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ORIGINAL RESEARCH

One of the Short-Chain Fatty Acids (SCFAs), Sodium Propionate, Can Reduce the Dosage of Sishen Pill in Regulating the Intestinal Microbiota in Diarrhea with Kidney-Yang Deficiency Syndrome

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Objective: To investigate the potential mechanisms underlying the combined therapeutic effects of sodium propionate and Sishen Pill and to provide experimental evidence supporting their mode of action.

Methods: The method utilized involved the induction of a mouse model of Diarrhea with Kidney-Yang Deficiency Syndrome by administering adenine combined with *Folium sennae*. After the successful establishment of the model, the mice in the model group were randomly assigned to one of the following treatment groups: natural recovery group, sodium propionate group, 75% Sishen Pill combined with 60 mg/kg sodium propionate group, or Sishen Pill group.

Results: The 75% Sishen Pill combined with 60 mg/kg sodium propionate demonstrated significantly better therapeutic effects compared to the Sishen Pill alone and sodium propionate alone groups. The combined treatment significantly improved the behavioral indices of mice ($p < 0.05$); increased the levels of MUC2 and sIgA ($p < 0.01$); reduced IL-6 levels ($p < 0.05$); and improved structural damage in the kidneys and small intestines. Intestinal microbiota analysis showed that 75% Sishen Pill combined with 60 mg/kg sodium propionate significantly increased beneficial bacteria such as *Lactobacillus* ($p < 0.05$), while *Prevotellamassili*a and *Maribacter* were significantly enriched in this group. Correlation analysis revealed that *Lactobacillus* and *Pediococcus* were positively correlated with MUC2 and sIgA, while negatively correlated with IL-6.

Conclusion: The 75% Sishen Pill combined with 60 mg/kg sodium propionate significantly alleviates symptoms related to Diarrhea with Kidney-Yang Deficiency Syndrome, enhances the efficacy of Sishen Pill by regulating the intestinal microbiota, boosts intestinal immune function, and reduces intestinal inflammation, providing a new approach for treating Diarrhea with Kidney-Yang Deficiency Syndrome.

Keywords: sodium propionate, kidney-yang deficiency syndrome, intestinal microbiota, intestinal inflammation, Sishen Pill

Introduction

Diarrhea is characterized by an increased frequency of bowel movements, the passage of loose stools, and, in severe cases, watery stools.[1] Diarrhea with Kidney-Yang Deficiency Syndrome, Clinically, the treatment approach focuses on warming the kidneys, dispersing cold, and astringing the intestines to stop diarrhea, with the representative prescription being Sishen Pill.[2]

Diarrhea with Kidney-Yang Deficiency Syndrome and intestinal microbiota imbalance often exhibit bidirectional interactions. With the deepening exploration of intestinal microbiota theory and advancements in modern technology, regulating the intestinal microbiota has emerged as a therapeutic strategy for managing diarrhea. This approach can improve symptoms by increasing beneficial bacteria and restoring the balance of the intestinal microbiome.[3,4] Zhang et al found that bacteria such as *Paraprevotella* have a significant intervention effect on diarrhea associated with spleen and stomach deficiency syndrome.[5]

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Intestinal microbiota dysbiosis, along with the translocation of microbial metabolites and endotoxins into the bloodstream, activates both local and systemic immune responses. This process is one of the key factors contributing to the low-grade inflammatory state observed in patients with Diarrhea with Kidney-Yang Deficiency Syndrome.[6] Studies have shown that the inflammatory response promotes intestinal microbiota imbalance, which in turn induces inflammatory reactions, creating a feedback loop. Short-chain fatty acids (SCFAs) play a role in regulating the intestinal microbiota, thereby improving the symptoms of Diarrhea with Kidney-Yang Deficiency Syndrome.[7]

SCFAs are metabolites produced by intestinal microbiota in the colon through the fermentation of unabsorbed carbohydrates. They are crucial for maintaining intestinal homeostasis and primarily include butyrate, propionate, and acetate.[8,9] Research has shown that SCFAs play a role in the treatment of diarrhea, as their effects are linked to the modulation of intestinal microbiota composition and the increased abundance of SCFA-producing bacteria.[10] Propionate, a non-steroidal anti-inflammatory agent, exerts its anti-inflammatory effects primarily by inhibiting cyclooxygenase in the arachidonic acid metabolism pathway.[11] A study by Ton et al demonstrated that propionate improves colitis induced by dextran sulfate sodium by enhancing intestinal barrier function and alleviating inflammation and oxidative stress.[12]

Sishen Pill, a well-known formula for treating kidney-yang deficiency syndrome. It directly targets the pathogenesis of ulcerative colitis (UC) associated with kidney and spleen yang deficiency, preventing excessive apoptosis of epithelial cells, promoting the repair of intestinal mucosal damage, modulating the intestinal microbiota, and exerting anti-inflammatory and antioxidant effects.[13] Sishen Pill has been shown to regulate energy metabolism in mice with Diarrhea with Kidney-Yang Deficiency Syndrome, improve kidney structure, and enhance the diversity of the intestinal mucosal microbiota. *Lactobacillus johnsonii* has been identified as a characteristic bacterium in mice treated with Sishen Pill for Diarrhea with Kidney-Yang Deficiency Syndrome.[14] Furthermore, Sishen Pill improves intestinal barrier function by promoting autophagy and inhibiting endoplasmic reticulum (ER) stress.[15] The combined use of traditional Chinese medicine and microecological agents has become a major research focus in contemporary studies. For example, a combination of 25% ultra-micronized Qiwei Baizhu San and 25% yeast formulation has been shown to effectively treat dysbiosis-induced diarrhea in mice.[16,17] Some studies have shown that sodium propionate and HMT-specific inhibitors may exhibit a synergistic effect in the treatment of colon cancer.[18] Therefore, this study will focus on exploring whether the combination of sodium propionate and Sishen Pill can alleviate diarrhea and repair intestinal function by modulating the composition of the intestinal microbiota, increasing the proportion of beneficial bacteria, and inhibiting the growth of pathogenic bacteria. The goal is to reduce intestinal inflammation and promote intestinal barrier repair.

Previous studies involving adenine combined with *Folium sennae* were performed to induce kidney yang deficiency-related diarrhea in mice.[19,20] This study focuses on the intestinal microbiota, detecting the levels of Mucin 2 (MUC2), Secretory immunoglobulin A (sIgA) in the intestinal mucosa, and Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-α) in the serum, analyzing the intestinal mucosal microbiome, and observing the pathological changes in the kidneys and small intestine. This study aimed to compare the therapeutic effects of SiShen Pill, sodium propionate, and the combination treatment to reveal the advantages of their combined application, providing a theoretical basis for the clinical treatment of Diarrhea with Kidney-Yang Deficiency Syndrome.

Materials and Methods

Materials

To eliminate the potential confounding effects of gender on the intestinal microbiota, this study exclusively utilized male mice.[21] A total of 70 male Kunming SPF-grade mice, aged 4 weeks and weighing between 18 and 22 g, were procured from Hunan Sleeks Experimental Co., Ltd. (Animal Quality Certificate No.: ZS-202404230013). The mice were housed in a specific-pathogen-free (SPF) environment at the Laboratory Animal Center of Hunan University of Chinese Medicine (License No.: SCXK(Xiang) 2019–0004), with controlled environmental conditions (temperature: 23–25°C, humidity: 50–70%, light/dark cycle: 12 h). They had unrestricted access to food and water, with the diet provided by the Laboratory Animal Center. The experimental protocol was approved by the Animal Ethics Committee of Hunan University of Chinese Medicine, and all procedures adhered to ethical guidelines for animal research (Ethical Approval No.: HNUCM21-2403-43).

Feed and Shielded Environmental Experimental Facilities

The breeding diet provided to the mice in this study was sourced from the Laboratory Animal Center of Hunan University of Chinese Medicine and manufactured by Beijing Hua Fu Kang Biotechnology Co., Ltd. The diet's composition included the following key nutrients: crude protein ≥20%, crude fat ≥4%, crude fiber ≤5%, crude ash ≤8%, moisture ≤10%, lysine ≥1.3%, calcium 0.6%-1.8%, phosphorus 0.6%-1.2%, and salt 0.3%-0.8%. The feed was guaranteed to be clean, uncontaminated, and of high quality, with the certification number Jing Shi Zheng (2024) 06076.[20]

Drugs and Reagents

Adenine (Changsha Yaer Biotechnology Co., Ltd., Lot No. EZ7890B179) was prepared at a concentration of 5 mg/mL and used immediately. *Folium sennae* (Maozhou Luqiao Pharmaceutical Co., Ltd., Lot No. 2111090022) were processed as follows: 300 mL of water was added to the *Folium sennae*, and the mixture was allowed to soak for 30 minutes. The mixture was then boiled and simmered for 30 minutes. Afterward, the liquid was filtered through gauze, and the remaining drug residue was re-extracted with 200 mL of water using the same boiling and simmering procedure. The two filtrates were combined and concentrated to yield a final *Folium sennae* decoction with a crude drug concentration of 1 g/mL.

Sishen Pill was composed of the following ingredients: *Psoraleae fructus* (Hunan Hengyue Traditional Chinese Medicine Co., Ltd., Lot No. 231001) 12 g, *Myristica semen* (Hunan Hengyue Traditional Chinese Medicine Co., Ltd., Lot No. 23083107) 6 g, *Euodiae fructus* (Hunan Rongkang Traditional Chinese Medicine Co., Ltd., Lot No. 230801) 3 g, *Schisandrae chinensis* (Maozhou Yonggang Decoction Pieces Co., Ltd., Lot No. A231031) 6 g, *Ziziphus jujuba* (Hunan Xinshen Zhilin Traditional Chinese Medicine Co., Ltd., Lot No. 2310001) 6 g, and *Zingiber officinale Roscoe* 6 g.

The preparation of the Sishen Pill decoction followed the same procedure as the *Folium sennae* decoction. The final decoction was concentrated to a concentration of 0.29 g/mL. Two distinct preparations of the decoction were made: one containing 75% of the full-dose Four Gods Pill decoction, and the other containing the full-dose decoction. Sodium propionate (Shanghai Aladdin Biochemical Technology Co., Ltd., Lot No. A2431246) was dissolved in sterile water to prepare solutions at concentrations of 480 mg/(kg·d) and 60 mg/(kg·d), respectively. All prepared drug solutions were stored at 4°C for future use.[22]

Reagents and Materials

The IL-6 ELISA kit (Lot No. JM-02446M2), TNF-α ELISA kit (Lot No. JM-02415M2), sIgA ELISA kit (Lot No. JM-02713M2), and MUC2 ELISA kit (Lot No. JM-11388M2) were all obtained from Jiangsu Jingmei Biotechnology Co., Ltd.

Modelling Method

After a 3-day acclimatization period, the mice were randomly allocated to two groups on a random number table: normal group (n = 10) and model group (n = 40). Following the protocol outlined in the literature, a mouse model of kidney-yang deficiency and diarrhea was established via a combination of adenine and senna leaves.[23] Mice in both model and treatment groups were administered a daily oral dose of a 50 mg/(kg·d) adenine suspension (0.35 mL per mouse) for 14 consecutive days.

Beginning on day 8, the treatment group also received a daily dose of *Folium sennae* decoction (10 g/(kg·d), 0.35 mL per mouse) for an additional 7 days. Mice in normal group were gavaged with an equal volume of sterile water at the same frequency. The successful establishment of the model was confirmed by the following clinical signs: loose or incomplete stools, cold extremities, arched back, reduced appetite, weight loss, and lethargy.[21]

Animal Grouping and Administration

Upon successful establishment of the model, the inducing factors ceased. Based on previous findings, 75% Sishen Pill combined with 60 mg/kg sodium propionate exhibits good efficacy. The mice in model group were subsequently randomized into four subgroups via a random number table: natural recovery group, sodium propionate group, 75% Sishen Pill combined with 60 mg/kg sodium propionate group, and Sishen Pill group, 10 mice per group. Upon successful establishment of the model, the modeling procedures were terminated. In sodium propionate group, the

animals were administered sodium propionate at a dose of 480 mg/(kg·d) via oral gavage. The 75% Sishen Pill combined with 60 mg/kg sodium propionate group received a mixture of 75% Sishen Pill combined with 60 mg/(kg·d) sodium propionate via oral gavage. In Sishen Pill group, the animals were given an equivalent dose of Sishen Pill decoction, corresponding to 5 g/(kg·d). The normal and natural recovery groups were orally given an equal volume of sterile water (0.4 mL/animal/dose) twice daily for 7 consecutive days.

Observation Indicators and Methods

Detection of Mouse Immune Organs

After the treatment, the mice were weighed, and the spleen and thymus weights were measured. The corresponding organ indices were calculated using the following formulas: Spleen index (%) = Spleen weight (g) / Mouse body weight (g) × 100%. Thymus index (%) = Thymus weight (g) / Mouse body weight (g) × 100%.[19]

Routine HE Stains of Kidney Tissue and Small Intestine Tissue

Fresh kidney and small intestine tissues were harvested and placed into embedding molds, followed by fixation in 4% paraformaldehyde. The specimens were subsequently dehydrated through a graded ethanol series and embedded in paraffin to form paraffin blocks. Tissue sections were then cut, deparaffinized, and rehydrated. Hematoxylin and eosin (H&E) staining was performed as part of routine histological preparation. Pathological examination of liver tissue was conducted using SlideViewer software, while small intestine tissue analysis was carried out using ImageJ software.[24]

Detection of Serum Biochemical Indices (IL-6, TNF-α)

The procedure was carried out according to the manufacturer's instructions for preparing the standard blank and sample wells. Following the addition of the samples, the enzyme was added, followed by incubation, washing, and color development. The concentration of each sample was measured at 450 nm using a microplate reader. Subsequently, the levels of IL-6 and TNF-α in the serum of mice from each group were quantified.[25]

Detection of MUC2 and sIgA Content in Colon Tissue

First, the mouse colon tissue was homogenized and processed according to the method specified in the literature, left to stand overnight, and then centrifuged at 3500 rpm for 10 minutes at 4°C. The supernatant was collected and analyzed following the instructions provided in the kit manual.[24]

DNA Extraction, 16S rRNA Gene Amplicon Sequencing and Sequence Analysis

Total genomic DNA was extracted from intestinal content samples via a bacterial DNA extraction kit (MP Biomedicals). The quantity and quality of the extracted DNA were assessed via a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the bacterial 16S rRNA gene V3+V4 regions was performed via region-specific primers, with the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For PCR product recovery and purification, the PCR products were first visualized via 2% agarose gel electrophoresis and subsequently purified via the Axygen® AxyPrep DNA Gel Extraction Kit. The purified PCR products were quantified via the Quant-iT PicoGreen dsDNA Assay Kit. The samples were pooled according to the sequencing requirements. Library construction was performed via the TruSeq Nano DNA LT Library Prep Kit (Illumina), and sequencing was conducted via the NovaSeq 6000 SP Reagent Kit v1.5 (500 cycles) on the Illumina NovaSeq 6000 platform, employing a paired-end sequencing strategy of 2×250 bp. Shanghai Pacino Biotechnology Co., Ltd., carried out sequencing.[26]

Bioinformatics Analysis

(1) Taxonomic Annotation: The raw sequencing data were denoised to obtain Amplicon Sequence Variants (ASVs) using the QIIME2 DADA2 pipeline. Taxonomic classification was performed using the Silva database (http://www.arb-silva.de/). Species annotation was conducted with the "classify-sklearn" algorithm in QIIME2 (https://github.com/QIIME2/q2-feature-classifier). For each ASV, default parameters in QIIME2 were applied, and the taxonomic assignment was carried out using a pre-trained Naive Bayes classifier.[27]

(2) Alpha Diversity: The non-rarefied ASV table was used to perform alpha diversity analysis with the "qiime diversity alpha-rarefaction" command, employing the following parameters: "–p-steps 10 –p-min-depth 10 –p-iterations 10". The minimum depth for rarefaction was set to 10, and the maximum depth ("–p-max-depth") was determined as 95% of the sample with the lowest sequencing depth. Ten evenly spaced rarefaction depths were selected between the minimum and maximum values, with each depth being rarefied 10 times. The alpha diversity indices were calculated at each rarefaction depth, and the final alpha diversity score was determined by averaging the values at the maximum rarefaction depth.[27]

(3) Rarefaction Curve: The alpha-rarefaction.qzv file was generated using the "qiime diversity alpha-rarefaction" command. This file can be visualized by uploading it to https://view.qiime2.org/.

(4) Beta Diversity: To assess beta diversity, the rarefied ASV table was used to compute the Bray-Curtis distance matrix with the "qiime diversity core-metrics-phylogenetic" command. The resulting distance matrix was subjected to Principal Coordinate Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS), with the output saved as QZV files. These files can be visualized by uploading them to the appropriate section of https://view.qiime2.org/.[28]

(5) Venn Diagram: A Venn diagram was created using the non-rarefied ASV table to visualize the shared and unique ASV across samples or groups.

(6) Dominant Microbiota Analysis: The composition and abundance of taxa at various taxonomic levels were determined using QIIME2. These results were then visualized in a bar chart to represent the dominant microbial communities present in the samples.

(7) Differentially Abundant Microbiota Analysis: The Linear Discriminant Analysis Effect Size (LEfSe) method was applied to identify microbiota that were differentially abundant between groups. The "classify_samples_ncv" function from the q2-sample-classifier plugin was used to perform Random Forest analysis on the non-rarefied ASV table.[29]

(8) Correlation Analysis: Spearman's rank correlation analysis was performed using the non-rarefied ASV table to examine the relationships between intestinal microbiota, inflammatory markers, and immune factors.

(9) Functional Prediction Analysis: Functional prediction of metabolic pathways was conducted using the PICRUSt2 pipeline (version 2.3.0), based on 16S rRNA gene sequences, with the KEGG database as the reference.[30]

Statistical Methods

The experimental data were analyzed using SPSS version 25.0 for statistical analysis. Data are presented as the mean ± standard deviation. For data that followed a normal distribution with homogeneity of variance, one-way analysis of variance (ANOVA) was employed for group comparisons. In cases where the data did not meet these assumptions, the Kruskal–Wallis *H*-test was used. A p-value of < 0.05 was considered statistically significant, while a p-value of < 0.01 was considered highly statistically significant.

Results

Effect of Combination of Sodium Propionate with Sishen Pill on the General Behavior Observation and Symptoms of Diarrheal Mice with Kidney-Yang Deficiency Syndrome

The normal group exhibited good health, with smooth fur and high energy. In contrast, the natural recovery group showed huddling, lethargy, and dull fur behaviors. The treatment group showed a normal mental state and restored fur shine (Figure 1A and B). As shown in Figure 2A, during the adaptation period, the body weights of the mice across all groups were comparable. However, at fourteen days of post-modeling, both the treatment and recovery groups presented significant reductions in body weight compared with normal group ($p < 0.01$ or $p < 0.05$). Following treatment, the body weights of the 75% Sishen Pill combined with 60 mg/kg sodium propionate and Sishen Pill groups were like those of normal group ($p > 0.05$). Still, they differed significantly from those of the natural recovery group ($p < 0.01$). Although the model group showed a gradual increase in body weight, it remained significantly lower than that of normal group ($p < 0.05$). These results suggest that the Sishen Pill decoction, as well as the combination of Sishen Pill and sodium propionate, can partially restore body weight in mice with kidney yang deficiency-induced diarrhea, bringing it closer to normal levels. In contrast, sodium propionate alone did not significantly increase body weight in these mice.

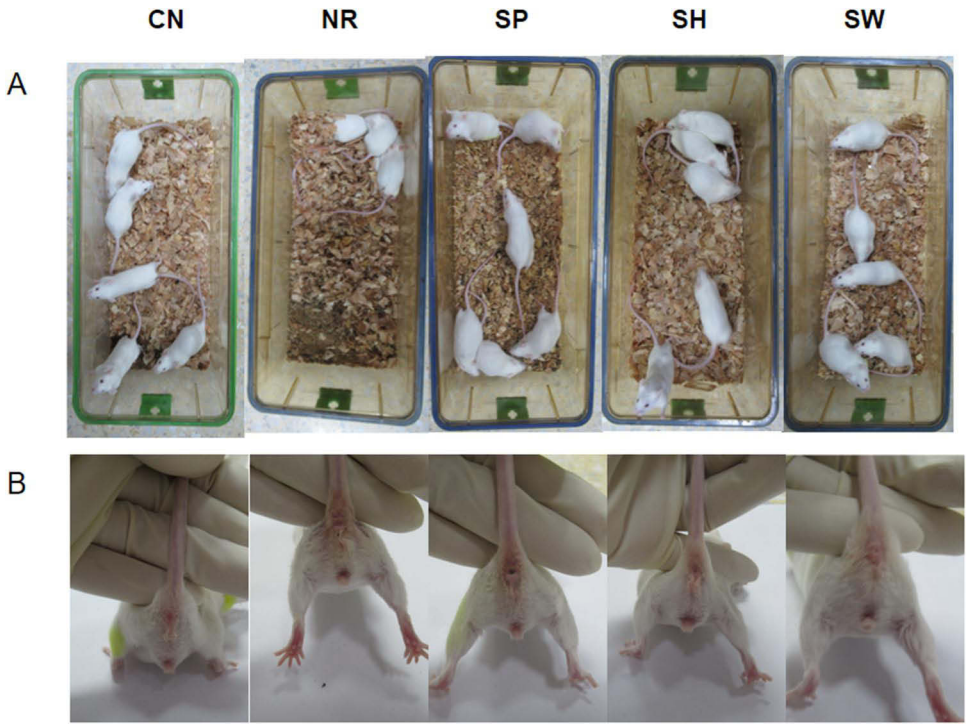

Figure 1 Effects of Sodium Propionate and Sishen Pill Combination on Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. (**A**) The general behavior of mice in each group. (**B**) Perianal cleanliness of mice in each group. The following figure and table are the same.
Abbreviations: CN, normal group; NR, natural recovery group; SP, sodium propionate group; SH, 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group; SW, Sishen Pill group.

Figure 2B shows that during the modeling phase, both the model and treatment groups presented a significant increase in fecal water content compared with the normal group ($p < 0.01$). As the treatment progressed, the fecal water content in the sodium propionate and 75% Sishen Pill combined with 60 mg/kg sodium propionate groups markedly decreased, with significant differences observed on day 18 compared with that in natural recovery group. Following the completion of treatment, the fecal water content in all the treatment groups returned to levels comparable to those in normal group ($p > 0.05$). In contrast, the model group presented a gradual decrease in fecal water content over time but remained significantly different from that of normal group ($p < 0.01$). These findings suggest that spontaneous recovery from the Effect of sodium propionate combined with Sishen Pill on the immune organs of mice with Diarrhea with Kidney-Yang Deficiency Syndrome in mice does not result in complete resolution of the condition. Both Sishen Pill and sodium propionate contribute to the restoration of normal fecal water content.

Effect of Sodium Propionate Combined with Sishen Pill on the Immune Organs of Diarrheal Mice with Kidney-Yang Deficiency Syndrome

Compared with those in normal group, the spleen and thymus indices in natural recovery group were significantly lower ($p < 0.01$). In contrast, both the spleen and thymus indices in the 75% Sishen Pill combined with 60 mg/kg sodium propionate group were significantly greater than those in the natural recovery group ($p < 0.01$ or $p < 0.05$). The spleen and thymus indices in the Sishen Pill group were significantly greater than those in the natural recovery group ($p < 0.05$), although the spleen index was significantly lower than that in the normal and 75% Sishen Pill combined with 60 mg/kg sodium propionate groups. The thymus index in the sodium propionate group was significantly greater than that in natural recovery group ($p < 0.05$), whereas no significant difference was detected in the spleen index between the sodium propionate and natural recovery groups ($p > 0.05$), as shown in Figure 2C and D.

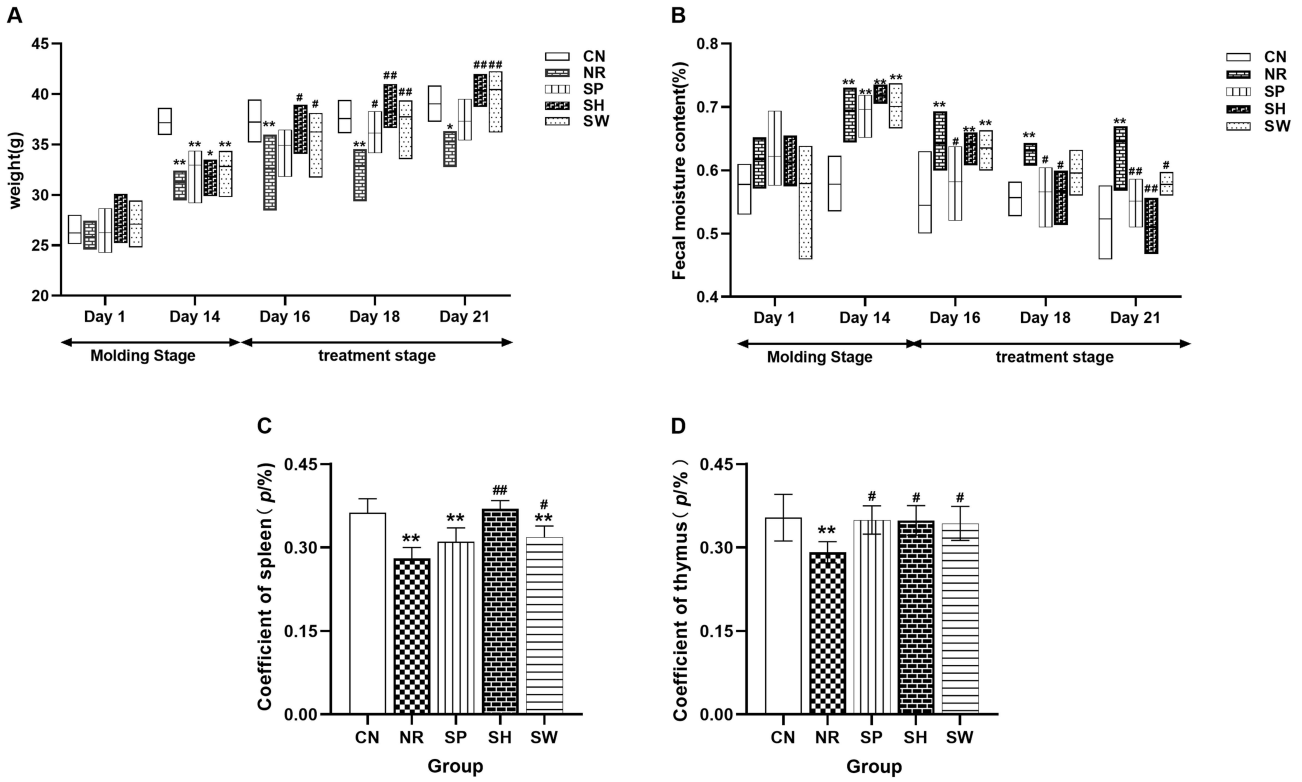

Figure 2 Effects of Sodium Propionate and Sishen Pill Combination on Mice with Diarrhea with Kidney-Yang Deficiency Syndrome: (**A**) Body weight, (**B**) Fecal moisture content, (**C**) Spleen index, (**D**) Thymus index. Compared with the CN group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the NR group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Graphs A, B, C, and D were created using GraphPad Prism 9.5.

Effect of the Combination of Sodium Propionate and Sishen Pill on Renal Pathology and Small Intestine Pathology in Diarrheal Mice with Kidney-Yang Deficiency Syndrome

Histological analysis via HE staining demonstrated that the renal tissue structure in normal group was well preserved, with no noticeable abnormalities. In contrast, natural recovery group exhibited significant pathological changes, including glomerular atrophy, sclerosis, inflammatory cell infiltration within the renal interstitium, and dilation of the renal tubules with enlarged lumens. Upon treatment, the mice in sodium propionate, 75% Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill groups presented a pronounced reduction in inflammatory cell infiltration, with the structural integrity of the glomeruli and renal tubules largely restored to normal, as depicted in Figure 3A.

Similarly, as shown in Figure 3B, the small intestine mucosal architecture in normal group was intact and well organized, without evidence of edema, inflammation, or lymphocytic infiltration, thus maintaining normal tissue structure. In contrast, natural recovery group displayed notable structural alterations, including a reduced number and sparse distribution of villi, accompanied by marked villous atrophy. Disruption of the mucosal structure was evident, with widespread lymphocytic infiltration and thinning of the mucosal muscular layer. Additionally, the crypts appeared round and exhibited an irregular arrangement. In sodium propionate and 75% Sishen Pill combined with 60 mg/kg sodium propionate groups, the small intestinal mucosa remained largely intact, with well-preserved layers showing no significant edema or inflammation and no evidence of lymphocytic infiltration. Mild lymphocytic infiltration was observed in the Sishen pill group. As presented in Figure 4, there were significant differences in small intestinal morphology between natural recovery group and normal, sodium propionate, 75% Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill groups ($p< 0.01$). Specifically, the villus height and the villus height/crypt depth ratio (Vh/Cd) in the natural recovery group were significantly lower than those in the normal, sodium propionate, 75% Sishen Pill combined with

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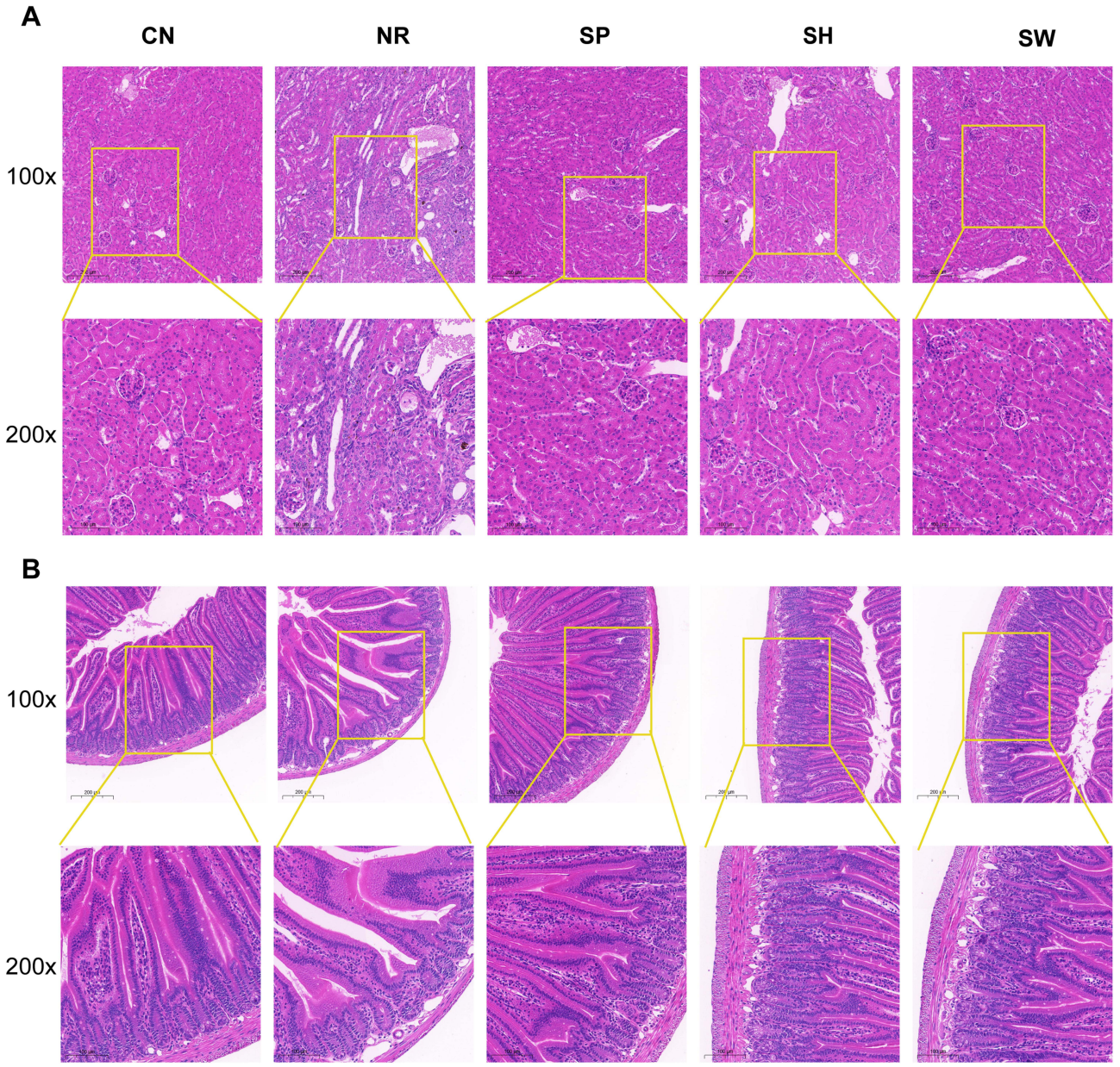

Figure 3 Effects of Sodium Propionate and Sishen Pill Combination on Kidney Tissue (**A**) and Small Intestinal Tissue Morphology (**B**) in Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. Graphs were constructed using SlideViewer.

60 mg/kg sodium propionate, and Sishen Pill groups ($p< 0.01$). Furthermore, significant differences in crypt depth were observed, with a marked disparity between the normal and natural recovery groups ($p < 0.01$).

Sodium Propionate Combined with the Sishen Pill for the Detection of IL-6 and TNF-α in Diarrheal Mice with Kidney-Yang Deficiency Syndrome

Compared with those in normal group, the serum IL-6 levels in natural recovery group were significantly elevated ($p < 0.05$), whereas the serum TNF-α levels were significantly reduced ($p < 0.05$). Conversely, the serum IL-6 levels in the 75% Sishen Pill combined with 60 mg/kg sodium propionate group were markedly lower than those in natural recovery group ($p < 0.01$), whereas the TNF-α levels were significantly greater ($p < 0.05$). Both the sodium propionate and Sishen Pill groups presented a decreasing trend in IL-6 levels, and notably, the serum TNF-α levels in both groups were significantly lower than those in normal group ($p < 0.01$ or $p < 0.05$). These findings are presented in Figure 5A and B.

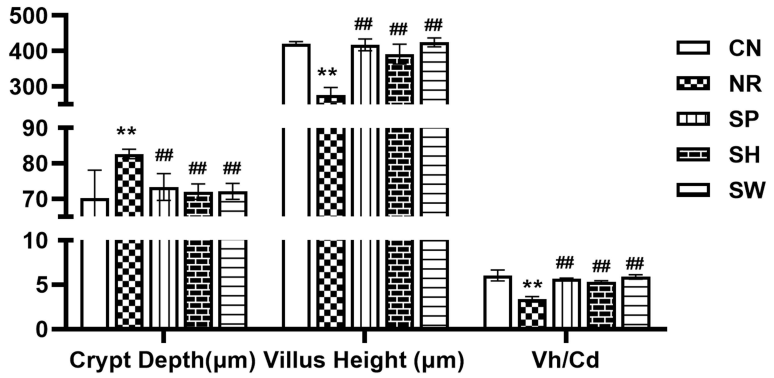

Figure 4 The pathological observation of HE staining in the small intestine tissue of mice in each group.
Notes: Compared with the CN group, $^{**}P < 0.01$; compared with the NR group, $^{\#\#}P < 0.01$. Analysis was performed using ImageJ.

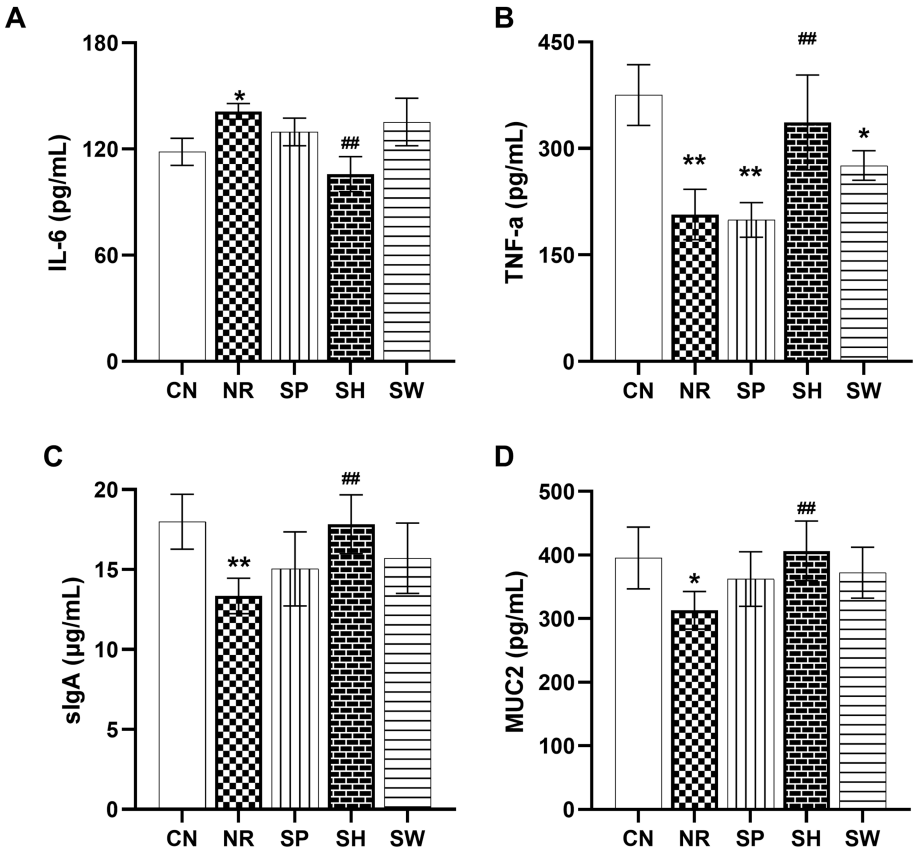

Figure 5 Effects of Sodium Propionate combined with Sishen Pill on IL-6 (**A**), TNF-α (**B**), sIgA (**C**), and MUC2 (**D**) in Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. Compared with the CN group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the NR group, $^{\#\#}P < 0.01$. Graphs A, B, C, and D were created using GraphPad Prism 9.5.

The Combination of Sodium Propionate and Sishen Pill Was Used to Detect the Contents of MUC2 and sIgA in Diarrheal Mice with Kidney-Yang Deficiency Syndrome

Compared with those in normal group, the levels of MUC2 and sIgA in the colons of the mice in natural recovery group were significantly lower ($p < 0.01$ or $p < 0.05$). Conversely, the MUC2 and sIgA levels in the colon of the mice in sodium propionate and Sishen Pill groups tended to increase relative to those in natural recovery group. Additionally, the levels of MUC2 and sIgA in the colon of the mice in 75% Sishen Pill combined with 60 mg/kg sodium propionate group were significantly greater than those in natural recovery group, and these differences were statistically significant ($p < 0.01$). These findings are depicted in Figure 5C and D.

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Effect of Sodium Propionate Combined with Sishen Pill on the Intestinal Microbiota of Diarrheal Mice with Kidney-Yang Deficiency Syndrome

Assessment of Sequencing Data Quality

As the sequencing depth increased, the rate of growth in the number of Amplicon Sequence Variants (ASVs) gradually declined. Both the Chao1 and Shannon rarefaction curves exhibited a plateau, indicating that the sequencing depth in this study was sufficient (Figure 6A and Supplementary Figure 1A). As the sample size increased, the species accumulation curves continued to rise, suggesting that the sequencing depth was adequate to capture the microbial diversity within the community samples (Supplementary Figure 1B). Following the denoising of the sequences via the DADA2 method, sequences with 100% similarity were clustered into a single ASV. Specifically, 837 ASVs were identified in normal group, of which 541 were unique; 3781 ASVs were identified in natural recovery group, with 3241 being unique; 1207 ASVs were identified in sodium propionate group, including 890 unique ASVs; 1828 ASVs were identified in 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group, with 1326 unique ASVs; and 614 ASVs were identified in Sishen pill group, with 479 unique ASVs. A total of 37 ASVs were shared across all five experimental groups (Figure 6B).

Analysis of Intestinal Microbiota Diversity

Alpha diversity is primarily used to assess the richness and diversity of the intestinal microbiota. Commonly used alpha diversity indices include the Chao1 estimator and the Shannon index. Compared with that in normal group, the Chao1 index in natural recovery group was significantly greater ($p < 0.01$). In contrast, the Chao1 index in sodium propionate and Sishen Pill groups was significantly lower than that in the natural recovery group ($p < 0.01$ or $p < 0.05$) (Figure 6C). Furthermore, the Shannon index in Sishen Pill group was significantly lower than that in natural recovery group ($p < 0.01$), whereas no significant differences in the Shannon index were detected between the other groups (Figure 6D).

Beta diversity is primarily used to examine the similarity of community composition across samples. PCoA revealed a significant difference in the intestinal microbiota composition between the normal and natural recovery groups, with PCo1 accounting for 26% and PCo2 accounting for 18.4% of the variation (Figure 6E). After treatment with sodium propionate and Sishen Pill, the intestinal microbiota composition of the sodium propionate, the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill tended to converge toward that of normal group. NMDS analysis (Figure 6F) revealed a stress value of 0.138, with a clear separation between the normal and natural recovery groups. These findings suggest that both sodium propionate, a 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill can improve the intestinal microbiota dysbiosis induced by Diarrhea with Kidney-Yang Deficiency Syndrome, promoting a shift in the intestinal microbiota composition of affected mice toward a healthier profile.

Analysis of the Relative Abundance of Intestinal Mucosal Flora

Intestinal microbiota analysis revealed the structural composition of microbial communities at the phylum and genus levels across different groups. At the phylum level, the dominant phyla in the intestinal mucosal microbiota were *Bacillota, Bacteroidetes, Actinomycetota*, and *Pseudomonadota*, with *Bacillota* being the most abundant (Figure 7A). Compared with those in the normal group, the *Bacillota/Bacteroidota* (F/B) ratio in the natural recovery group was significantly lower, whereas the ratios in the sodium propionate, 75% Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill groups approached normal levels (Figure 7C).

At the genus level, the dominant genera included *Clostridium, Lactobacillus*, and *Pediococcus*, with *Clostridium* being the most prevalent (Figure 7B). Compared with those in normal group, the abundances of *Lactobacillus* and *Clostridium* were significantly lower in natural recovery group. In contrast, the abundance of *Lactobacillus* in the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate groups and the abundance of *Clostridium* in the 75% dose of Sishen Pill combined with 60 mg/kg propionate and Sishen Pill groups increased and approached normal levels (Figure 7D and Supplementary Figure 2A). Additionally, compared with that in normal group, the abundance of *Pediococcus* was significantly lower in both natural recovery group and 75% Sishen pill combined with 60 mg/kg sodium propionate group ($p < 0.05$), whereas the abundance of *Pediococcus* in the sodium propionate group was significantly greater than that in natural recovery group (Supplementary Figure 2B). The abundances of *Parabacteroides* and *Porphyromonas* in natural recovery group were significantly greater than those in normal, sodium propionate, and Sishen Pill groups ($p < 0.01$) (Supplementary Figure 2C and D).

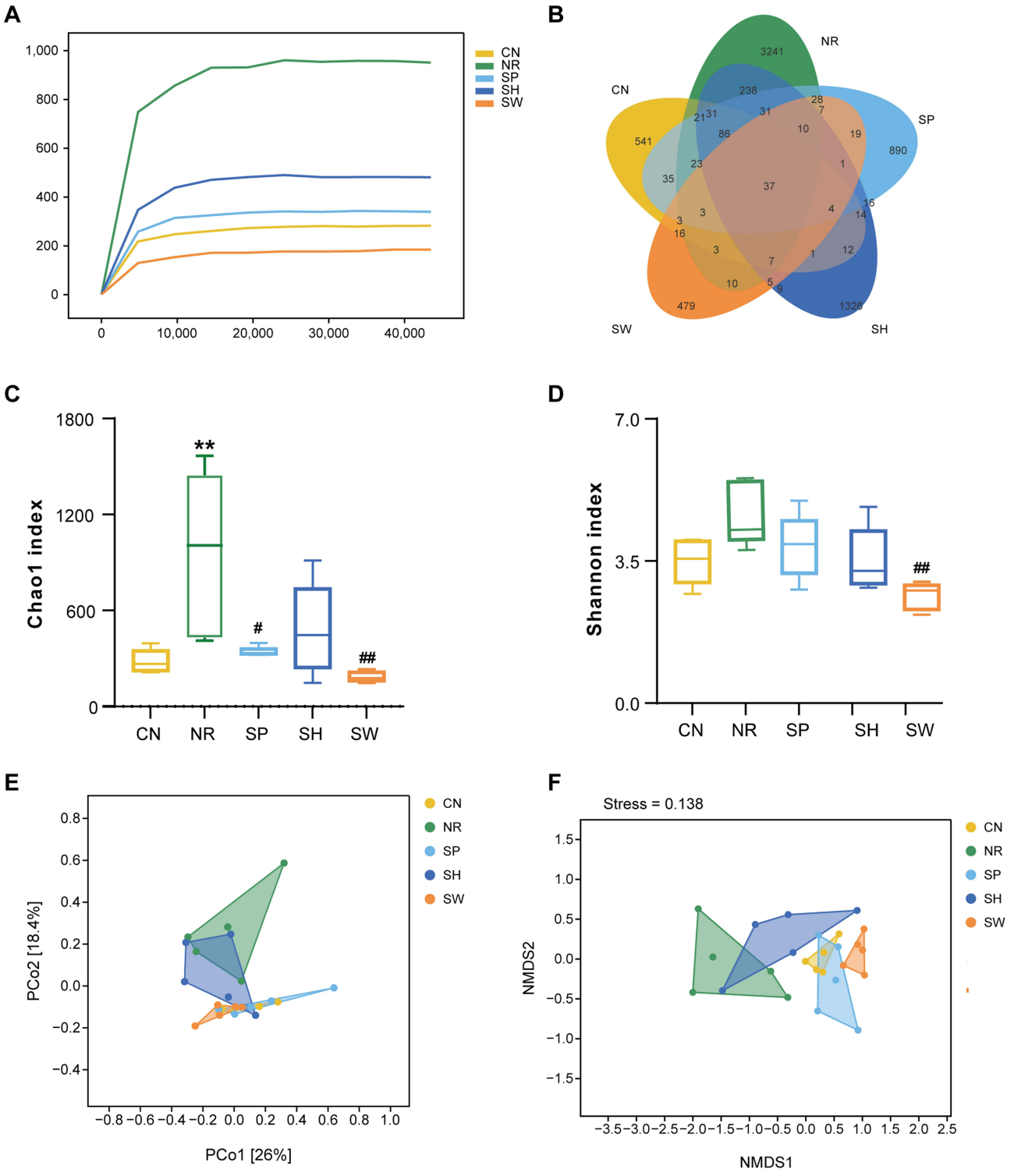

Figure 6 Effects of Sodium Propionate Combined with Sishen Pill on the Intestinal Microbiota in Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. (**A**) Chao1 rarefaction curve of the intestinal mucosal microbiota; (**B**) Venn diagram: ASV distribution of the intestinal mucosal microbiota; (**C**) Chao1 index; (**D**) Shannon index; (**E**) pCoA analysis; (**F**) NMDS analysis. Compared with the CN group, $^{**}P < 0.01$; compared with the NR group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Analysis was performed using GenesCloud (https://www.genescloud.cn/login).

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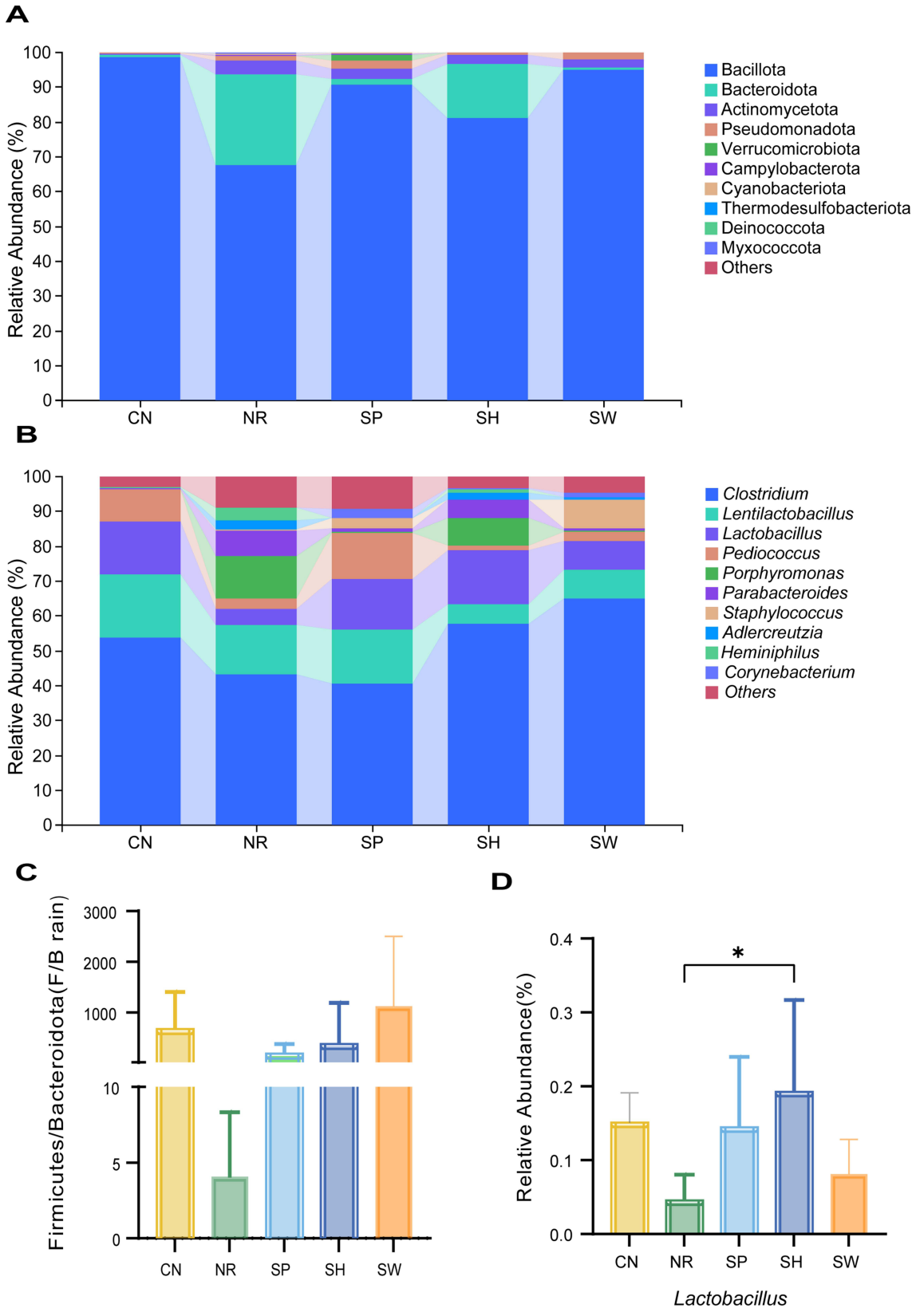

Figure 7 Effects of Sodium Propionate Combined with Sishen Pill on the Composition of the Intestinal Mucosal Microbiota in Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. (**A**) Phylum-level intestinal mucosal microbiota; (**B**) Genus-level intestinal mucosal microbiota; (**C** and **D**) Differential bacteria at genus and species levels in the intestinal mucosa. Compared with the CN group, $^{*}P < 0.05$. Graphs A and B were created using GenesCloud (https://www.genescloud.cn/login), and Graphs C-H were created using GraphPad Prism 9.5.

Characteristic Microflora Analysis

LEfSe analysis was performed with a threshold of 3.2 to assess the taxonomic relationships of key microbial taxa from phylum to genus (from the innermost to the outermost circle) in the sample communities. As shown in Figure 8A, differences in microbial abundance were observed between normal and natural recovery groups, with 28 bacterial taxa identified as key discriminators. In normal group, *Lactobacillus, Haemophilus*, and *Pediococcus* were significantly enriched, whereas in the natural recovery group, genera such as *Porphyromonas* and *Parabacteroides* were significantly enriched. The normal, sodium propionate, 75% Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill groups also presented distinct microbiota compositions. The normal group had one characteristic bacterium, the sodium propionate group had two, the 75% Sishen Pill combined with 60 mg/kg sodium propionate group had four, and Sishen Pill group had one. Specifically, *Levilactobacillus* and *Latilactobacillus* were significantly enriched in the sodium propionate

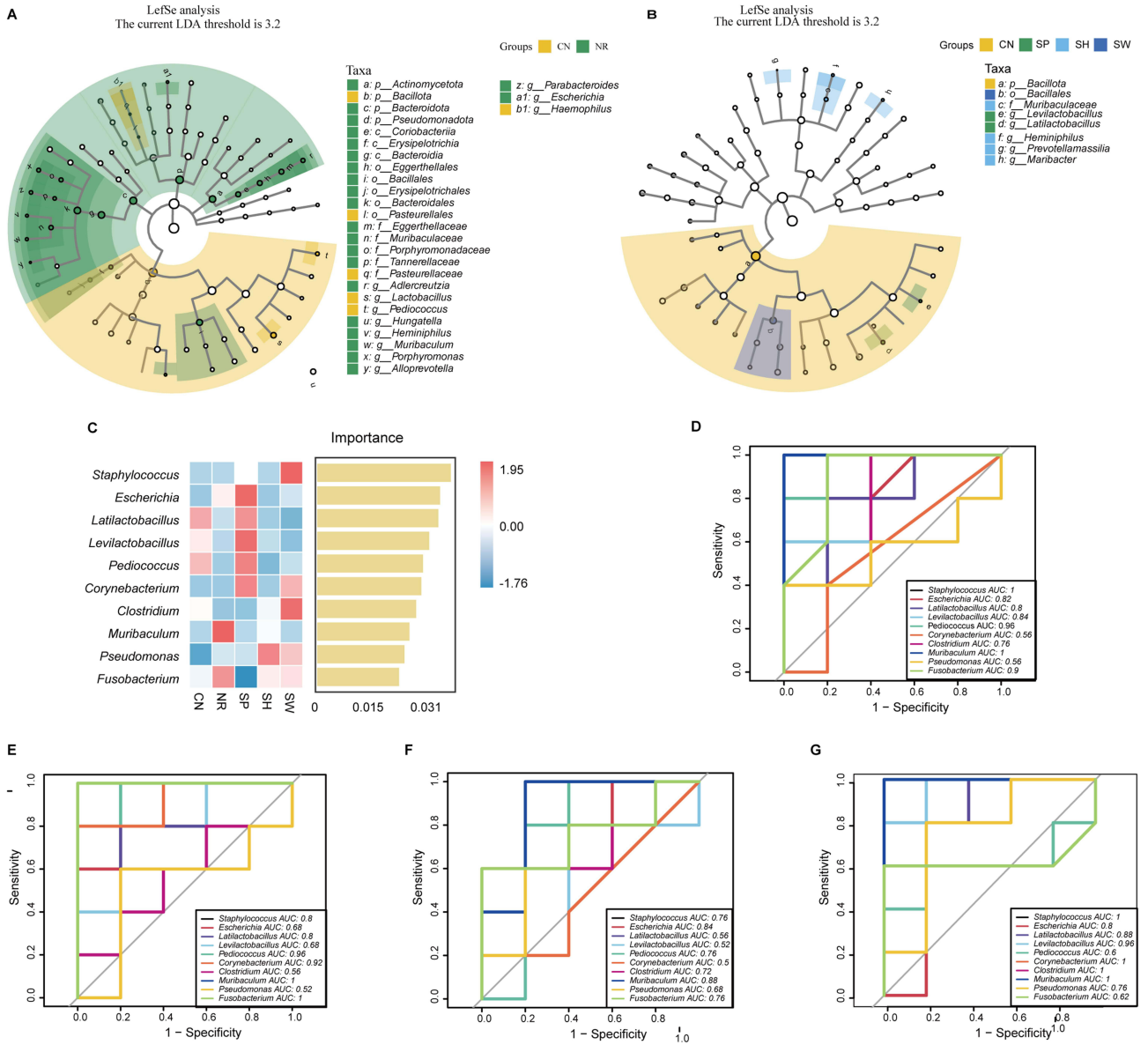

Figure 8 Effects of Sodium Propionate Combined with Sishen Pill on the Characteristic Intestinal Mucosal Microbiota in Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. (**A**) Phylogenetic tree of species in the CN and NR groups; (**B**) Phylogenetic tree of species in the CN, SP, SH, and SW groups; (**C**) Random forest analysis at the genus level; (**D**) ROC diagnostic curve for the CN and NR groups (**E**) ROC diagnostic curve for the NR and SP groups; (**F**) ROC diagnostic curve for the NR and SH groups; (**G**) ROC diagnostic curve for the NR and SW groups. Graphs were created using GenesCloud (https://www.genescloud.cn/login) and the OmicStudio platform from Lianchuan Biotechnology (https://www.omicstudio.cn/tol) for graphs D-G.

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group, *whereas Prevotella* and *Maribacter* were significantly enriched in the 75% Sishen Pill combined with 60 mg/kg sodium propionate group (Figure 8B). In combination with random forest analysis, the top 10 genera in the intestinal mucosal microbiota were selected, and genus-level characteristic bacteria were selected for ROC curve analysis (Figure 8C–G). The results indicated that the characteristic bacteria for distinguishing normal and natural recovery groups were *Staphylococcus* and *Pediococcus* (AUC = 1), which demonstrated high diagnostic potential between the two groups. For distinguishing natural recovery and sodium propionate groups, *Fusobacterium* (AUC = 1) showed a strong diagnostic value. Similarly, *Escherichia* (AUC > 0.8) was identified as the key discriminator between the natural recovery and the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate groups, whereas *Staphylococcus* (AUC = 1) was found to have significant diagnostic value for distinguishing the natural recovery and Sishen pill groups.

Analysis of the Metabolic Function of Intestinal Mucosal Microbiota

Based on the KEGG database, we used PICRUSt2 analysis to predict the metabolic pathways associated with the intestinal mucosal microbiota. This approach further allowed us to assess the impact of combined sodium propionate and Sishen Pill treatment on the metabolic function of the intestinal mucosal in Diarrheal mice with Kidney-Yang Deficiency Syndrome. The KEGG metabolic pathways were categorized into six major functional classes, with 26 subcategories. The median value of the top three categories was greater than 534.6156 (Figure 9A). Among these, the metabolic

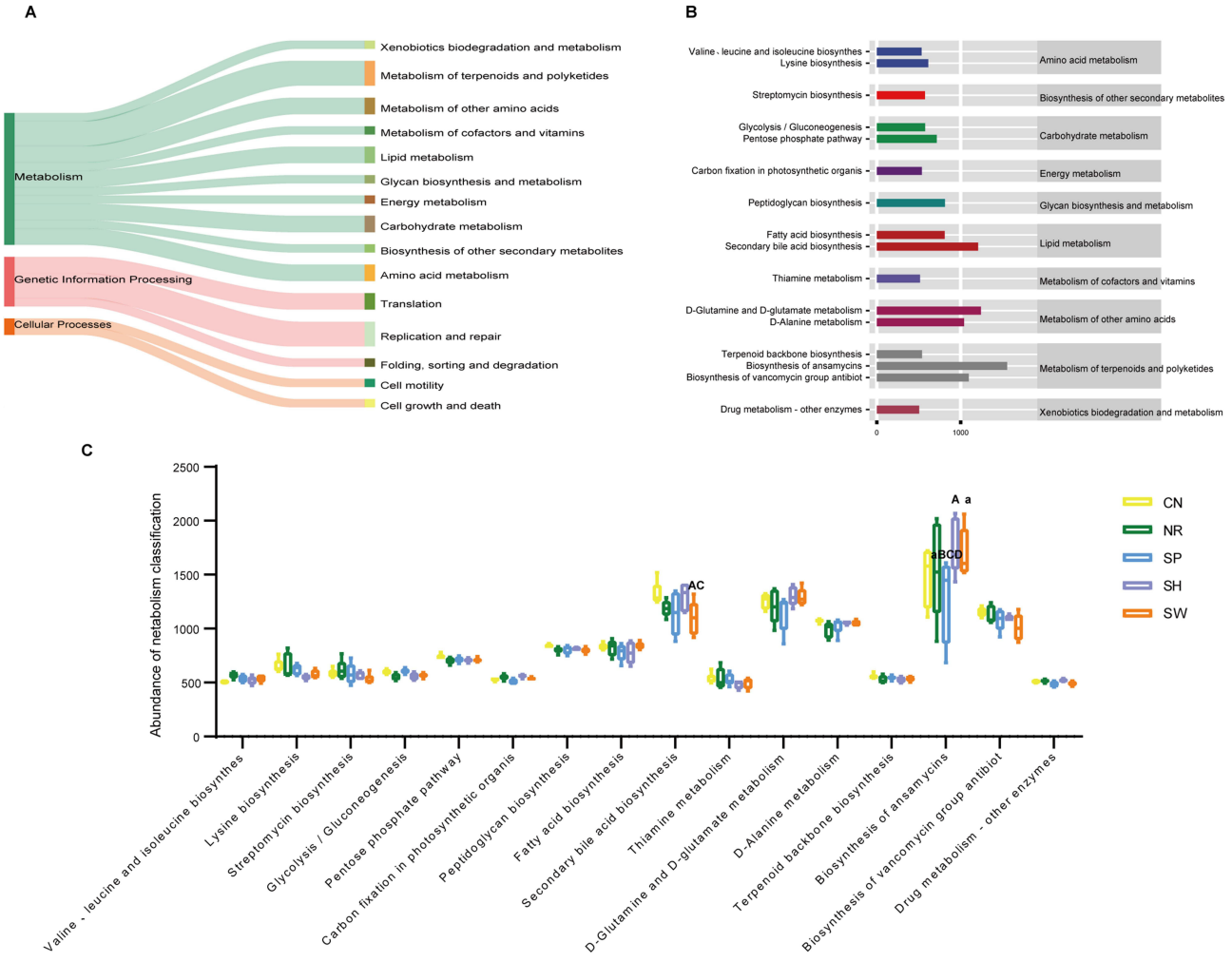

Figure 9 Effects of Sodium Propionate Combined with Sishen Pill on the Metabolic Function of Intestinal Mucosal Microbiota in Mice with Diarrhea with Kidney-Yang Deficiency Syndrome. (**A**) First and second-level metabolic pathways based on the KEGG database; (**B**) Metabolic histogram; (**C**) Inter-group comparison of metabolic functions (third level). a: $P < 0.05$, (**A**) $P < 0.01$; compared with NR group, b: $P < 0.05$, (**B**) $P < 0.01$; compared with SH group, c: $P < 0.05$, (**C**) $P < 0.01$; compared with SW group, d: $P < 0.05$, (**D**) $P < 0.01$. Graphs A and B were created using GenesCloud (https://www.genescloud.cn/login), and graph C was created using GraphPad Prism 9.5.

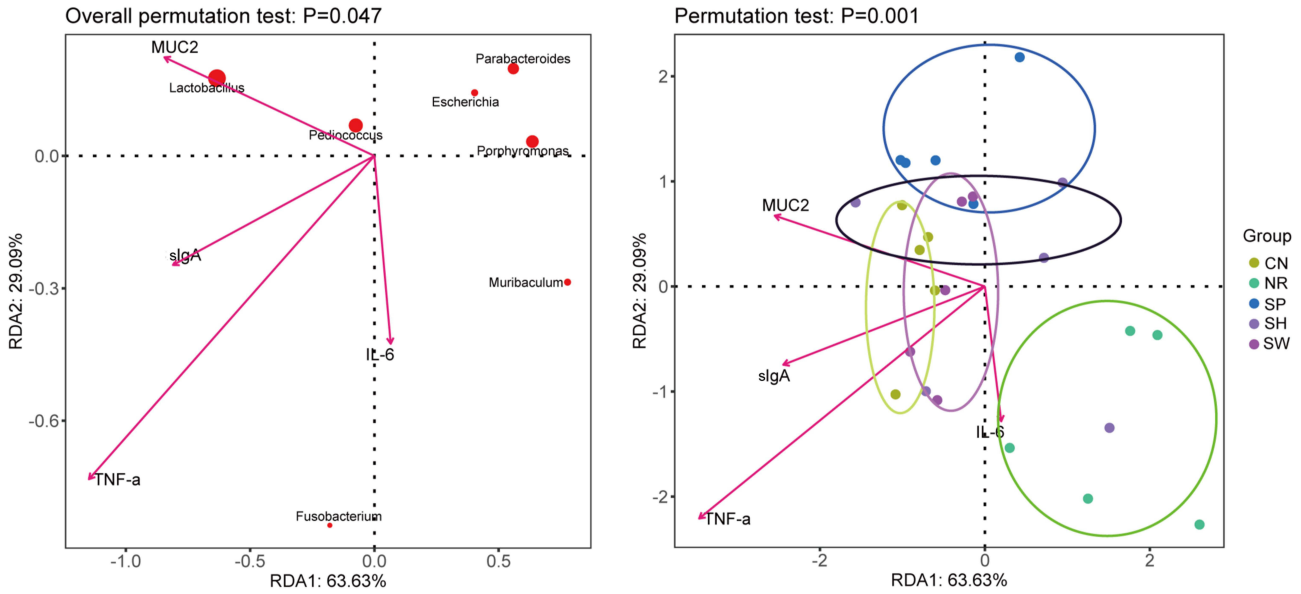

Figure 10 RDA Analysis of Characteristic Microbiota and Related Indicators. The graph was created using BioinCloud (https://www.bioincloud.tech/).

functional class accounted for 61.5% of the total abundance. The primary metabolic functions affected included amino acid metabolism, terpenoid and polyketide metabolism, lipid metabolism, and carbohydrate metabolism (Figure 9B). Further analysis of the third-level metabolic categories revealed that compared with normal group, natural recovery and Sishen Pill groups presented a decreasing trend in metabolic activity related to secondary bile acid biosynthesis. In contrast, the 75% Sishen Pill combined with 60 mg/kg sodium propionate group presented increased activity, approaching that of normal group. Additionally, concerning natural recovery, the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate and Sishen pill group presented an increasing trend in the metabolic activity of ansamycin biosynthesis compared with normal group, with statistically significant differences observed between the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate and Sishen pill group and normal group (Figure 9C).

Correlation Analysis Between Intestinal Mucosal Microbiota and Indicators

Correlation analysis revealed a significant negative correlation between IL-6 and *Lactobacillus* and *Pediococcus* and a positive correlation with *Fusobacterium, Porphyromonas*, and *Muribaculum*. Additionally, MUC2 and sIgA were positively correlated with *Pediococcus* and *Lactobacillus*, whereas TNF-α, MUC2, and sIgA were negatively correlated with *Porphyromonas, Escherichia*, and *Parabacteroides* (Figure 10). The microbiota compositions of sodium propionate, 75% Sishen Pill combined with 60 mg/kg sodium propionate, and Sishen Pill groups were similar to those of normal group, with Sishen Pill group exhibiting the highest similarity to the normal group. Furthermore, the natural recovery group presented a significant positive correlation with IL-6.

Discussion

The observation of animal behavioral manifestations provides a critical foundation for the development of syndrome models in traditional Chinese medicine (TCM) through experimental research.[30] Diarrhea with Kidney-Yang Deficiency Syndrome in mice results in symptoms such as loose stools, perianal contamination, and weight loss. Previous studies have demonstrated that mice with Diarrhea with Kidney-Yang Deficiency Syndrome exhibit significant renal damage, including prominent injury to the glomeruli and renal tubules, interstitial edema, and the accumulation of inflammatory cells.[31,32] Following combined treatment with sodium propionate and Sishen Pill, a marked increase in body weight was observed in both the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group and Sishen Pill group. These findings suggest that the primary effect of Sishen Pill decoction is to increase nutrient absorption, thereby facilitating weight recovery. In contrast, sodium propionate alone did not significantly affect weight gain. Notably, the

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75% dose of Sishen Pill combined with 60 mg/kg sodium propionate effectively alleviated Diarrhea with Kidney-Yang Deficiency Syndrome in the mice. These results align with previous findings from our research group, which indicated that the combination of sodium propionate and Sishen Pill can, to some extent, influence body weight, anal temperature, and fecal moisture content in mice with kidney yang deficiency syndrome and diarrhea.

The thymus plays a pivotal role in producing a substantial number of thymocytes, which contribute to the body's antitumor and antimicrobial defense mechanisms.[33] It is essential for the development of immune function and the restoration of immune regulatory capabilities following immune dysfunction.[34] The spleen, which serves as a key indicator of immune organ status, is an integral component of the body's nonspecific immune system. In natural recovery group of mice post-modeling, both the spleen and thymus indices were significantly lower. However, after intervention with sodium propionate and Sishen Pill, there was a notable increase in both the spleen and thymus indices. The 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate resulted in the most pronounced recovery among the various treatment groups. These results suggest that the combination of sodium propionate and Sishen Pill exerts a synergistic effect, enhancing immune function.

The regulation of pro-inflammatory cytokine (IL-6) and anti-inflammatory cytokine expression levels plays a crucial role in intestinal inflammation and immune responses, with both cells and cytokines driving tissue damage.[35] TNF-α exerts various roles in modulating developmental and immune processes, including inflammation, differentiation, lipid metabolism, and apoptosis, and is implicated in several diseases.[36] As the inflammatory response persists, negative feedback mechanisms within the body may be activated, leading to a reduction in TNF-α levels. TNF-α is effectively suppressed, particularly through myokines or proteins produced by muscle cells, such as IL-6.[37] MUC2, the major component of the intestinal mucus layer, protects the intestine from mechanical, chemical, and biological insults and contributes to the maintenance of intestinal homeostasis.[38] sIgA, the most abundant immunoglobulin secreted in the intestine, serves as the primary defense against pathogen adhesion and colonization at the intestinal mucosal surface. It prevents pathogens from adhering to and penetrating the intestinal barrier, thus modulating immune homeostasis.[39,40] Studies have shown that sodium propionate significantly stimulates the secretion of IL-8 and TNF-α, while having no significant effect on the secretion of IL-18 and IL-6.[41] In the present study, compared with the normal group, the natural recovery group exhibited a downward trend in TNF-α, MUC2, and sIgA, and an upward trend in IL-6. However, after intervention with sodium propionate and Sishen Pill, the differences in TNF-α, MUC2, sIgA, and IL-6 between the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group and normal group were significantly reduced. Pathological examination of kidney and small intestine tissues revealed that, in the natural recovery group, the glomeruli in the kidneys were markedly damaged, and inflammatory cell infiltration in the renal interstitium was observed. In the small intestine, the mucosal structure was disrupted, with shortened villi, increased crypt depth, and varying degrees of inflammation. These findings suggest that Diarrhea with Kidney-Yang Deficiency Syndrome leads to immune dysfunction, exacerbated inflammatory responses, and damage to the intestinal mucosal barrier in mice, consistent with previous research. Both sodium propionate group and 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group were effective in repairing the damaged intestinal mucosal tissue and inhibiting the inflammatory response. Among these, the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group significantly alleviated inflammation, tissue damage, and diarrhea symptoms in the Diarrheal mice with Kidney-Yang Deficiency Syndrome, restored immune function, and repaired the intestinal mucosal barrier.

The intestinal microbiota is recognized as a crucial regulator of both pathological and physiological processes in the host.[42] Several bacterial species within the intestinal microbiome, particularly those belonging to the *Clostridiales* family, play an essential role in the biosynthesis of propionate. Sodium propionate has been shown to exert variable effects on intestinal physiological functions, contributing to metabolic regulation in the intestinal and being closely associated with specific physiological activities of intestinal cells.[43] Studies indicate that traditional Chinese medicine (TCM) can significantly modulate the composition and function of the intestinal microbiota under diseased conditions, with the microbiota playing a pivotal role in the metabolic transformation of TCM.[44] The therapeutic effects of TCM are characterized by the involvement of multiple components, targets, and pathways, with the intestinal microbiota acting as one of the primary targets. As such, TCM extensively and complexly interacts with herbal medicines to restore intestinal balance.[45] For example, research has demonstrated that Sishen Pill can modulate the intestinal microbiota by

increasing the abundance of *Bifidobacterium, Lactobacillus*, and *Bacteroides* while decreasing the abundance of *Enterococcus* and *Enterobacteria*.[9] Moreover, Sishen Pill has been found to protect the intestinal mucosa through modulation of the abundance of *Proteobacteria* (downregulated) and *Clostridium* (upregulated), suggesting its potential to restore balance within the intestinal microbiota.[3,10] The intestinal serves as a vital innate barrier that helps maintain the internal environment by preventing the invasion of pathogenic microbes and toxins.[45] In conditions such as celiac disease, inflammatory bowel disease, and irritable bowel syndrome, damage to the intestinal barrier is associated with low-grade inflammation of the small intestinal mucosa.[46] In this study, the natural recovery group of mice exhibited a higher number of unique amplicon sequence variants (ASVs) in the intestinal mucosa compared to normal group, while sodium propionate, the 75% dose of Sishen Pill combined with the 60 mg/kg sodium propionate, and Sishen Pill groups displayed lower ASV counts than natural recovery group. Additionally, the natural recovery group showed better separation from the treatment groups. Compared to normal group, the Alpha diversity indices of the natural recovery group displayed a downward trend. Specifically, the Chao 1 index of the sodium propionate and Sishen Pill groups was significantly lower than that of the natural recovery group, and the Shannon index also showed a declining trend in all treatment groups. These findings suggest that natural recovery, along with interventions using sodium propionate and Sishen Pill, induced changes in the intestinal mucosal microbiota. The data imply that Diarrhea with Kidney-Yang Deficiency Syndrome may lead to increased intestinal mucosal diversity. In contrast, both sodium propionate and Sishen Pill are capable of restoring this diversity to normal levels. However, the combined use of both treatments did not result in a significant enhancement in the restoration of intestinal mucosal diversity.

The Firmicutes-to-Bacteroidetes (F/B) ratio is widely recognized as a marker of intestinal homeostasis. Deviations in this ratio, whether an increase or decrease, are commonly indicative of intestinal dysbiosis.[47] *Lactobacillus* plays a pivotal role in maintaining microbial equilibrium by competitively excluding pathogenic bacteria and exhibiting antimicrobial activity, which in turn stimulates innate immune responses.[48] These immune-regulatory effects are partially mediated by *Lactobacillus*-derived metabolites, such as short-chain fatty acids (SCFAs), notably propionate, acetate, and butyrate. These postbiotics interact with specific receptors on intestinal epithelial cells, thereby suppressing the pro-inflammatory activity of neutrophils and macrophages while promoting the immunosuppressive functions of regulatory T cells (Tregs).[49] *P. pentosaceus* B49 has demonstrated efficacy in alleviating constipation in mice, positioning it as a potential therapeutic agent for this condition.[50] Beneficial *Clostridia* are integral to a range of physiological functions, including metabolism, immune regulation, and the maintenance of intestinal microbial balance, all of which are essential for human health.[51] In our experimental results, the F/B ratio, along with the abundance of *Lactobacillus, Pediococcus*, and *Clostridium*, was significantly reduced in natural recovery group compared to normal group. Conversely, the F/B ratio and *Clostridium* abundance showed an upward trend in the treatment groups, with the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group exhibiting *Lactobacillus* levels comparable to those of normal group. These findings suggest that during the natural recovery process from Diarrhea with Kidney-Yang Deficiency Syndrome, the intestinal mucosa of the mice becomes increasingly susceptible to pathogenic invasion. However, both sodium propionate and Sishen Pill appear to exert a synergistic effect, enhancing immune function and restoring intestinal microbial balance, thereby improving the intestinal's resistance to pathogenic invaders. Previous studies have indicated that *Porphyromonas* is linked to an elevated risk of gastrointestinal cancer, while *Parabacteroides* possess both pathogenic and probiotic potential.[52] Oral administration of *P. distasonis* to wild-type mice exacerbated DSS-induced colitis, potentially heightening the risk of inflammatory bowel disease.[53] In our study, the abundance of *Porphyromonas* and *Parabacteroides* was markedly elevated in the natural recovery group. In contrast, both sodium propionate and Sishen Pill significantly reduced the abundance of these pathogens. These results suggest that Diarrhea with Kidney-Yang Deficiency Syndrome leads to an overgrowth of harmful bacteria in the intestinal mucosa, contributing to inflammation. Sodium propionate and Sishen Pill both appear to mitigate this dysbiosis by decreasing pathogenic bacteria and inflammatory factors, thereby improving the inflammatory response. The combined administration of sodium propionate and Sishen Pill may have exerted complementary effects, possibly enhanced the probiotic potential of *Parabacteroides*, and contributed to therapeutic benefits. LEfSe analysis and random forest models further confirmed that the abundance of the harmful bacterium *Porphyromonas* was elevated in the natural recovery group, while beneficial bacteria such as *Prevotellamassilia* and *Maribacter* were enriched in the 75% dose of Sishen Pill combined with 60 mg/kg sodium

propionate group. These findings support the hypothesis that sodium propionate and Sishen Pill act synergistically to reduce pathogenic bacteria, increase beneficial bacteria, and regulate intestinal microbial homeostasis.

In the metabolic pathways, intervention with sodium propionate and Sishen Pill in Diarrhea with Kidney-Yang Deficiency Syndrome primarily mediates the therapeutic effects through the modulation of amino acid metabolism, terpenoid and polyphenol metabolism, lipid metabolism, and carbohydrate metabolism. In the 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group, the activity of secondary bile acid metabolism was significantly restored to near-normal levels. Both primary and secondary bile acids play crucial endocrine roles and are involved in the regulation of host metabolism, indicating that the combination of sodium propionate and Sishen Pill may facilitate the improvement of secondary bile acid metabolism.[51] However, the specific metabolic functions of these intestinal microbes require further exploration in future studies.

The results of the correlation analysis revealed a significant negative correlation between *Lactobacillus* and *Pediococcus* with IL-6, as well as a positive correlation between *Fusobacterium* and *Porphyromonas*. Furthermore, MUC2 and sIgA were positively correlated with *Pediococcus* and *Lactobacillus*, whereas TNF-α, MUC2, and sIgA were negatively correlated with *Porphyromonas, Escherichia*, and *Parabacteroides*. We speculate that Lactobacillus and Pediococcus can inhibit IL-6 secretion and enhance the secretion of MUC2 and sIgA. The mucosal microbiota interacts with inflammatory factors and immune-protective factors.

However, this study has some limitations in exploring the effects of sodium propionate combined with Sishen Pill on the intestinal mucosal barrier and microbiota in Diarrheal mice with Kidney-Yang Deficiency Syndrome. One such limitation is the choice of mouse strain. Different strains of mice may exhibit varying intestinal microbiota structures and metabolic characteristics, which could lead to differences in their responses to Sishen Pill and sodium propionate. Therefore, to improve the credibility and applicability of the research findings, future studies should consider using multiple standardized mouse strains for the experiments.

Conclusion

We found that 75% Sishen Pill combined with 60 mg/kg sodium propionate exhibited significantly better therapeutic effects compared to the Sishen Pill alone and sodium propionate alone groups. The 75% Sishen Pill combined with 60 mg/kg sodium propionate significantly alleviated symptoms related to Diarrhea with Kidney-Yang Deficiency Syndrome, and it enhanced intestinal immune function and reduced intestinal inflammation by modulating the intestinal microbiota. Further analysis indicated that sodium propionate could enhance the efficacy of Sishen Pill by regulating the intestinal microbiota, reducing the required dosage of Sishen Pill, and thereby minimizing potential side effects. This study provides new evidence for the modernization of traditional Chinese medicine, revealing the synergistic effect of Sishen Pill and sodium propionate, and highlighting the important role of the intestinal microbiota in regulating Diarrhea with Kidney-Yang Deficiency Syndrome.

Abbreviations

SP, Sodium Propionate; CN, normal group; NR, natural recovery group; SH, 75% dose of Sishen Pill combined with 60 mg/kg sodium propionate group; SW, Sishen Pill; ELISA, enzyme-linked immunosorbent assay; ASVs, amplicon sequence variants; PCoA, principal coordinate analysis; LEfSe, linear discriminant analysis effect size.

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA1177613.

Ethics Approval and Informed Consent

The experiment was approved by the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine and was conducted in accordance with the Guidelines for Humane Endpoint Review of Animal Experiments. (Ethics Number: HNUCM21-2403-43).

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Disclosure

The authors report no conflicts of interest in this work.

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