Exploring the Mechanism of *Astragalus membranaceus* in the Treatment of Post-Infectious Irritable Bowel Syndrome Based on Random Walk with Restart Algorithm and Experimental Validation

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**Abstract**

**Background:** This study aims to screen target genes with high relevance by running the random walk with restart (RWR) algorithm on the target background network, and to explore the mechanism of *Astragalus membranaceus* (AM) mediating targets in the treatment of post-infectious irritable bowel syndrome (PI-IBS) by experimental validation. **Methods:** AM active ingredients were retrieved from TC MSP and HERB databases, of which target genes were predicted by SwissTargetPrediction. The disease genes were obtained by retrieving irritable bowel syndrome (IBS) from disease databases, and integrated to AM target genes to obtain “AM-IBS” intersection genes. The RWR algorithm was used to identify “AM-IBS” crossover genes, and target genes were screened based on correlation scores. The rat model of PI-IBS was established by multiple compound stimulation, followed by treatment with different doses of AM (0.2, 1, 2 g/(kg·d)). Intestinal function of rats was assessed by measuring fecal water content and body weight. Sucrose preference test and open-field test were performed to assess changes in behaviors of rats during the experiment. The expression of Toll-like receptor 4 (TLR4) in colon tissues was detected by immunohistochemistry, and the co-localized expression pattern of polymerase I and transcript release factor (PTRF) /TLR4 was observed by immunofluorescence. **Results:** At the end of the modeling, the body weight of PI-IBS rats was decreased, but fecal water content, levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), as well as depressionanxiety-like behaviors were increased, all of which were improved to varying degrees after AM treatment. Moreover complex stimulation of rats with EPSPD, TNBS and CUMS induced higher levels of anxiety and depression, while AM treatment reduced anxiety and depression levels. Notably, AM treatment could inhibit the overexpression of TLR4. **Conclusion:** *Astragalus membranaceus* has a certain therapeutic effect on PI-IBS and may improve PI-IBS symptoms by regulating the PTRF/TLR4-related pathway.
1 Introduction

Post-infectious irritable bowel syndrome (PI-IBS) is a highly prevalent gastrointestinal disease, and its symptoms are likely to be induced or aggravated by nervousness, infection and visceral hypersensitivity in clinical cases [1]. The morbidity of PI-IBS is relatively higher in young and middleaged females, which cannot be neglected [2]. In recent years, the number of PI-IBS patients has been increasing with the changes of living habits and the rise of competitive pressure. At present, there are a lot of drugs for PI-IBS, but their therapeutic effects are not ideal. Notably, PI-IBS as an advantageous disease treated by traditional Chinese medicine (TCM), TCM has a good therapeutic effect on it [3].

*Astragalus membranaceus* (AM) is the root of leguminous plant mongolia astragaloside or *Astragalus membranaceus* Bge [4]. AM is known as “the master of tonic” in ancient times and commonly used for the improvement and treatment of diseases, with a long history in TCM [5]. AM has the effects of tonifying qi, lifting yang as well as promoting diuresis for eliminating swelling [6]. As a modern pharmallogical study demonstrated [7,8], AM has the vital effects such as protecting gastroenteric function, repressing and ameliorating peritoneal complications, promoting immune function, antioxidation and anti-inflammation, which is beneficial to the rehabilitation of human body.

Due to the lack of pre-clinical animal models, it is difficult to assess the therapeutic effect of drugs on PI-IBS. In the preparation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) via autoclaved sterilization, the success rate of PI-IBS modeling can reach over 95% [9,10]. PI-IBS is a frequently occurred gastrointestinal disease, which has widely been perceived as a functional disease. Excessive pressure and mental factors are deemed to play a pivotal role in irritable bowel syndrome (IBS) [11]. In previous research, the PI-IBS rat model not only has the symptoms of immune-mediated intestinal dysfunction, but also has the pathological mechanism of depression- and anxiety-like diseases, which is established by the compound stimulus of early postnatal sibling deprivation (EPSD), TNBS and chronic unpredictable mild stress (CUMS) [12]. However, the mechanism of AM in treating model rats induced by compound stimulus of EPSD, TNBS and CUMS is still unclear.

The random walk with restart (RWR) algorithm is modified on the basis of random walk algorithm, by which the proximity of genes, namely the distance starting from a certain gene node in a graph to a randomly selected adjacent gene node or the distance returning from the randomly selected adjacent gene node to the starting gene node, can be calculated in this research. RWR algorithm can predict the multifaceted relationships between two nodes to estimate the proximity between them [13].

This study determines the target genes of AM and IBS with high relevance by RWR algorithm, and constructs an animal model of PI-IBS for further validation, with the intention of exploring and analyzing the mechanism of AM in the treatment of PI-IBS.

2 Materials and methods

2.1 Databases and analytical software

The databases used in this study were listed below:

TCMSP (http://lsp.nwu.edu.cn/tcmsp.php),
HERB (http://herb.ac.cn),
GeneCards (https://www.genecards.org/),
DrugBank (https://go.drugbank.com),
OMIM (https://www.omim.org),
PharmGKB (https://www.pharmgkb.org),
TTD (https://db.idrblab.net/ttd/full-data-download),
STRING (http://string-db.org),
PubChem (https://pubchem.ncbi.nlm.nih.gov/) and
SwissTargetPrediction (http://swisstargetprediction.ch/).
2.2 Prediction of AM targets
In HERB database, relevant targets of AM were searched and reserved. In TCMSp database [14], the keyword “Astragalus membranaceus” was input for retrieval of its active ingredients, with filter criteria of drug-likeness (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30%. The obtained active ingredients were imported to the PubChem database to obtain corresponding SMILE number. The predicted targets of ingredients acquired from SwissTargetPrediction were integrated to the targets obtained from HERB database to obtain the relative targets for AM.

2.3 Screening of IBS-related targets
IBS-related target genes were obtained by retrieval of “irritable bowel syndrome” in GeneCards, DrugBank, OMIM, PharmGKB and TTD databases. Then, these genes were subjected to further intersection for removal of the duplicate ones.

2.4 Construction of STRING background network
AM-related targets obtained above were integrated with IBS-related targets, and the duplicated genes were reserved to obtain the “drug-disease” targets related to AM and IBS. Then, AM-IBS targets were imported to STRING database to obtain the protein-protein interaction (PPI) network.

2.5 Running of RWR algorithm
By running RWR algorithm in the obtained STRING background network, the correlation score of pairwise gene nodes was acquired, based on which 50 targets with the top correlation score were singled out as the key targets for AM treatment of IBS. RWR algorithm was executed through running the dnet package in R software, where the restart probability was set to the default value of 0.75.

2.6 Annotation of biological processes and analysis of metabolic pathways
Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed on AM-IBS targets through R software [15]. GO functional annotation was performed in target genes. Then, the major metabolic pathways involved in the enriched target genes in KEGG database were annotated and screened. Pathway enrichment analysis was set at p-value = 0.05.

2.7 Experimental validation

2.7.1 Experimental drugs
AM granules (batch number: 0510084, specification: 4.0 g/bag, equivalent to 10 g of crude drug, Guangdong, China). Pinaverium bromide (PB; batch number: H20120127, Abbott, French).

2.7.2 Experimental animals
Adult pregnant Sprague Dawley (SD) rats (weight: 190-210 g; animal license number: SCXK(Yu)2019-0001) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd. This study was approved by Animal Care and Use Committee of Wannan Medical College (approval number: LLSC-2022-216). During the whole research, rats were kept under the controlled room temperature of 23 °C ± 1 °C with relative humidity of 55% ± 5% in a 12 h/12 h day/night cycle, and had free access to water (except for CUMS test and sucrose preference test).

2.7.3 Reagents and instruments
The following reagents and instruments were used in this study: 5% TNBS (T97750, Jizhi Biochemical, Shanghai, CN), anti-PTRF antibody (PA5-110243, Thermo Invitrogen, Waltham, MA, USA), anti-TLR4 antibody (PA5-23123, Thermo Invitrogen), 4% paraformaldehyde (PH0427, Dekang Biological, Ningbo, China), rat interleukin-6 (IL-6) enzyme-linked immunosorbent assay ELISA kit (RK00020, ABclonal, Wuhan, China), tumor necrosis factor-alpha (TNF-α) ELISA kit (RK00029, ABclonal), and microplate reader
(SPARK, Tecan Trading AG, Switzerland).

2.7.4 Grouping and modeling of PI-IBS rats

Considering that PI-IBS was an inflammatory-immune disease, this study merely used the newly born male rats. According to previously published studies [16,17], a PI-IBS rat model was constructed by the compound stimulus of EPSD, TNBS and CUMS. From 9:00 a.m. to 11:00 a.m. between the 2nd day to the 14th day post the partum, the immature rats were moved from their mothers’ cages to the adjacent cages. Then, the rats were continuously reared for 2 weeks, and divided into normal, model, AM-H, AM-M, AM-L and PB groups. After being anesthetized by pentobarbital, rats were subjected to intrarectal injection of 0.8 mL TNBS (20 mg/rat) dissolved in 50% alcoholic 28 days post the partum, according to the previously published method [18]. Rats in normal group were injected with the same volume of 50% alcoholic. As for TNBS modeling, a soft catheter lubricated by paraffin wax was inserted into the rat colon at a depth of about 8 cm from anus, via which TNBS was injected. 2 weeks after the recovery of rats from TNBS modeling, stress tests were daily carried out in rats for 21 days, including 24h fasting for solids and liquids, reversal of the diurnal cycle, cold stimulation in 4 °C ice water for 5 min, hot stimulation in a 45 °C thermostat for 5 min, and pain stimulation by tail pinch, as shown in Figure 1.

![Figure 1 Experimental protocol.](image)

2.7.5 Drug administration of PI-IBS rats

Except for rats in normal group and model group, rats in PB group were given PB via intragastric administration. In a nutshell, PB was dissolved in sterile distilled water until reaching the concentration of 2.7 mg/mL, and then intragastrically administered to rats as per 2.7 g/(kg-d) [19]. Rats in AM high dose group (AM-H), AM medium dose group (AM-M) and AM low dose group (AM-L) groups were given AM at the high, medium, and low doses of 2, 1 and 0.2 g/(kg·d), respectively. The drug administration was continuously conducted for 14 days.

2.7.6 Open-field test

The rats were separately placed in the center of open-field equipment (height: 40.0 cm, width: 100.0 cm, length: 100.0 cm) between 8:00 a.m. to 11:00 a.m. The bottom of the open field consisted of 25 squares (20 × 20 cm). According to a prior study [20], the frequency of rats crossing the square grids within 10 min was calculated. When the four legs of rats moved from one quadrant to another quadrant, the frequency of rats crossing the square grids was calculated as a measuring standard for the amount of exercise. The open-field equipment was cleaned prior to new trial.

2.7.7 Sucrose preference test

One week before and after drug administration, sucrose preference test was conducted [21]. Briefly, each rat was separately reared. Following 24-h fasting for solids and liquids, rats had free access to the solution in two bottles (one for tap water, and another for 1% saccharose solution) within 1 h. The consumption of saccharose solution was recorded, and
its percentage was calculated with the formula below: the percentage of consumed saccharose solution (%) = (sucrose intake/total intake) × 100%.

### 2.7.8 Immunohistochemical staining

One week after the sucrose preference test, rats were anesthetized by intraperitoneal injection of pentobarbital at the rate of 30 mg/kg, blood was taken via the abdominal aorta, serum was separated for biochemical indexes using a centrifuge, and colon tissue was intercepted, and fixed in 4% formalin and embedded in paraffin. Then, tissue sections at 5 μm thickness were prepared for immunohistochemical staining, followed by reaction with primary antibody against TLR4. Finally, Image J software was applied for analysis of optical density.

### 2.7.9 Immunofluorescence

The paraffin-embedded colon tissues were sectioned, followed by dewaxing, hydration and antigen retrieval. Next, 5% bovine serum albumin (BSA) was added to incubate the sections for 1 h. The sections were further incubated with antibodies against PTRF and TLR4 at 4 °C overnight. Then, 4′,6-diamidino-2-phenylindole (DAPI) was utilized to stain cell nuclei. After being sealed, the sections were placed under the optical microscope for observation.

### 2.7.10 ELISA

The content of TNF-α and IL-6 in rat serum was measured in accordance with the instructions of ELISA kits.

### 2.7.11 Statistical analysis

Image J and GraphPad Prism were applied for data analysis and processing. The results were expressed as mean ± standard deviation. The comparisons among multiple groups were made by one-way analysis of variance (ANOVA), followed by Tukey’s test for multiple comparisons. A nonparametric test was used to compare band density values between the groups. The difference was statistical significant at $p < 0.05$.

### 3 Results

#### 3.1 AM-IBS targets

A total of 532 AM targets were obtained by sorting out TCMSP and HERB databases. Likewise, 3417 IBS-related genes were acquired through retrieval in multiple disease gene databases (Figure 2A). Finally, 327 AM-IBS targets were determined after integration of AM targets and IBS-related genes while reserving the same genes (Figure 2B).

#### 3.2 Interaction network diagram of AM-IBS targets

AM-IBS targets were imported to STRING database, where the species was set as “Homo sapiens”, and the confidence was kept as more than 0.4. After removal of free nodes, a PPI network was obtained (Figure 2C), with 324 nodes and 7413 edges.

#### 3.3 Correlation score of AM-IBS targets

The correlation score of AM-IBS targets was obtained by running RWR algorithm in R software (Figure 3), with CREBBP, JAK2, BAX, TLR4 and RELA raking in the top. Notably, TLR4 was a vital protein involved in non-specific immunity, and PI-IBS was highly correlated with immune dysfunction. Research has uncovered that TLR4 can activate the body to initiate immune response when the mucosa is damaged, playing an indispensable role in autoimmune disease [22].
Figure 2 Network pharmacology analysis (A. IBS-related genes; B. AM-IBS targets; C. AM-IBStargets PPI network).

Figure 3 50 genes with top AM-IBS correlation score.

3.4 GO and KEGG pathway enrichment analyses

GO and KEGG pathway enrichment analyses were carried out after import of AM-IBS targets to DAVID online platform (https://david.ncifcrf.gov/) (Figure 4). Data of GO analysis revealed that AMIBS targets were mainly implicated in biological processes such as cell differentiation, inflammatory response and glucose homeostasis. In terms of molecular function, AM-IBS targets were chiefly correlated with zinc ion binding, enzyme binding and steroid binding. With regard to cell components, AM-IBS targets primarily acted on nucleus, cytoplasm and endoplasmic reticulum membrane. Results of KEGG analysis uncovered that AM-IBS targets were mainly enriched in lipid, atherosclerosis, prostate cancer, AMPK signaling pathway, neutrophil formation, toll-like receptor signaling pathway, TNF signaling pathway, arachidonic acid metabolism and apoptotic signaling pathways.

3.5 In vivo experimental results

3.5.1 Effects of AM treatment on the weight and fecal water content of PI-IBS rats

To determine the therapeutic effect of AM on PI-IBS rats, the body weight and fecal particle weight of rats were detected to assess whether the PI-IBS model was successfully established. Following EPSD, TNBS and CUMS stimulation, model rats showed lighter body weight and higher fecal water content relative to normal rats, suggesting successful construction of the PI-IBS model. Compared with those in PI-IBS group, the body weight of rats was markedly increased, yet the fecal water content was decreased in AM group (Figure 5). These results indicated that the symptoms of PI-IBS rats were ameliorated after AM treatment.
Figure 4 Enrichment analysis of AM-IBS targets (A. GO enrichment analysis; B. KEGGenrichment analysis).

Figure 5 Effect of AM treatment on body weight and fecal water content of rats (A. Changes in body weight of rats in each group were observed one day before and after drug administration. B. Comparison of fecal water content in each group was made at CUMS and one day after drug administration). Note: Data were expressed as mean ± standard deviation. Compared with PI-IBS group: * p < 0.05; ** p < 0.01.
3.5.2 Effects of AM treatment on anxiety-like behaviors of PI-IBS rats induced by open-field test and sucrose preference test

The results showed that EPSD, TNBS and CUMS stimulation had significantly negative effects on the behaviors of PI-IBS rats during open-field test (Figure 6B). Notably, there was an increasing trend towards the frequency of AM group rats staying in the central area (Table 1 and Figure 6A). The sucrose preference of rats was overtly reduced after EPSD, TNBS and CUMS stimulation, while being raised post AM treatment, suggesting that the symptoms of PI-IBS rats such as anxiety and depression were improved after AM treatment. These findings hinted that the compound stimulus of EPSD, TNBS and CUMS could induce the higher levels of anxiety and depression in rats, and AM could reduce these levels.

Table 1 Effect of AM treatment on rats in the open-field test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg·d)</th>
<th>Total distance (cm)</th>
<th>Distance in center (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>23320.89 ± 2422.50</td>
<td>1141.34 ± 387.15 **</td>
</tr>
<tr>
<td>PI-IBS</td>
<td>-</td>
<td>20836.77 ± 3814.92</td>
<td>497.19 ± 170.50</td>
</tr>
<tr>
<td>PI-IBS+AM 0.2</td>
<td></td>
<td>19406.50 ± 4554.48</td>
<td>583.11 ± 212.41 **</td>
</tr>
<tr>
<td>PI-IBS+AM 1</td>
<td></td>
<td>18876.20 ± 3272.52</td>
<td>700.83 ± 104.87 **</td>
</tr>
<tr>
<td>PI-IBS+AM 2</td>
<td></td>
<td>23363.10 ± 6266.73</td>
<td>727.25 ± 146.10 **</td>
</tr>
<tr>
<td>PI-IBS+PB 2.7</td>
<td></td>
<td>20427.40 ± 2173.56</td>
<td>795.21 ± 184.69 **</td>
</tr>
</tbody>
</table>

Note: Data were expressed as mean ± standard deviation. Compared with PI-IBS group: ** p < 0.01.

Figure 6 Effects of AM treatment on anxiety- and depression-like behaviors of rats (A. Effects of open-field test on the rats in each group. B. Comparison of sucrose consumption between groups the day before and after drug administration). Note: Data were expressed as mean ± standard deviation. Compared with PI-IBS group: * p < 0.05; ** p < 0.01.
3.5.3 Effects of AM treatment on the protein expression of TLR4 in colon tissues of PI-IBS rats

Immunohistochemical results uncovered that the protein expression of TLR4 was apparently increased in rat colon tissues post stimulation of EPSD, TNBS and CUMS, yet was reduced to varying degrees after AM treatment (Figure 7), indicating that TLR4 expression was significant for the recovery of AM-treated PI-IBS rats.

3.5.4 Effects of AM treatment on co-expression of TLR4/PTRF in colon tissues of PI-IBS rats

Double-labeling immunofluorescence was performed for testing TLR4 and PTRF, where PTRF was denoted as red color, TLR4 was indicated as green color, and cell nuclei dyed by DAPI were displayed as blue color. The results uncovered that TLR4 and PTRF had stronger co-localization pattern. Compared with normal rats, model group rats presented obviously increased number of cells having colocalization with TLR4 and PTRF. Relative to model group, the number of cells showing co-localization with TLR4 and PTRF was reduced in AM group (Figure 8), indicating that the expression levels of TLR4 and PTRF were declined in colon tissues of PI-IBS rats after AM treatment. In other words, AM may improve the symptoms of PI-IBS rats by TLR4/PTRF signaling pathway.

3.5.5 Effects of AM treatment on expression levels of TNF-α and IL-6 in PI-IBS rat serum

The expressions of TNF-α and IL-6 were assessed in rats of each group. In model group, the expressions of TNF-α and IL-6 were notably higher than those in normal group (Figure 9). Compared with those in model group, the content of TNF-α and IL-6 in AM group was reduced to varying degrees (Figure 9). These findings suggested that the expressions of TNF-α and IL-6 were elevated in PI-IBS rat serum, and AM treatment could diminish the expressions of TNF-α and IL-6, and mitigated the inflammatory response induced by PI-IBS.

Figure 7 Effect on TLR4 protein expression in rat colon tissues and quantitative data (scale bar = 200 μm). Note: Data were expressed as mean ± standard deviation. Compared with PI-IBS group: * p < 0.05; ** p < 0.01.
Figure 8 Merged confocal images of PTRF (red), TLR4 (green) and DAPI (blue) nuclear staining overlays and quantitative data of cells (scale bar = 200 μm). Note: Data were expressed as mean ± standard deviation. Compared with PI-IBS group: * p < 0.05; ** p < 0.01.

Figure 9 Expressions of TNF-α and IL-6 in rat serum. Note: Data were expressed as mean ± standard deviation. Compared with PI-IBS group: * p < 0.05; ** p < 0.01.
4 Discussion

PI-IBS is clinically manifested as abdominal distension, abdominal pain or abdominal discomfort, with which patients may experience changes in bowel habits. At present, the pathogenic mechanism of PI-IBS has yet to be fully elucidated. It is mostly recognized that the abnormal interaction of brainintestinal axis and changes in the bowel habits can be attributed to joint participation and action of multiple factors. Currently, there is no specific therapy for this disease in the clinic. The commonly used treatment means include patient education, consolation and first-line therapy (e.g., fiber laxative or osmotic laxative for constipation, opioid for diarrhea, spasmolytic for pain and management for relative psychological disorders). For patients with no response to the treatment of PI-IBS, detection of specific dysfunction is required for a minority of PI-IBS patients. In AM, there are a variety of effective ingredients with wide range of pharmacological effects, playing a vital role in assorted aspects such as anti-inflammation, anti-oxidation, anti-tumor and anti-depression [23-25]. Based on RWR algorithm, this study determined the targets with high relevance, and obtained the main targets of AM in treating IBS. The PI-IBS model constructed by the compound stimulus of EPSD, TNBS and CUMS could show the clinical symptoms closest to PI-IBS patients. By dint of its reliability and repeatability, the colon perfusion of TNBS has been widely applied in the construction of ulcerative colitis models, where the chemical drug TNBS can induce intestinal infection, thereby causing inflammatory response. The experimental results uncovered that AM could improve the symptoms such as the decrease of body weight and the increase of fecal water content. At the same time, AM treatment increased the frequency of rats entering the central area during sucrose preference test and open-field test, indicating that AM ameliorated depression- and anxiety-like behaviors of PI-IBS rats. In addition, AM treatment relieved the inflammation of PI-IBS by down-regulating the expression levels of IL-6 and TNF-α in the serum [26,27]. Additionally, studies indicate that PTRF impaired formation of the TLR4/Myd88 complex, while LPS strengthened the co-localization and interaction between PTRF and TLR4 in lipid rafts [28]. Further studies indicate that PTRF was required for LPS-Mediated iNOS induction and NO Production in Colorectal Epithelial Cells, which trigger the downstream signal cascades, including ERK, p38, and JNK [12]. Polymerase I and transcript release factor (PTRF), also known as cacin-1, is a structural protein regulating cave. Research uncovers that the expression levels of pro-apoptotic and proinflammatory genes are significantly raised in adipose tissues of cavin-1-deficient rats, thereby leading to cell apoptosis and inflammatory response [29]. A study has found that AM inhibits the transcriptional activity of nuclear factor kappa B (NF-κB) in lipopolysaccharides (LPS)-activated RAW26.4 cells to reduce the release of cytokines IL-6 and TNF-α [30]. In addition, AM can improve the clinical symptoms of colitis, reconstruct immune balance and relieve the colonic mucosal injury [31,32]. TNF is a pleotropic inflammatory cytokine. In the clinical practice, anti-TNF agent has been intensively applied in treatment of inflammation in intestinal diseases. The clinical observation of Eun et al. [33] revealed that the serum level of TNF-α in patients with colitis was significantly up-regulated, and could return to normal after AM treatment. A previous experiment by a research group has demonstrated that PTRF knockout results in the decreased interaction of TLR4 and PTRF in PI-IBS rats, and the expression of TLR4 downstream products is weaken due to the deficiency of PTRF [28]. PTRF can affect the coexpression of TLR4 and PTRF as well as downstream signal transduction to mitigate the intestinal inflammatory response [34], and simultaneously reduce the expressions of cytokines TNF-α and IL-6 to improve
intestinal inflammation [35]. These findings suggest that inhibition of PTRF can repress the activation of TLR4 pathway and diminish the secretion of inflammatory factors, thus ameliorating the symptoms of PI-IBS. The experimental results in this study mirrored that the application of AM markedly down-regulated the levels of IL-6 and TNF-α, indicating that AM could improve the colitis by suppressing the expression levels of inflammatory factors IL-6 and TNF-α. Meanwhile, our study discovered that the expressions of PTRF and TLR4 were obviously elevated in rats after modeling, while being remarkably reduced after interference of AM drugs. These data collectively hinted that AM may improve PI-IBS symptoms by regulating PTRF/TLR4 signaling pathway.

In summary, this study analyzes the potential mechanism of AM treatment, obtains the main targets using RWR algorithm, and finally validates the role of AM in improving PI-IBS symptoms through experiments, systematically revealing the pharmacologic mechanism of AM in treating PI-IBS. With a great detail, this study finds that the effect of AM on treating PI-IBS may be realized by regulating PTRF/TLR4 signaling pathway to protect colonic mucosal tissues.

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Conflicts of Interest
All authors disclosed no relevant relationships.

Author Contributions
J.Y.: Investigation, writing-original draft; Z.W.: Conceptualization, data curation, review & editing; K.C.: Investigation, validation; X.Z.: Investigation; C.L.: Investigation, methodology; B.L.: Conceptualization, methodology, data curation; writing-review & editing. Y.Z.: Conceptualization, data curation, visualization, writing-original draft, supervision, review & editing, project administration. J.Y. and K.C. contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate
Adult pregnant Sprague Dawley (SD) rats (weight: 190-210 g; animal license number: SCXK(Yu)2019-0001) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd. This study was approved by Animal Care and Use Committee of Wannan Medical College (approval number: LLSC-2022-216).

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Availability of Data and Materials
The data presented in this study are available on request from the corresponding author.

Supplementary Material
Not applicable.

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