Research article

Mechanism of action of FoxiangSan in diabetic gastroparesis: Gut microbiota and cAMP/PKA pathway

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A B S T R A C T

Diabetic gastroparesis, a common complication of type 2 diabetes (T2DM), presents a significant treatment challenge. *FoxiangSan* is emerging as a potential therapy. *FoxiangSan* is a traditional Chinese medicine formula with the potential for treating diabetic gastroparesis by modulating gut microbiota and cAMP/PKA signaling pathways. This study explores the mechanisms behind *FoxiangSan*'s effects on T2DM-induced gastroparesis, focusing on its impact on gut microbiota and the cAMP/PKA pathway. A rat model of type 2 diabetic gastroparesis was established through a high-fat diet and streptozotocin (STZ) injection, and the effects of *FoxiangSan* were assessed. Additionally, protein expression related to the cAMP/PKA pathway was examined, and *FoxiangSan*'s influence on gut microbiota was studied using 16S rRNA sequencing. *FoxiangSan* significantly alleviated hyperglycemia, improved gastric pathology in rats with gastroparesis, enhanced the expression of 5-HT4, cAMP, PKA, and pPKA in the gastric antrum, and rebalanced gut microbiota. *FoxiangSan* demonstrates the therapeutic potential for T2DM-associated gastroparesis by modulating the cAMP/PKA pathway and gut microbiota.

1. Introduction

Type 2 diabetes mellitus (T2DM) presents a significant global health challenge [1–3]. Data from the World Health Organization (WHO) underscores a notable increase in T2DM cases over recent decades, with projections indicating a continued rise in the future [4, 5]. Beyond the economic burden, T2DM brings a plethora of complications that severely affect patients' quality of life [6–8]. Diabetic gastroparesis, a common T2DM complication, has garnered significant attention from researchers [9–12]. This condition not only causes digestive discomfort and hampers nutrient absorption but also elevates the risk of other diabetes-related complications [13,14].

Presently, the treatment approach for diabetic gastroparesis primarily centers on blood sugar management and symptomatic relief. Although various drugs have been employed in clinical practice, certain therapeutic challenges persist [5,15,16]. Some medications may induce side effects, and their long-term effectiveness remains uncertain [17]. In managing diabetic gastroparesis, symptom relief alone falls short. It is imperative to elucidate the mechanisms driving the development and progression of this condition, thereby facilitating the establishment of practical therapeutic approaches [18,19].

FoxiangSan, formulated by National TCM Master Professor Lu Renhe, comprises lemon fruit (*Citrus medica* L.*var.socdactylis*

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Swingle), lemon peel (*Citrus medica* L.), nutgrass rhizome (*Cyperus rotundus* L.), tangerine peel (*Citrus Reticulatae Pericarpium*), chry-santhemum powder (*Inula japonica Thunb.*), Schisandra fruit (*Lindera aggregata(Sims)Kosterm.*), and Prince Ginseng (*Pseudostellaria beterphylla(Miq)Pax ex Pax et Hofm.*) in a ratio of 1:1:1:1:1:1:1:3. Recent years have witnessed a growing interest in the application of traditional Chinese medicine for managing T2DM and its complications, especially gastric atony [20–23]. Previous research conducted by our team has shown that *FoxiangSan* effectively alleviates relevant symptoms. Nonetheless, further investigation is required to delineate its specific mechanism of action.

In recent years, the role of gut microbiota in human health and disease has been progressively unveiled [24–26]. Numerous studies have indicated a potential link between gut microbiota imbalance and the occurrence and progression of various diseases, such as T2DM and gastroparesis. Extracts from Alpinia officinarum Hance have been reported to improve diabetic gastroparesis by modulating the SCF/c-kit signaling pathway and balancing gut microbiota [27]. The cAMP/PKA signaling pathway is considered a crucial mechanism in the pathogenesis of gastroparesis. Research has shown that CNP inhibits the activity of the PKC family in the smooth muscle of rats through the PKG/PKA-PLCβ pathway, reducing levels of IP4 and DG, which results in muscle contraction inhibition and may be a key pathogenic factor in diabetic gastroparesis [28]. Therefore, it is hypothesized that the regulation of gut microbiota and the cAMP/PKA signaling pathway may bear significant relevance in ameliorating the comorbidity of T2DM and gastroparesis.

Based on the preceding context, this study endeavors to elucidate the mechanism of action of *FoxiangSan* in gastric atony in T2DM, with a specific focus on its influence on gut microbiota and the cAMP/PKA pathway. The objective of this study is to provide innovative strategies and directions for the treatment of diabetic gastroparesis, promote the clinical use of *FoxiangSan*, and establish a theoretical foundation for its application in related disorders.

2. Materials and methods

2.1. Experimental animals

Seventy healthy male Sprague-Dawley (SD) rats weighing 170 ± 20 g were used in this study. The rats were obtained from Beijing Huafu Kang Biotechnology Co., Ltd. (license number: SCXK [Beijing] 2020-0004). The rats are reared in a controlled non-laboratory environment, where the temperature is maintained at $23\,°C \pm 2\,°C$, the relative humidity is maintained at 35 % $\pm$ 5 %, and a light-dark cycle of 12 h is provided, along with natural ventilation. The rats were properly fed by the feeding regulations before the experiment commenced and were given one week to acclimate to the environment. This study received approval from the Animal Care and Use Committee of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (approval number 2022B186).

The high-fat and high-sugar feed comprises 20 % protein, 35 % carbohydrates, and 45 % fat. This feed is purchased from Beijing Huafu Kang Biological Technology Co., Ltd., with SCXK [Beijing] 2020-0004 license number.

2.2. Drugs and reagents

The Chinese herbs used in *FoxiangSan* were sourced from the Commercial Pharmacy of China Academy of Chinese Medicine Sciences in Beijing, China. Professor Yang Xiaohui then authenticated these herbs at Dongzhimen Hospital of Beijing University of Chinese Medicine. The prescription and manufacturing process have been patented (supplementary materials). The formulation used in this study is ten times more concentrated than the prescription used in the pilot study. Five milliliters of volatile oil should be extracted from three components of bergamot (*Citri Sarcodactylis Fructus, Fructus Citri, Cyperi Rhizoma*), as well as from another five components (*Citri Reticulatae Pericarpium, Pinelliae Rhizoma, Flos Inulae, Radix Linderae, and Radix Pseudostellariae*). To a 4-liter solution of herbal medicine with a density of 2.75 g/mL, add volatile oil in a ratio of 1 mL per 0.8 L and mix well. The preparation is conducted weekly and stored in a refrigerated environment to inhibit the evaporation of volatile oils. The nimesulide acid is sourced from Lunan Biotech Co., Ltd. (Approval Number: National Medical Approval H19990317).

The following reagents were purchased from The Tykome Biotech Company in Beijing: Streptozotocin (Sigma, USA, S0130-1G), cAMP ELISA kit (ab cam, ab136947), PVDF membrane (0.22 μ m) (Millipore, America, ISEQ00010), Protein extract (MDL, MDL91201), Protease inhibitor (MDL, MD912893), BCA Protein Assay Kit(MDL, MD913053), SDS-PAGE kit (MDL, MD911919), Middle-RangedProteinMarker (Thermo, 26617), Secondary antibody (MDL, MD912565); P-pka (a bcam, ab75991), PKA(AffinitY, AF7746); 5-HT4(AffinitY, DF3503), TRIZOL(Invitrogen, 10296028), DEPC(MDL, MD911875), UltraPure Agarose(ABI-invitrogen, 16500100), SuperScript III RT reverse transcription kit (ABI-invitrogen, 11752050), Sybr qpcr mix(ABI-invitrogen, 4472920).

2.3. Preparation and quality control of Foxaingsan

Methanol and acetonitrile of HPLC grade were acquired from Fisher Scientific Co. in Loughborough, UK, while formic acid of HPLC grade was obtained from Sigma-Aldrich in St. Louis, MO, USA. The Milli-Q system (Millipore, MA, USA) was utilized to purify deionized water (with a resistivity of 18.2 MΩ). The reference materials were purchased from Shanghai Yuanye Biotechnology Co., Ltd.

After washing, the samples were placed in a forced-air drying oven set at 55 °C for 24 h. After drying, the sample was homogenized using the Retsch MM400 mixer manufactured by Retsch GmbH company in Hain City, Germany. Next, 100 mg of fine powder in 2 mL of methanol were suspended and the liquid was extracted using an ultrasonic extractor for 30 min. The extraction solution is centrifuged at 12,000 g for 10 min using the Eppendorf 5810R centrifuge at Eppendorf GmbH in Hamburg, Germany. When filtering the upper clear liquid, it is recommended to use a needle filter with a pore size of 0.22 μm. Introduce a 1-μL sample solution into the

UPLC/Q-TOF MS system for analysis.

UPLC separation was conducted using the UPLC-i-Class system from Waters Corporation, Milford, USA, employing an HSS T3 column (100 × 2.1 mm, 1.8 μm) for chromatographic analysis. The column temperature is 40 °C, and the flow rate is 0.5 mL/min. Mobile phase A comprises a 0.1 % aqueous solution of formic acid, while mobile phase B is composed of a 0.1 % solution of formic acid in acetonitrile. The gradient elution program consists of the following steps: at 0 min, the mobile phase B concentration is 5 %; at 16 min, it increases to 85 %; at 18 min, it reaches 100 %; and maintains 100 % until 20 min. The sample injection volume is 1 μL. TOF mass spectrometry experiments were conducted using the Xevo G2-S Q-TOF mass spectrometry system by Waters Corporation, situated in Milford, USA. The data collection mode utilizes continuous MSE and centroid samples for control. The experiment was conducted using ESI (negative ion mode). The source temperature is 100 °C, the dissolution temperature is 450 °C, and the gas flow rate for dissolution is 900 L per hour. The capillary voltage is set to 2 kV, while the cone voltage is set to 40 V. The collision energy for low-energy scanning is set at 6 electron volts in the trap, whereas high-energy scanning employs a ramp ranging from 35 to 60 electron volts. The range of data collection spans from 50 to 1500 Da. Leucine enkephalin was used as a reference substance at 200 pg/μL concentration and a flow rate of 10 μL/min. In ESI (positive ion mode), the *m/z* value was determined to be 556.2766, while in ESI (negative ion mode), it was found to be 554.2620. The supplementary materials present the typical peak intensities (BPI) chromatograms of *FoxiangSan* and the reference substances (hesperidin, naringin, and rutin), illustrating the characteristic fragment ions of these reference substances [29].

2.4. Establishment of diabetic gastroparesis animal model and animal grouping

After one week of adaptive feeding, ten Sprague-Dawley (SD) rats were chosen as the control group (CG), and a diabetes gastroparesis model was induced in sixty SD rats, drawing from previous research. Following a 12-h fasting period, we measured fasting blood glucose (FBG) concentration in rats with a diet high in fat and sugar (with 45 % fat content) for 2 weeks. The animals then undergo a 12-h fasting period without water. After weighing, the animals were intraperitoneally injected with a dose of 50 mg/kg of STZ. STZ was dissolved in a 0.1 mol/L citrate buffer solution (pH = 4.4) and kept on ice for 10 min before the injection. In the CG group, an intraperitoneal injection of normal saline at the same dose was administered. After a period of normal feeding lasting 8 weeks, tail docking was performed along with blood sampling to determine the concentration of FBG. Select Sprague-Dawley rats that meet the criteria for the diabetic gastroparesis model, which include a fasting blood glucose (FBG) concentration of ≥16.7 mmol/L, as well as symptoms like abdominal distention and weight loss [30]. The rats were randomly divided into 5 groups: the model group (MG), the western medicine group (WMG) treated with mosapride citrate, and the low, medium, and high-dose traditional Chinese medicine (FoxiangSan) groups referred to as LDG, MDG, and HDG, respectively. Throughout the modeling process, 8 animals died: 3 in the MG group, 1 in the WMG group, 3 in the LDG group, and 1 in the HDG group. The causes of death included three cases of gastric medication errors, two cases of animal bites, and three cases of intestinal obstruction.

After establishing the model, the medication was administered at a dosage that *was* seven times the adult dose (5 mL/kg per day) for eight weeks. The control group (CG) received daily intraperitoneal injections of a standardized quantity of distilled water. The model group (MG) received daily intragastric injections of equivalent distilled water. The Western medicine group (WMG) received daily intragastric injections of a standardized mosapride citrate solution. The group receiving Chinese medicine, which consisted of 30 STZ-induced diabetic rats, was given *FoxiangSan* at a human and animal equivalent dose. Each rat received an average dosage of 5 mL/kg/d, equivalent to 13.75 g/kg/d of dried herbal medicine. The high, medium, and low dose groups (LDG, MDG, HDG) receive doses that are double, equal to, and half those given to humans and animals, respectively.

2.5. Western blot

Total proteins were extracted from tissues and cells using high-efficiency RIPA lysis buffer (Catalog number R0010, Solarbio, Beijing, China) according to the provided instructions. Nuclear and cytoplasmic proteins were separated using a nuclear-cytoplasmic separation reagent kit (Catalog No. NUC201, Sigma-Aldrich, USA), following the manufacturer's instructions. After incubating at 4 °C for 15 min, the samples were centrifuged at 15,000 rpm/min for 15 min. The supernatant was collected, and the protein concentration of each sample was assessed using the BCA assay kit (catalog number: 20201ES76, Yisheng Biotechnology Co., Ltd., Shanghai, China). Quantification was conducted using various concentrations. The proteins separated by polyacrylamide gel electrophoresis were subsequently transferred to a PVDF membrane through a wet transfer method. After transfer, the membrane was blocked with 5 % BSA at room temperature for 1 h. The primary antibodies for 5-HT4, PKA, *p*-PKA, and β-actin were diluted and subsequently added. Incubate overnight at 4 °C on a rocking platform. The membrane was washed three times for 5 min each using TBST. Following the washes, the membrane was incubated with HRP-conjugated goat anti-rabbit IgG (catalog number: ab205718, diluted 1:20000, Abcam, UK) at room temperature for 1 h. The samples were washed with TBST for 5 min, repeated 3 times. Next, the developing solution was added, and the samples were developed. Protein quantification analysis was conducted using ImageJ 1.48 software (National Institutes of Health). It was achieved by analyzing the grayscale values of each protein and calculating the ratio relative to the reference grayscale value for quantification [31]. The experiment is repeated three times.

2.6. qRT-PCR

Total RNA extraction was performed using the Trizol reagent kit (10296010, Invitrogen, Thermo Fisher, USA). RNA quality and concentration can be determined using ultraviolet–visible spectrophotometry (ND-1000, Nanodrop, Thermo Fisher, USA). The reverse transcription was performed using the PrimeScript™ RT-qPCR Kit (RR086A, TaKaRa, Mountain View, CA, USA). The real-time

quantitative reverse transcription polymerase chain reaction (RT-qPCR) was conducted using the SYBR Premix Ex TaqTM (DRR820A, TaKaRa) on the LightCycler 480che system (Ro Diagnostics, Pleasanton, CA, USA). Using GAPDH as the internal reference for mRNA. Shanghai General Biosciences Co., Ltd designed the amplification primers. The primer sequences are as follows: 5-HT4 (CAGCCAA-GACTTTATGTGTCA, CCTGAATTGATATAGCCAAGCC) and PKA (GCCGAACTTGGACCTTGTGT, ACCAGCAGCCATCTCGTAG). The quantity $2^{-\Delta\Delta Ct}$ signifies the fold change in expression of the target gene in the experimental group relative to the control group. The formula is as follows: $\Delta\Delta CT = \Delta Ct$ (experimental group) - ΔCt (control group), where ΔCt represents the difference between the Ct values of the target gene and the reference gene [32].

2.7. 16S rRNA sequencing and data analysis

Collect fecal samples from the abovementioned rats, with 6 rats in each group. The general workflow of 16S rRNA sequencing involves extracting total DNA from microbial samples. The 16S rRNA gene segment was amplified using specific primers and polymerase chain reaction (PCR). Subsequently, a library was constructed, and high-throughput sequencing was carried out.

The samples were evaluated using multiQC and kneaddata, available at https://github.com/biobakery/biobakery/wiki/kneaddata. multiQC was employed for sequence quality control, whereas kneaddata was utilized to remove host and contaminant sequences. The microbial species tree can be visualized, and differences can be annotated using GraPhlAn (https://github.com/biobakery/graphlan.git) to obtain the relative abundance of microbial taxa. Alpha diversity analysis uses indices like Chao1 to evaluate the species' complexity. We calculated the beta diversity distance matrix and conducted a Principal Coordinates Analysis (PCoA) [33].

Create differential abundance bar plots using LDA Effect Size (LEfSe, http://huttenhower.sph.harvard.edu/lefse/). Set the linear discriminant analysis (LDA) score threshold to 3.5. LDA scores indicate the level of influence of differentially abundant species between groups. Higher scores indicate significant differences in features between the two groups.

The QIIME data were transformed using the R package Compositions. Then, the phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt)2 was utilized to predict metagenome pathways for each primer set, employing the MetaCyc database. This study involves statistical analysis and visualization of unstratified results using the STAMP (v2.1.3) software. The Welch's *t*-test is typically employed to compare compositional differences between functional groups [34,35].

2.8. Statistical analysis

All data were analyzed using GraphPad Prism 8.0. Quantitative data were expressed as mean ± standard deviation (Mean ± SD). Non-paired t-tests were conducted to compare two groups, while one-way analysis of variance (ANOVA) should be used for comparisons among multiple groups. We tested the homogeneity of variance using the Levene test. If homogeneity of variance was found, we performed pairwise comparisons using Dunnett's T3 and LSD-t tests. The Dunnett's T3 test is employed when there are unequal variances [36]. A significance level of $P < 0.05$ indicates a statistically significant difference in comparison between the two data groups.

3. Results

3.1. A comparison was made between the blood glucose levels and gastric motility indices in each group of rats

We observed a significant decrease in gastric residue levels in the normal group (CG group) compared to the other five groups. In comparison to the model group (MG group), the Western medicine group (WMG group), as well as the high, medium, and low dose groups (HDG group, MDG group, LDG group), exhibited a decreasing trend in gastric residue levels, indicating their therapeutic effect on diabetic gastroparesis (Table 1, Fig. 1).

3.2. FoxiangSan can significantly activate the 5-HT/cAMP/PKA pathway

In this study, we also investigated the impact of Foxiangsan on the 5-HT/cAMP/PKA pathway. Stomach tissues were collected from each group of rats, and indicators related to the 5-HT/cAMP/PKA pathway were assessed using qRT-PCR and Western blot analysis. The results revealed significant reductions in the levels of 5-HT4, PKA, and pPKA in the model group (MG group) compared to the

Table 1
6 Comparison of gastric residues and blood glucose parameters among groups.

Group	CG	MG	WMG	LDG	MDG	HDG
The gastric residue (%)	0.3187 ± 0.0808	0.5314 ± 0.0835*	0.3758 ± 0.0635#	0.3703 ± 0.0349#	0.3506 ± 0.0759#	0.3703 ± 0.0349#
Blood Glucose (mmol/L)	8.7144 ± 1.2657	30.7271 ± 7.7573*	31.8686 ± 7.4250*	31.1714 ± 6.6543*	30.5125 ± 3.9120*	30.8389 ± 5.7849*

Note: Data are presented as mean ± standard deviation. *Compared with the CG, P < 00.05. #Compared with the MG, P < 00.05. CG, control group; MG, model group; WMG, western medicine group; LDG, low-dose group; MDG, mid-dose group; HDG, high-dose group.
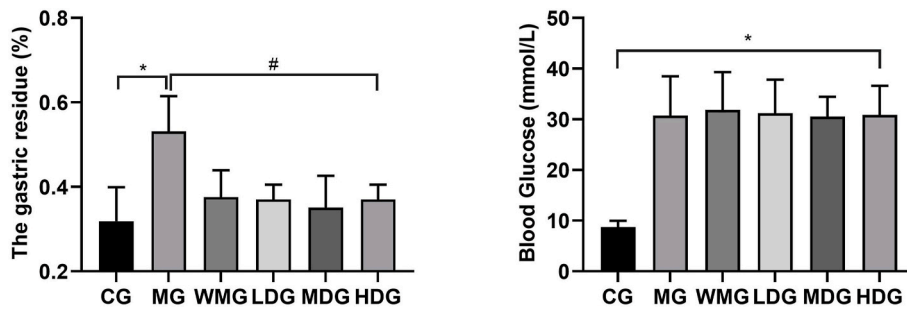
Fig. 1. 6 Comparison of gastric residues and blood glucose parameters among groups. Note: Data are presented as mean ± standard deviation. *Compared with the CG, P < 00.05. #Compared with the MG, P < 00.05. CG, control group; MG, model group; WMG, western medicine group; LDG, low-dose group; MDG, mid-dose group; HDG, high-dose group.

control group (CG group). Conversely, compared to the model group (MG group), the levels of these indicators markedly increased in the Western medicine group (WMG group) as well as the low, medium, and high doses of the traditional Chinese medicine groups (LDG group, MDG group, HDG group). Significantly, the high dose group (HDG) exhibited the most pronounced increase in the indicators above among LDG and HDG. The results in the high-dose group were similar to those of the positive control 5-hydroxytryptamine group. The Ketanserin group showed lower levels of 5-HT4, PKA, and pPKA compared to the model group, with Western blot analysis not detecting any protein bands and the presence of minimal mRNA of 5-HT4 and PKA (Fig. 2A–C). The results suggest that *FoxiangSan* can effectively activate the 5-HT/cAMP/PKA pathway, potentially leading to therapeutic benefits for gastric atony.

3.3. FoxiangSan can modify the species diversity of intestinal microbiota in rats with diabetic gastroparesis

An imbalance in gut microbiota is strongly linked to the onset and progression of diabetes. However, its impact on diabetic gastroparesis remains uncertain. This study investigated the influence of *FoxiangSan* on the species diversity of the intestinal microbiota in rats through 16S rRNA sequencing.

Core analysis can elucidate the extent of species overlap across all samples. This study observed a gradual decrease in the total number of projects and shared projects within each group, eventually reaching a plateau. It suggests that the sample sizes in each group were sufficient to evaluate species richness and core species (Fig. 3A). Alpha diversity is a method used to calculate the species composition within samples, considering both abundance and richness. Commonly employed algorithms for evaluating species diversity in samples include Chao1, Shannon index, and α-rarefaction curve. Like Shannon's diversity index, this curve can reflect the diversity of microbial communities, and its value shows a positive correlation with community diversity. We observed that all groups of curves showed a flattening trend, suggesting that the data size of each group met the sequencing requirements, allowing for a comprehensive reflection of the microbial diversity information for each group of samples (Fig. 3B–D).

Beta diversity can be used to examine variations in species composition among communities. Weighted UniFrac distances were utilized to compute the Beta diversity distance matrix. Principal Coordinate Analysis (PCoA) was conducted (Fig. 4A). Adonis analysis demonstrated a substantial differentiation of gut microbiota communities among the five groups (Fig. 4B).

The results above indicate significant differences in gut microbiota diversity between the normal and model groups. *FoxiangSan* can alter the species diversity of gut microbiota in rats with diabetic gastroparesis.
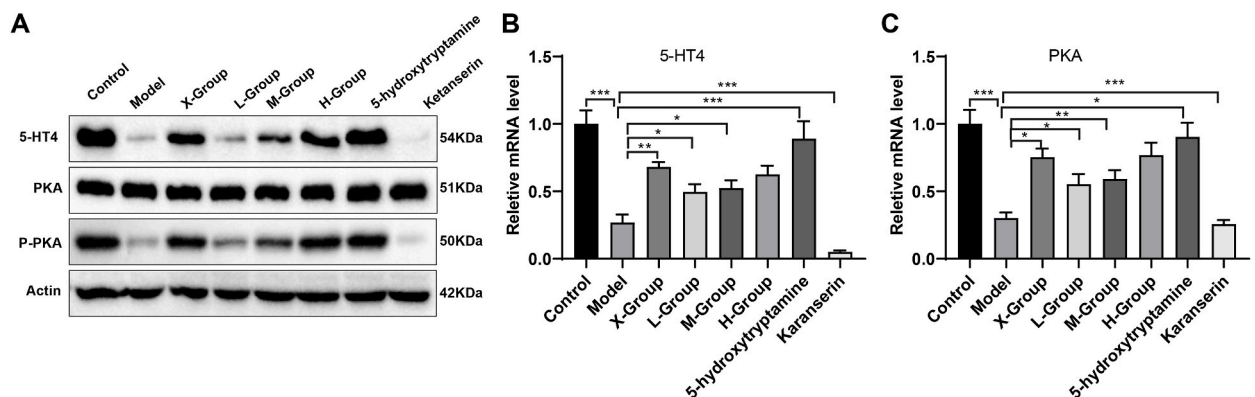
Fig. 2. The effects of *FoxiangSan* on the 5-HT/cAMP/PKA pathway. Note: (A) Western blot analysis of each group's 5-HT4, PKA, and pPKA levels. (B) qRT-PCR analysis of 5-HT4 and (C) PKA levels in each group. p < 0.01, p < 0.001, ***p < 0.0001.
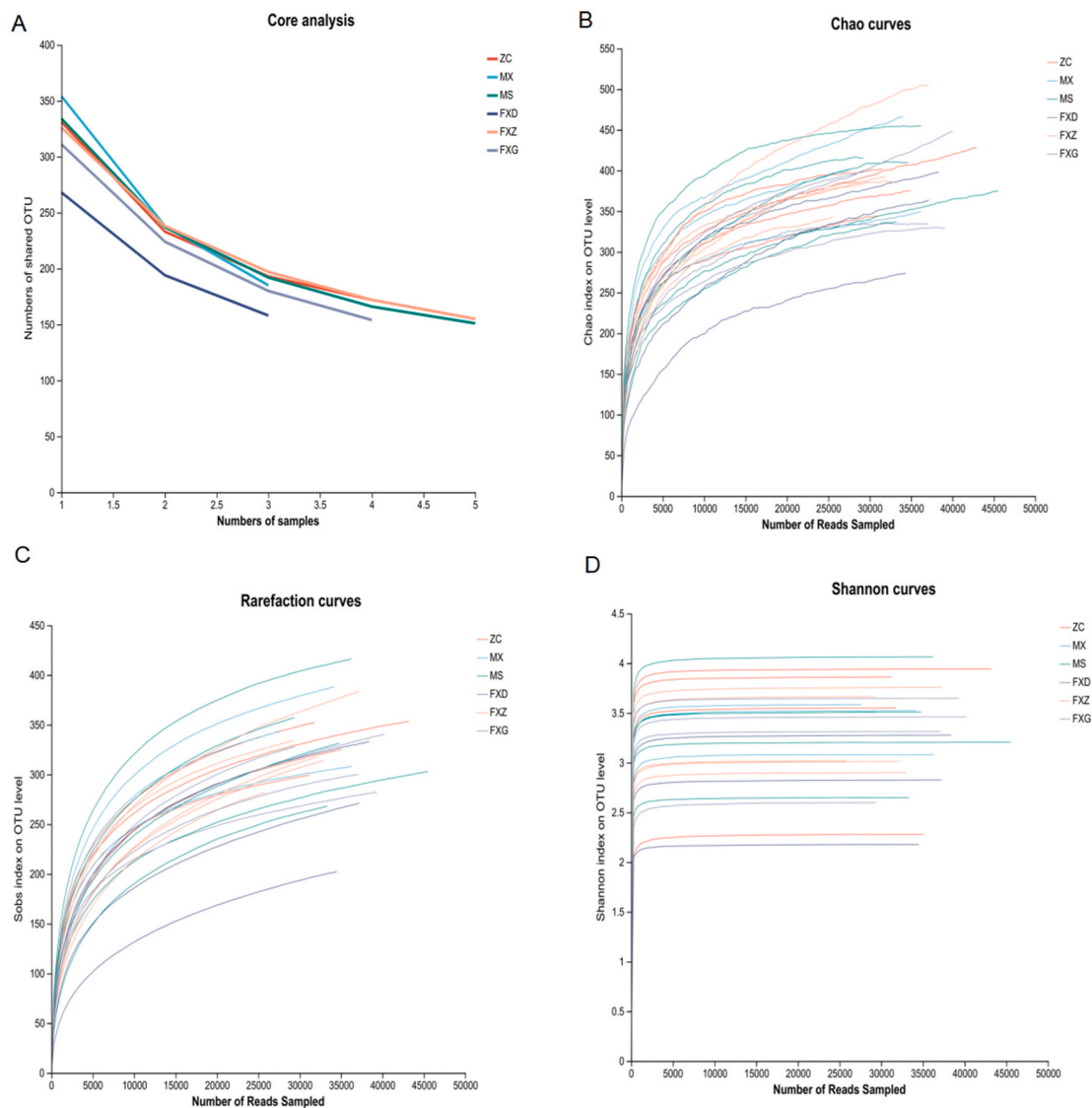
Fig. 3. Annotation and evaluation of intestinal microbiota in each group of rats. Note: (A) Core analysis of the number of shared species among all samples. (B) Chao curves. (C) Rarefaction Curves. (D) Shannon curves. Each group consists of 6 rats.

3.4. FoxiangSan can modify the species composition of the gut microbiota in rats with diabetic gastroparesis

The abundances of intestinal microbiota at the phylum level exhibit significant differences among distinct rat groups (P < 0.01). According to the figure, the abundance of Firmicutes was significantly lower in MG compared to CG. Conversely, the abundance of Actinobacteria, Proteobacteria, and Firmicutes was significantly higher (Fig. 5A). These results indicate that Actinobacteria, Proteobacteria, and Firmicutes are the dominant bacteria in the gut of rats with diabetic gastroparesis. Compared to the MG group, the abundances of Bacteroidetes were significantly lower in the LDG, MDG, and HDG groups, indicating that *FoxiangSan* can reduce the abundance of Actinobacteria in the gastrointestinal microbiota of rats with diabetic gastroparesis (Fig. 5B).

To explore the inter-group differences in the species composition of gut microbiota, we conducted LEfSe analysis. The results revealed that using a threshold of LDA score (log10) > 3.5 and P < 0.05, compared to ZC, MX exhibited significant decreases in f_Ruminococcaceae and g_Lachnospiraceae_UCG-001, while being enriched in p_Bacteroidetes, g_Clostridium_sensu_stricto_1, p_Proteobacteria, and g_Desulfovibrio. Furthermore, compared to MX, HDG showed an increase in f_Eubacteriaceae, g_Lachnospiraceae_NC2004_group, g_Subdoligranulum, and decreases in f_Muribacella, f_Rikenellaceae, g_Alistipes, and g_Helicobacter (Figs. S1–2; Fig. 6A–B).

The results above confirmed that *FoxiangSan* can modify the species composition of the gut microbiota in rats with diabetic gastroparesis, where Actinobacteria are identified as the primary bacterial population.
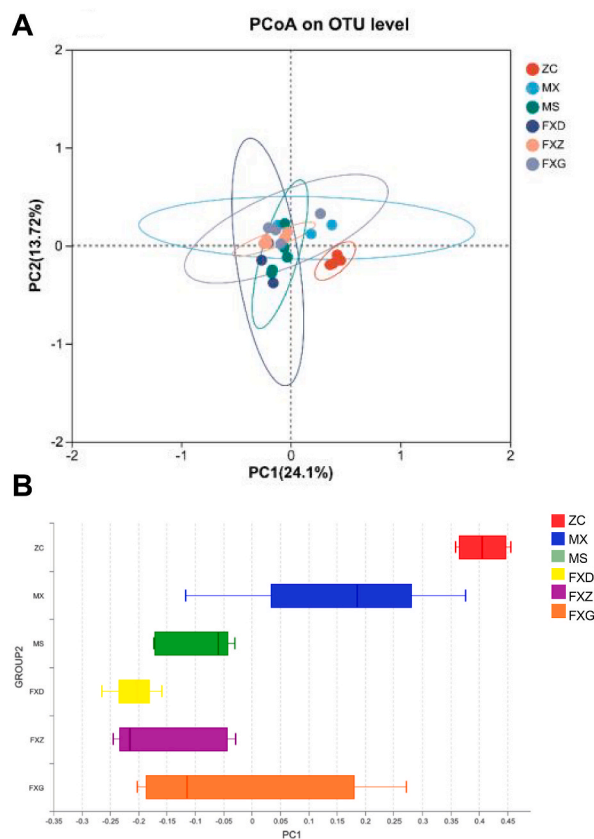
Fig. 4. Beta diversity analysis of intestinal microbiota in each group of rats. Note: (A) Principal coordinate analysis (PCoA) plot of beta diversity of gut microbiota. (B) Bar plot of beta diversity of gut microbiota. Each group consists of 6 rats.

3.5. The regulation of the intestinal microbiota by FoxiangSan in diabetic gastroparesis rats may potentially involve specific metabolic pathways

The results of the PICRUSt2 functional prediction showed that the abundance of several MetaCyc pathways was reduced in MG compared to CG. These pathways include the non-oxidative branch of the pentose phosphate pathway, pyruvate fermentation to acetate and lactate II, the superpathway of pyrimidine nucleobases salvage, peptidoglycan maturation (meso-diaminopimelate containing), CDP-diacylglycerol biosynthesis II, and CDP-diacylglycerol biosynthesis I. However, intervention with *FoxiangSan* was found to reverse these effects (Fig. 7A–D). This result implies that *FoxiangSan* may affect the intestinal microbiota in diabetic gastroparesis rats through pathways associated with phosphoglucitol.

4. Discussion

Diabetic gastroparesis, a common complication of diabetes, has emerged as a pressing clinical concern necessitating immediate attention [9,37–39]. In recent years, there has been a growing public awareness of the therapeutic potential of traditional Chinese medicine in addressing various ailments [20–23]. In light of this backdrop, we conducted a comprehensive study to explore the potential therapeutic role of *FoxiangSan*, a traditional Chinese herbal remedy, in managing diabetic gastroduodenal dysfunction.

Our data reveals a significant advantage of *FoxiangSan* in reducing blood glucose levels in diabetic rats. Moreover, *FoxiangSan* exhibits a positive therapeutic impact on gastric atony, specifically in terms of gastric dynamics. Conventional treatment approaches often fall short of providing sustained relief for gastric paresis [16,40–42]. *FoxiangSan* may present a novel and promising therapeutic alternative for this condition.

The serotonin (5-HT)/cAMP/PKA pathway plays a pivotal role in regulating gastrointestinal function, sensation, and food retention [43–45]. FoxiangSan ability lies in its clear activation of this key pathway, providing new insights into its mechanism for treating gastroparesis. Anomalies in the 5-HT/cAMP/PKA pathway among diabetic patients may be one of the primary causes of gastroparesis [18,46,47]. Therefore, the regulatory effect of FoxiangSan on this pathway holds significant clinical importance. Existing studies have reported a positive correlation between the 5-HT/cAMP/PKA signaling pathway and intestinal motility [48]. Modulating the 5-HT/cAMP/PKA signaling pathway and intestinal microbiota has been shown to improve constipation [43]. Thus, we believe that the 5-HT/cAMP/PKA signaling pathway is closely related to intestinal microbiota.

Community barplot analysis


Community barplot analysis
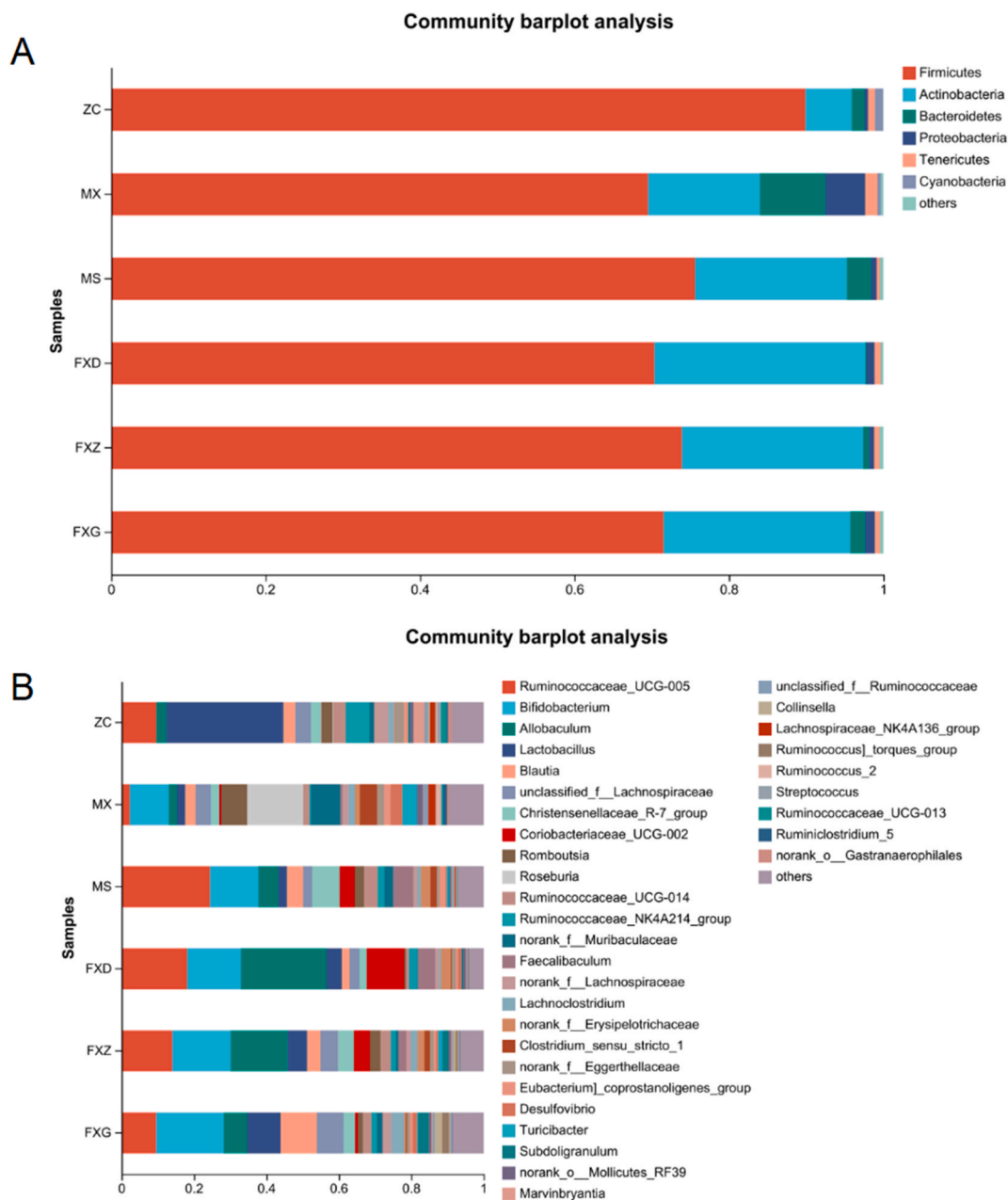
Fig. 5. Analysis of species composition at the phylum level and differences in intestinal microbiota among groups of rats. Note: (A) Dominant bacterial species and their relative abundances in each group. (B) Differences in community abundance at the phylum level among groups. Six rats in each group.

The composition of gut microbiota plays a pivotal role in the development of various diseases, particularly metabolic disorders [49–53]. FoxiangSan has demonstrated significant potential in modulating the gut microbiota of diabetic gastroparesis rats. Previous research has shown that Alpinia officinarum Hance can improve symptoms of diabetic gastroparesis in mice by regulating the gut microbiota [27]. This effect may be related to a decrease in the abundance of Bacteroidetes and an increase in the abundance of Firmicutes, aligning with the findings of this study. Additionally, the prevalence of *Helicobacter pylori* infection in diabetic gastroparesis patients is 74.6 %, significantly higher than in the diabetic-only and normal control groups. Following administration of *Foxiangsan* in this study, there was a notable decrease in the abundance of *Helicobacter pylori* [54].

Previous research has shown that an increase in the gut microbiota probiotic Lachnospiraceae is beneficial for improving symptoms of gastroparesis in diabetes patients [27]. This study reveals an elevation of Lachnospiraceae_UCG-001 in the model group, while the HDG group exhibited an increase in Lachnospiraceae_NC2004_group. Additionally, existing literature indicates that the reduction of
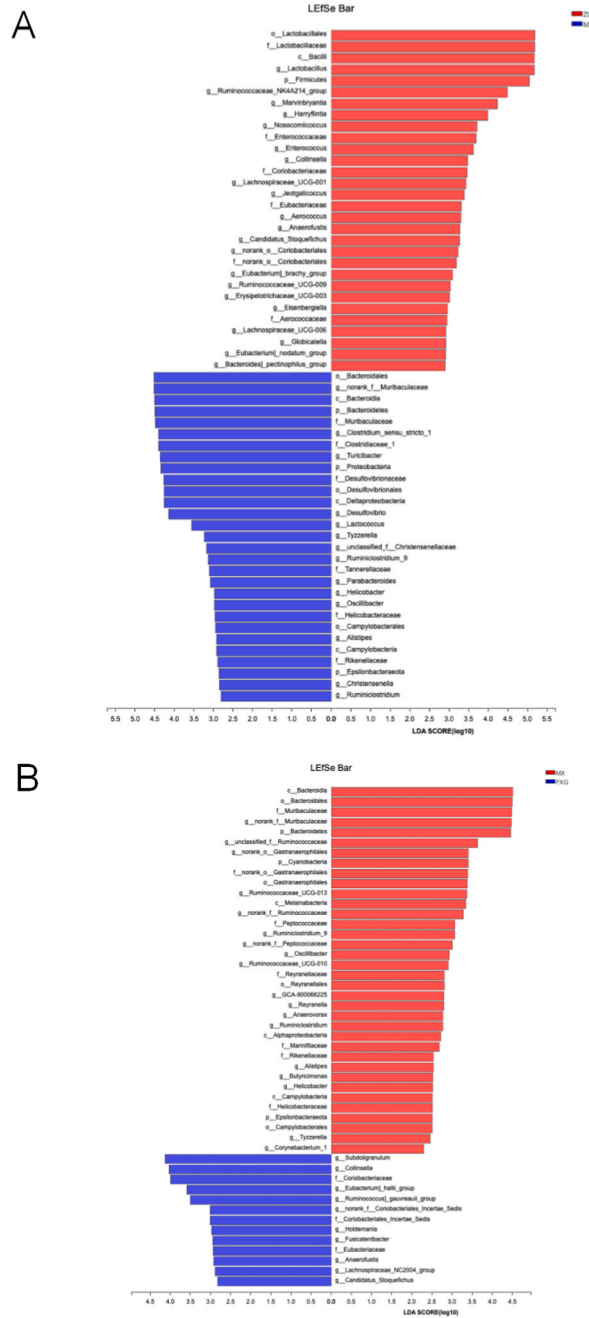
Fig. 6. Differences in species composition among groups analyzed by LEfSe. Note: (A) Bar chart of LDA values distribution of intestinal microbiota species abundance compared between groups ZC and MX; (B) Bar chart of LDA values distribution of intestinal microbiota species abundance compared between groups MX and FXG; with 6 rats in each group.

pathogenic bacteria such as Alistipes and Helicobacter is advantageous for the restoration of gut microbiota in T2DM [55]. In the high-dosage group of *Foxiangsan* in this study, Alistipes and Helicobacter were lowered.

Actinobacteria plays a crucial role in maintaining the balance of gut microbiota [56–58]. Therefore, the regulation of its abundance by *FoxiangSan* raises questions about its connection to therapeutic effects on gastric atony. The LEfSe analysis conducted in this study has additionally illuminated *FoxiangSan*'s regulatory effect on specific bacterial species, providing a comprehensive research direction to explore the interaction between *FoxiangSan* and these particular bacterial species. The pentose phosphate pathway is pivotal in numerous cellular physiological processes and significantly contributes to the progression of diabetes [59–61]. *FoxiangSan*'s impact on this pathway may significantly contribute to its therapeutic effect on diabetic gastric paresis. In future research, further investigation
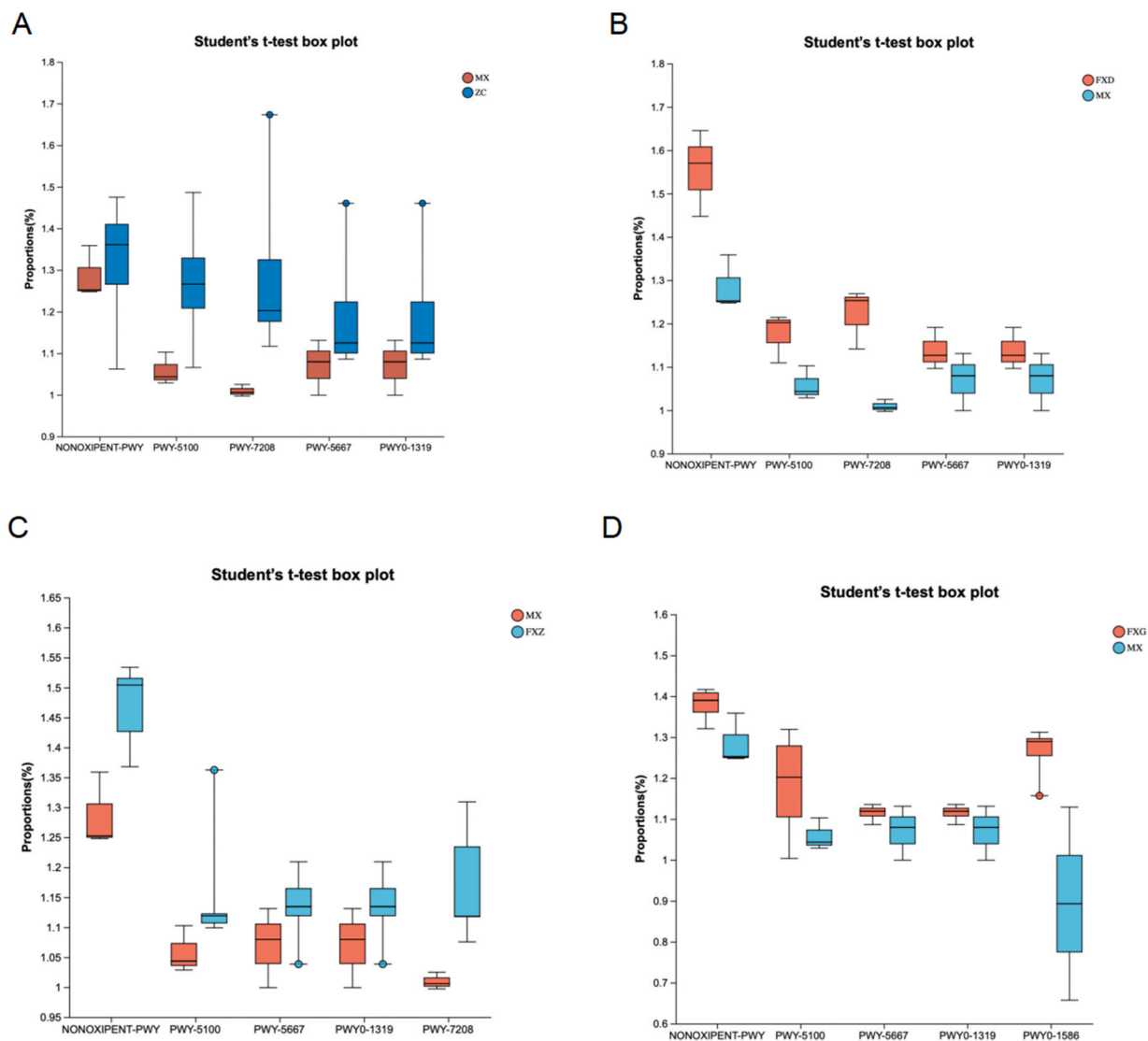
Fig. 7. Results of PICRUSt2 functional prediction of MetaCyc pathways abundance. Note: (A–D) Differences in the abundance of MetaCyc pathways in pairwise comparisons. Each group consists of 6 rats.

into the relationship between *FoxiangSan* and these key metabolic pathways will provide additional insights.

Actinobacteria play an essential role in maintaining gut microbiota balance [56–58]. The LEfSe analysis in this study further elucidated the regulatory effect of FoxiangSan on specific bacterial species, opening up deeper research pathways that may involve interactions with these particular species. Previous studies have indicated a correlation between actinomycetes in the gut microbiota and the expression of cAMP and PKA (https://doi.org/10.26599/FSHW.2022.9250119). Furthermore, lactate metabolism by gut microbiota can participate in fatty acid metabolism and inflammatory responses through the cAMP-PKA cascade reaction. In a study on the comorbidity of irritable bowel syndrome (IBS) and depression, Yu et al., found that resveratrol improved depressive behavior and modulated gut microbiota in rats by regulating the 5-HT1AR-dependent PKA/CREB/BDNF signaling pathway within the GBA. These studies undoubtedly demonstrate the close connection between cAMP-PKA and gut microbiota [62]. The pentose phosphate pathway plays a pivotal role in various cellular physiological processes and is significantly involved in the progression of diabetes [59–61], suggesting a potential close association between FoxiangSan and the improvement of this pathway within the gut microbiota. Future research aimed at deepening the understanding of the relationships between FoxiangSan and these critical metabolic pathways will provide further insights.

In summary, *FoxiangSan* demonstrates significant therapeutic effects on diabetic gastroparesis through multiple mechanisms (Fig. 8). This research not only introduces a new treatment approach but also enhances our understanding of the therapeutic potential of herbal medicine. While further verification and research are necessary, *FoxiangSan* undeniably offers new perspectives for addressing diabetic gastroparesis, providing potential for more effective clinical therapy options.
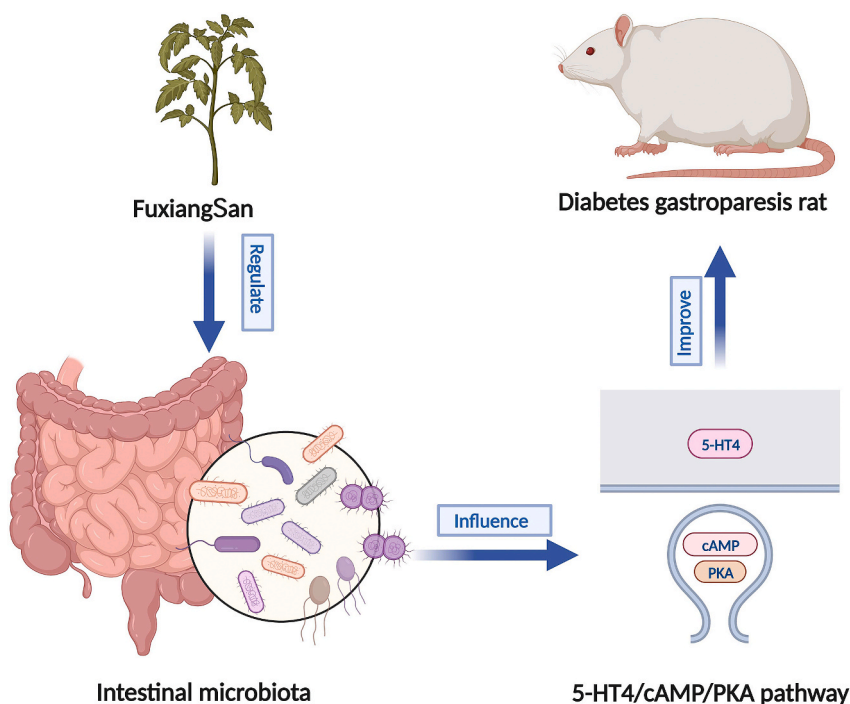
Fig. 8. The diagram depicting the mechanism illustrates how *FoxiangSan* alleviates gastroparesis symptoms related to Type 2 Diabetes Mellitus (T2DM), by modulating both the cAMP/PKA signaling pathway and the gut microbiome.

Ethics approval and consent to participate

This study received approval from the Animal Care and Use Committee of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (approval number 2022B186).

Consent for publication

Not applicable.

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Availability of data and materials

The data that supports the findings of this study are available on request from the corresponding author.

CRediT authorship contribution statement

Jukai Huang: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Yaling Song:** Writing – review & editing, Visualization, Validation, Supervision. **Shuli Cheng:** Writing – review & editing, Software, Resources, Project administration, Methodology. **Xiaohui Yang:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35558.

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