group. The intake of different doses of traditional Chinese medicine significantly increased the relative abundance of *Lactobacillus*, enhanced the symbiotic relationships of *Lactobacillus* with other intestinal flora, reduced the production of constipation, there was no significant difference in the total copies of intestinal bacteria between the constipated rats and the control group. The total copies of intestinal bacteria decreased after taking the traditional Chinese medicine. It provided a theoretical basis for the treatment and mechanism of constipation.

**Supplementary material**

**Declarations**

**Author Contributions**

*Dr. Sihan Li* - He has design whole experiment and contribution to data correction and formal analysis; *Mr. Youcheng He* - He has design whole experiment and contribution to data correction and formal analysis; *Dr. Haiou Zhang* - He put some contribution to data correction and formal analysis, *Mr. Rong Zheng* - He put some contribution to data correction and formal analysis; *Mr. Ruoying Xu* - He put some contribution to data correction and formal analysis; *Mr. Qihong Liu* - He has design whole experiment and contribution to data correction and formal analysis; *Dr. Shuihua Tang* - He put some contribution to data correction and formal analysis; *Prof. Xiao Ke* - He has conceptualization, design and supervision of review and also writing - original draft this article. In addition, funding acquisition and project administration; and *Prof. Minghan Huang* - Prof. Huang has conceptualization, design and supervision of review and also writing - original draft this article. In addition, funding acquisition and project administration. All authors have read and approved the final manuscript.
Funding

This work was supported by the school management project of Fujian University of traditional Chinese Medicine (X2019027-discipline) and (X2019028-discipline).

Acknowledgments

English revision by Dr. M.J. Gill is acknowledged.

Conflicts of interest

Conflicts of Interest: The authors declare no conflict of interest.

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Figures
Figure 1

Changes in the diversity of the gut microbiota: (A-D) the goods coverage, Chao1 index, observed species and Shannon index of 6 groups; (E) Cluster based on analysis of hierarchical clustering using unweighted pair group method with arithmetic mean to study the similarity between different samples; (F) Non-metric multidimensional scaling method (NMDS) is to simplify the research objects (samples or variables) in multidimensional space to low dimensional space for positioning, analysis and classification. The samples in the same group are all in a circle, which means that the differences between groups are not obvious, while the non-intersection between groups means that there are certain differences between groups; (G) PCoA plots based on Spearman distances are colored by time point. The significant differences between groups were calculated by analysis of similar (ANOSIM) tests. The data were analyzed by one-way ANOVA (*p <0.05; **p < 0.01). FC: constipation model group, MO: mosapride group, DI: low dose traditional Chinese medicine group; ZH: middle dose traditional Chinese medicine group; GH: high dose traditional Chinese medicine group; KO: control group. n=8/group.
Figure 2

Composition of the gut microbiota: (A) The relative contribution of the top 8 phyla in each group; (B) Relative abundance of Firmicutes in 6 groups, the data were analyzed by one-way ANOVA (*p < 0.05; **p < 0.01); (C) Relative abundance of Bacteroidetes in 6 groups, the data were analyzed by one-way ANOVA (*p < 0.05; **p < 0.01); (D) The relative contribution of the top 30 genera in each group; (E) Relative abundance of Lachnospiraceae in 6 groups, the data were analyzed by one-way ANOVA (*p < 0.05; **p < 0.01); (F) Relative abundance of Lactobacillus in 6 groups, the data were analyzed by one-way ANOVA (*p < 0.05; **p < 0.01).
Figure 3

Major differential microbial species: (A-B) Taxonomic cladogram obtained from LEfSe at 6 groups. Biomarker taxa are highlighted with colored circles and shaded areas. Each circle's diameter reflects the abundance of those taxa in the community.
Figure 4

Co-occurrence network and functional prediction analysis: (A) Co-occurrence network of the control group KO; (B) Co-occurrence network of the FC group; (C) Co-occurrence network of supplementation with high-dose dose of Chinese medicine. Blue indicates positive correlation, red indicates negative correlation. The numbers of nodes and links indicate the tightness of the connection between the bacteria and others, indicating the strength of the symbiotic relationship; (D) Functional gene abundance for the intestinal microbiota; (E) Analysis of gene difference between FC and GA groups.

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