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Intervention effect of *Potentilla discolor-Euonymus* alatus on intestinal flora of type 2 diabetes mellitus rats

S.-Y. HE[1], X.-M. QIU[2], Y.-Q. WANG[1], Z.-Q. SU[1], B.-Y. ZHANG[1], Z. WEN[1], Y.-F. YANG[1], B.-F. XING[1], M. HONG[1], R. LIAO[1]

[1]The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong Province, China
[2]The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong Province, China

Shaoying He and Xinmei Qiu contributed to the work equally and should be regarded as co-first authors

Abstract. – OBJECTIVE: With this study, we aimed at exploring the regulation mechanism of *Potentilla discolor-Euonymus alatus* on intestinal flora of T2DM (Type 2 Diabetes Mellitus) rats induced by high-fat diet combined with streptozotocin.

MATERIALS AND METHODS: T2DM rats were induced by high-fat diet combined with streptozotocin. There were normal control group, model group, metformin group, high-dose Chinese medicine group and low-dose Chinese medicine group. Each group included 10 rats. Normal control group: normal feeding, no modeling, ordinary feed, and gavage of 0.9% normal saline. Model group: T2DM rats, high-fat diet, and gavage of 0.9% normal saline. Metformin group: T2DM rats, high-fat diet and fed with metformin solution. High-dose Chinese medicine group: T2DM rats, high-fat diet, and gavage of concentrated Chinese medicine at a dose of 6 times the clinical dose. Low-dose Chinese medicine group: T2DM rats, high-fat diet, and gavage of concentrated Chinese medicine at a dose twice the clinical dose. The general situation of T2DM rats was observed, and the changes of intestinal flora were observed with 16SrDNA sequencing.

RESULTS: The T2DM rats induced by high-fat diet combined with streptozotocin were molded. After intervention, at the class level, the ratio of *γ-proteobacteria* was 22.30% in the model group, 11.97% in the metformin group, 3.24% in the high-dose Chinese herbs group and 1.72% in the low-dose Chinese herbs group; the ratio of *Erysipelothrix insidiosa* was 4.73% in the model group, 4.68% in the metformin group, 3.93% in the high-dose Chinese herbsgroup and 2.92% in the low dose group; the ratio of *Lactinobacillus* was 2.30% in the model group, 0.01% in the metformin group, 0.00% in the high-dose Chinese herbs group, and 0.00% low-dose Chinese herbs group; at the portal level, the *Firmicutes/Bacteroides* was 0.88 in the normal control group, 3.40 in the model group, 1.71 in the metformin group, 2.74 in high-dose Chinese medicine group, and 1.34 in low-dose Chinese medicine group; at the genus level, the relative abundance of *Lactobacillus* in the model group was 3.28%, that of *Akkermansia* was 1.99%, that of *Shigella coli* was 22.08%, and that of *Vibrio phaseus* was 7.67%. All of them were improved after the intervention of metformin and traditional Chinese medicine.

CONCLUSIONS: *Potentilla discolor-Euonymus Alatus* could improve the composition and structure of intestinal flora in T2DM rats and regulate the diversity of intestinal flora. The ratio of Firmicutes/Bacteroidetes was adjusted, mainly to increase the number of Bacteroides; the flora related to intestinal barrier was adjusted, mainly to increase the number of *Lactobacillus* and *Akkermansia* bacteria.

Key Words:
Type 2 diabetes mellitus, Potentilla discolor, Euonymus alatus, Intestinal flora.

Introduction

The treatment of obesity and diabetes is a long-term process. Traditional hypoglycemic drugs, such as insulin, increase the weight of patients to a certain extent, which in turn increase the insulin resistance of diabetic patients causing more disordered blood sugar metabolism. Statistics have shown that the weight loss of 20 jin can reduce the risk of death of T2DM patients by 25%[1]. At present, there are many control strategies for T2DM, mainly including weight management, medical nutrition therapy, exercise therapy, smoking cessation, blood sugar monitoring, diabetes education, oral hypoglycemic drug therapy (metformin, sul-

Corresponding Author: Rui Liao, MD; e-mail: rayliao1985@163.com

fonylureas, glinides, TZD, etc.), insulin therapy, etc. In addition, there are also some new therapeutic drugs[2]. The above drugs have achieved certain curative effect in lowering blood sugar, but at the same time, they also bring some adverse reactions[3], which greatly affect the treatment effect and patient compliance. Therefore, the treatment of T2DM patients should have a definite effect in lowering blood sugar, reducing weight, and at the same time should avoid causing hypoglycemia, and protect the function of islet beta cells.

More and more evidence shows that obesity, insulin resistance (IR) and T2DM are all related to the changes of intestinal flora. The abundance of *Lactobacillus* and *Enterococcus* in *firmicutes* is significantly increased, that of *Bacteroides* in *Bacteroides* is low, and that of *Bifidobacterium* and *Roche* in *Actinomycetes* is low. At the same time, in T2DM patients, the abundance of *Clostridium* and other butyrate-producing bacteria decreased, and the oxidative stress resistance function of microorganisms increased[4]. The abundance of four *lactobacillus* strains in T2DM patients increased, while that of five *Clostridium* strains decreased, and indicated that intestinal flora is very important in the metabolic changes related to T2DM[5,6].

In this study, taking T2DM as the breakthrough point, flora 16S rDNA sequencing method was used, and the mechanism and treatment of T2DM after the intervention of the traditional Chinese medicine pair *Potentilla discolor-Euonymus alatus* were observed. Finally, the scientific connotation of T2DM treatment with traditional Chinese was clarified.

Materials and Methods

Drugs and Reagents

Drug preparation

1. Took 800 g of *Euonymus alatus* and *Potentilla discolor*, refluxed and extracted for 2 hours for the first time, and used 6,400 ml of 75% ethanol; refluxed continuously for the second time for 1 hour, used 4,800 ml of 75% ethanol, and mixed the extractive solutions twice. After filtering, concentrated the extractive solution into 80 ml of concentrated solution (1 ml/10 g) by vacuum rotary evaporator.
2. Metformin hydrochloride, Aladdin, article number: M107827. Used 0.9% normal saline to prepare the suspension. The gavage volume was 10 ml/kg.

Animals Model

50 rats were divided into 5 groups, 10 rats fed with normal diet and 40 rats fed with high-fat and high-sugar diet on unrestricted diet. Normal feed was purchased from Guangdong Laboratory Animal Center, and high-fat feed was purchased from Botai Hongda Biological Company.

Establishment of Type 2 Diabetes Rat Model

After 4 weeks of feeding with high-fat diet: (1) Fasting and water for 12 hours before modeling; (2) Prepare 0.1 mol/L citric acid (solution A)-sodium citrate buffer (solution B). The preparation method is as follows: add 21 g of citric acid to 1,000 ml of double-distilled water in liquid A, add 29.4 g of sodium citrate to 1,000 ml of double-distilled water in liquid B, and mix liquid A and liquid B at a ratio of 1:1 to make the pH value 4.2-4.5. Then, the citric acid buffer (0.1 mol/L, PH 4.2-4.5) was prepared. (3) Take an appropriate amount of STZ in the dark as far as possible, dissolve it in citric acid buffer on ice, and prepare a solution with a concentration of 1%. Protect from light, place on ice, and use up within 15 minutes after preparation. (4) According to the body weight of the rat and to the STZ of 40 mg/Kg, the STZ solution was injected into the left lower abdomen of the rat to induce type 2 diabetes. They received a high-fat diet immediately after STZ injection. (5) Criteria for successful type 2 diabetes model: collected blood from the tail vein for 3 consecutive days, and blood glucose was detected. If the blood glucose level was higher than 16.6 mmol/L for 2 consecutive days, the modeling was considered to be successful.

Grouping and Dosing

There were 4 groups: model group, metformin group, high-dose Chinese herbs group, and low-dose Chinese herbs group. On the second day after successful modeling, the rats were given intragastric administration for 12 consecutive weeks of continuous intervention. Blood glucose and body weight were measured every week. The intragastric dose of rats was calculated according to 10 ml/kg body weight, and the intragastric dose of each rat was adjusted according to the body weight.

Animal Material

Before the experiment, the rat was fixed, the perianal area was sterilized, the tail was lifted, the abdomen of the rat was gently pressed

Table I. Changes in fasting blood glucose levels of rats in each group ($\bar{x} \pm s$).

	Normal control group	Model group	Metformin group	High-dose Chinese medicine group	Low-dose Chinese medicine group-
1 week	4.53 ± 0.47	24.53 ± 4.40	23.91 ± 4.58	22.36 ± 2.67	22.05 ± 2.17
2 weeks	4.66 ± 0.53	23.06 ± 4.44	20.99 ± 2.64	21.87 ± 2.29	23.52 ± 3.15
3 weeks	3.40 ± 0.98	24.11 ± 3.19	18.62 ± 1.46	19.94 ± 1.40	20.92 ± 2.47
4 weeks	3.87 ± 0.89	23.39 ± 3.75	18.00 ± 1.47	19.84 ± 1.57	20.76 ± 1.83
5 weeks	3.99 ± 0.79	23.08 ± 3.02	17.56 ± 1.49	19.71 ± 1.09	20.91 ± 1.49
6 weeks	3.62 ± 1.00	24.56 ± 2.72	14.50 ± 1.49	16.44 ± 1.92	20.24 ± 2.47
7 weeks	4.00 ± 1.20	25.68 ± 2.71	9.47 ± 0.75	11.66 ± 2.18	16.46 ± 1.11
8 weeks	3.64 ± 1.04	27.73 ± 2.71	7.87 ± 1.10	9.25 ± 1.02	14.25 ± 1.41
9 weeks	4.21 ± 0.75	24.91 ± 1.79	7.99 ± 0.59	8.60 ± 0.68	13.24 ± 2.09
10 weeks	4.05 ± 0.89	24.29 ± 4.72	7.46 ± 0.61	7.83 ± 0.72	12.94 ± 1.53
11 weeks	4.30 ± 0.60	25.43 ± 4.54	6.38 ± 0.93	7.11 ± 0.85	11.29 ± 1.80
12 weeks	4.07 ± 0.69	26.30 ± 3.86	6.20 ± 0.65	6.89 ± 0.81	11.17 ± 1.24

to promote defecation, and the rat feces were collected in a sterile cryopreservation tube, which was then labeled, stored at -80°C and shipped on dry ice. 16s rRNA was analyzed by Hangzhou Lianchuan Biotechnology Co. (Hangzhou, China).

Statistical Analysis

The measurement data of the experimental results were expressed as mean±standard deviation, and SPSS 23.0 statistical software (IBM Corp., Armonk, NY, USA) was used for analysis. Single factor analysis of variance was used for comparison of multiple groups of means, and LSD method was used for comparison between pairs. $p<0.05$ indicated that the difference was statistically significant.

Results

General Results

Before modeling, the rats were in a good mental state, their fur was shiny, and their defecation and water consumption were normal. After modeling, the rats were obviously depressed, with polydipsia, polyphagia, polyuria, and dull fur. After gavage of metformin and traditional Chinese medicine, the rats' spirits improved, and their mobility was more active than that of the model group. Due to the different tolerance of rats to STZ, three rats died in the model group, one rat died in the metformin group, two rats died in the high-dose Chinese medicine group and three rats died in the low-dose Chinese medicine group.

Effect of Potentilla Discolor-Euonymus Alatus on Fasting Blood Glucose of T2DM Rats

After modeling, the fasting blood glucose of rats in each group was significantly higher than that of the normal control group ($p<0.05$). Compared with the model group, after intervention, the fasting blood glucose of rats in the metformin group and in the Chinese medicine group began to decrease ($p<0.05$) after 4 weeks, especially in the metformin group, which indicated that *Potentilla discolor-Euonymus alatus* had hypoglycemic effect on the fasting blood glucose of T2DM rats (as shown in Table I and Figure 1).

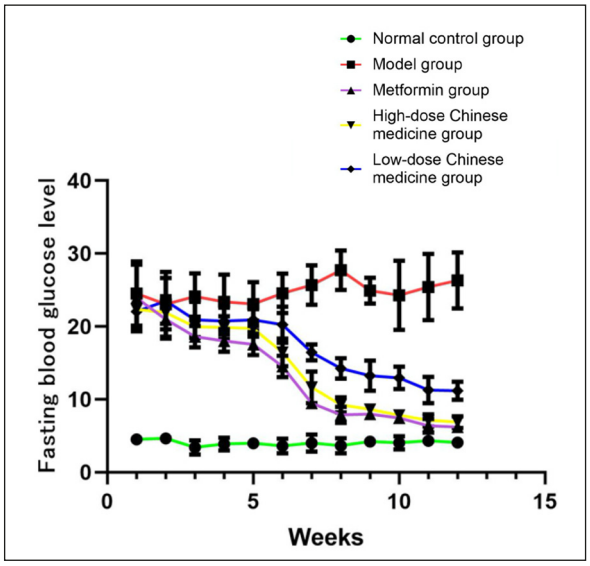

Figure 1. Changes in fasting blood glucose levels of rats in each group.

Table II. Alpha diversity index of each group.

Shannon	Simpson	Observed otus	Chao1
4.13 ± 6.22	0.91 ± 0.02	42.67 ± 2.16	44.50 ± 5.87
4.81 ± 0.45	0.93 ± 0.05	77.75 ± 1.50	77.50 ± 1.50
4.96 ± 0.37	0.94 ± 0.03	79.00 ± 3.39	79.00 ± 3.39
5.04 ± 0.28	0.95 ± 0.02	76.00 ± 2.55	76.00 ± 2.86
4.94 ± 0.41	0.94 ± 0.04	75.20 ± 4.44	75.80 ± 4.60

Analysis of the Diversity of Intestinal Flora α in T2DM Rats by Potentilla Discolor-Euonymus Alatus

The α diversity index of intestinal flora was used to evaluate the diversity of microbial flora among groups. In this study, indexes including observed species, Shannon, Simpson and Chao1 were used to evaluate the α diversity of microbiota. Simpson index refers to the diversity and evenness of species distribution in flora. Chao1 index is used to estimate the total number of species contained in flora samples. In our study, the Shannon and Simpson values of metformin group and traditional Chinese medicine group increased in different degrees. It shows that the diversity of flora is increased, and the distribution is more uniform. Therefore, *Potentilla discolor-Euonymus alatus* could maintain the diversity of intestinal microbial flora in T2DM rats (as shown in Table II).

Analysis of the Effect of Potentilla Discolor-Euonymus Alatus on the β Diversity of Intestinal Flora in T2DM Rats Treated

The β diversity is a measure of the similarity of flora composition among different samples, and the difference of flora composition among different samples is observed. In this study, NMDS analysis, PCoA analysis and ANOSIM analysis were used for β diversity analysis.

According to NMDS analysis, certain differences among groups were detected. This indicated that the intestinal flora of T2DM rats was abnormal after modeling. And the intestinal flora had recovered to some extent after the intervention of metformin and traditional Chinese medicine (as shown in Figure 2).

Analysis of Flora Composition at the Class Level

At the class level, there were *Bacteroides, Clostridium, Coriobacteriaceae, proteobacteria, Bacillus, Gamma Proteus, Firmicuteria, Verrucomicrobia, erysipelothrix, Firmicutes, Actinomycetes* and *Campylobacter* in each group. Among them, the *γ-proteobacteria* in the model group was 22.30%, significantly higher than that in the model group ($p<0.05$). After drug intervention, the *γ-proteobacteria* level decreased significantly ($p<0.05$), with 11.97% in the metformin group, 3.24% in the high-dose Chinese herbs group and 1.72% in the low dose group. The rate of *erysipelothrix* in the model group was 4.73%, which was significantly higher than that in the normal control group ($p<0.05$). After intervention, it was 4.68% in the metformin group, 3.93% in the high-dose Chinese herbs group and 2.92% in the low-dose Chinese herbs group. *Actinobacillus* in the model group was 2.30%, significantly higher than that in the normal control group ($p<0.05$). After intervention, it was 0.01% in the metformin group, 0.00% in the high-dose Chinese medicine group and 0.00% in the low-dose Chinese medicine group. All of them were significantly lower than those in the model group ($p<0.05$). This

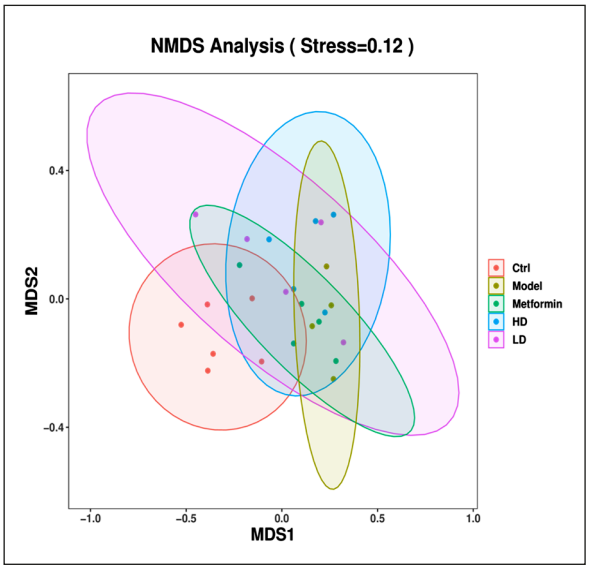

Figure 2. β-diversity NMDS analysis chart of each group.

indicated that *Potentilla discolor-Euonymus alatus* herb pair could regulate the class-level flora of intestinal floras in T2DM rats (as shown in Figure 3).

Analysis of Phylum Horizontal F lora Composition

At the phylum level, the intestinal floras of rats in each group included: *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia* and *Epsilonbacteraeota*. The *Firmicutes/Bacteroides* was 0.88 in the control group and 3.40 in the model group, showing a significant increase compared with that in the normal control group ($p<0.05$). However, after the intervention of metformin and traditional Chinese medicine, the *Firmicutes/Bacteroides* decreased significantly ($p<0.05$), being 1.71 in the metformin group, 2.74 in the high-dose Chinese herbs group and 1.34 in the low-dose Chinese herbs group. This shows that *Potentilla discolor-Euonymus alatus* can effectively regulate the intestinal flora disorder of T2DM rats (as shown in Figure 4).

Analysis of the Composition of Genus Level Flora

At the genus level, the intestinal floras of rats in each group included: the uncategorized genus *Murmurus, Corisella, Lactobacillus, Shigella Escherichia coli, Bacteroides, Vibrio phasianus, Akkermansia, Lachnospiraceae* NK4A136_group, *Heteroplasma, Desulfovibrio, Rumatococcaceae* NK4A214 group, *Eubacterium, Desulfovibrio, Biliophilus, Romebuthiella., Heterobacterium, Crestanaceae* R-7 group, *Firmicutes, spirillum, Allantozoon parapsilosis*, and other genera. The relative abundance of *Lactobacillus* and *Akkermansia* decreased by 3.28% and 1.99% in the model group ($p<0.05$). The relative abundance of *Shigella Escherichia coli* and *Vibrio phaseus* increased by 22.08% and 7.67% ($p<0.05$). After the intervention of metformin and traditional Chinese medicine ($p<0.05$), they were all improved to a certain extent. This showed that *Potentilla discolor-Euonymus alatus* could improve the intestinal flora of T2DM rats (as shown in Figure 5).

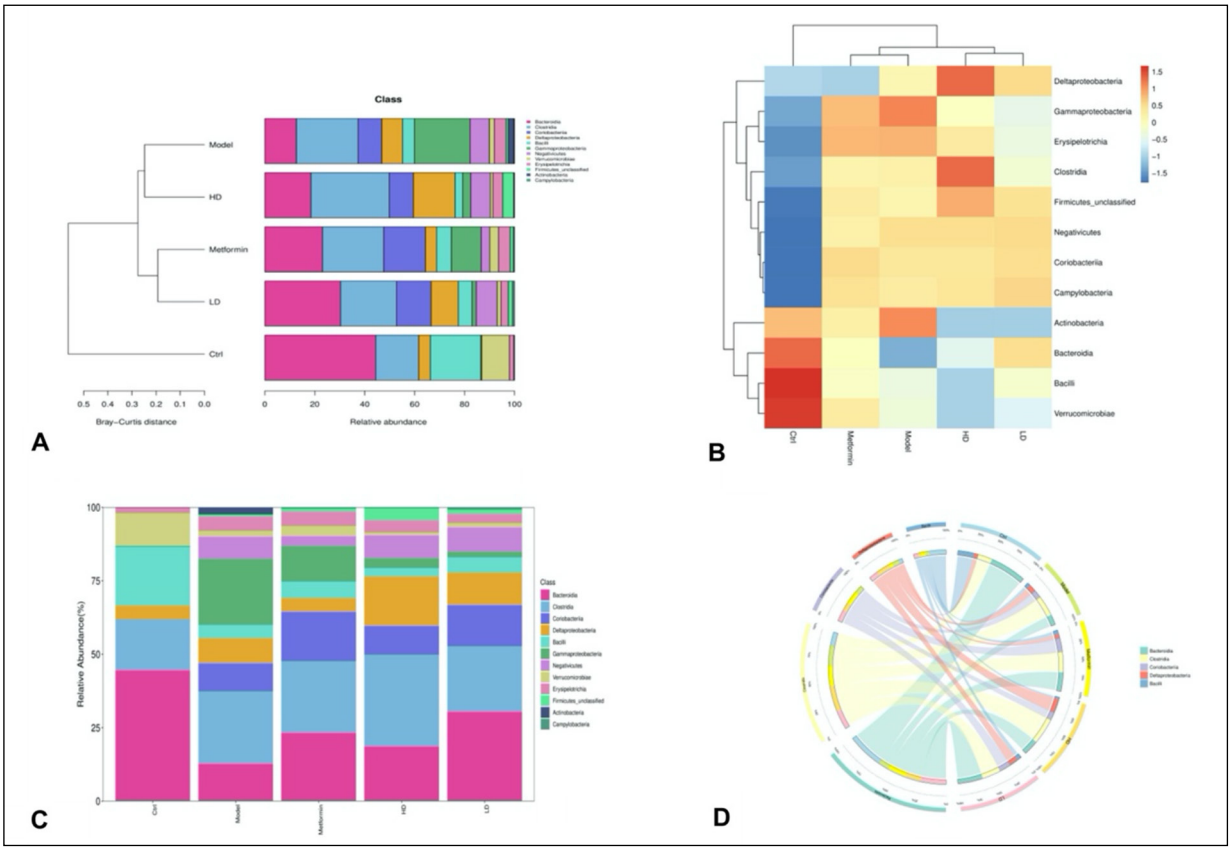

Figure 3. Analysis of flora composition at the class level. **A**, Colony change clusters in each group at the class level; **B**, Heat map of colony changes in each group at the class level; **C**, Histogram of colony changes in each group at the class level; **D**, Groups of Circos diagrams at the class level.

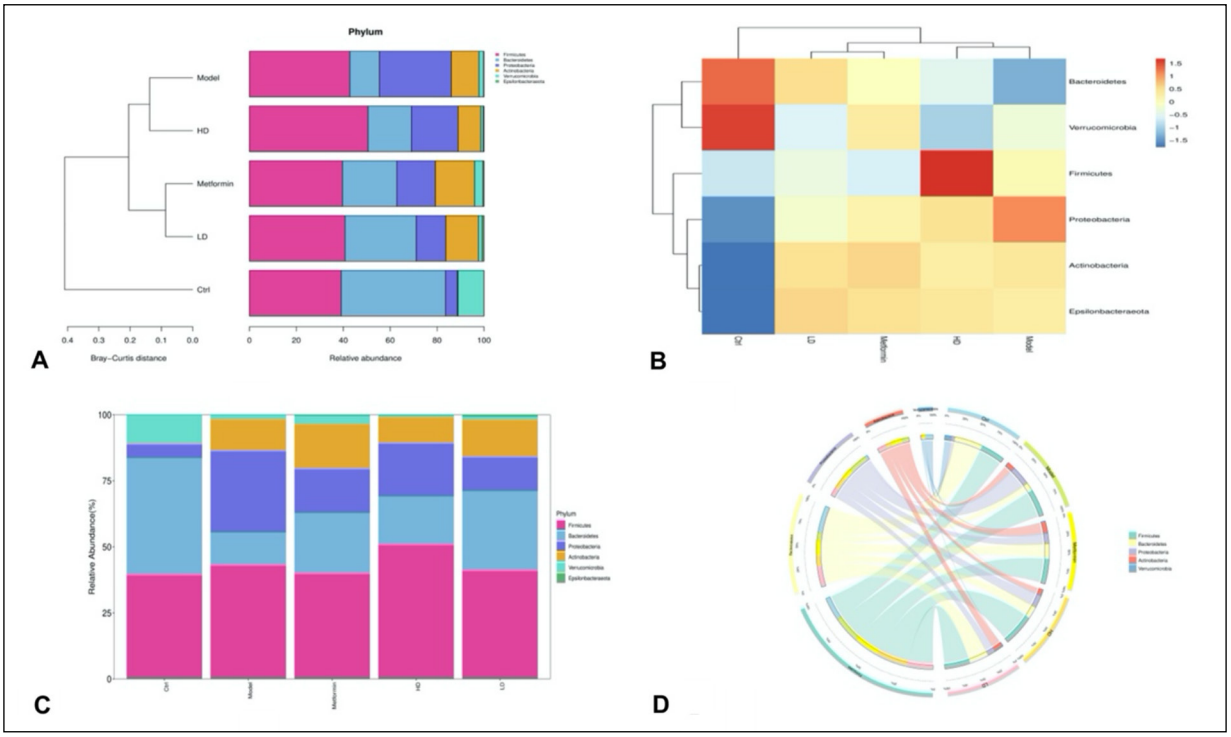

Figure 4. Analysis of phylum horizontal flora composition. **A**, Cluster map of colony changes in each group at the phylum level; **B**, Heat map of colony changes in groups at the phylum level; **C**, Histogram of colony changes in each group at the phylum level; **D**, Groups of Circos diagrams at the gate level.

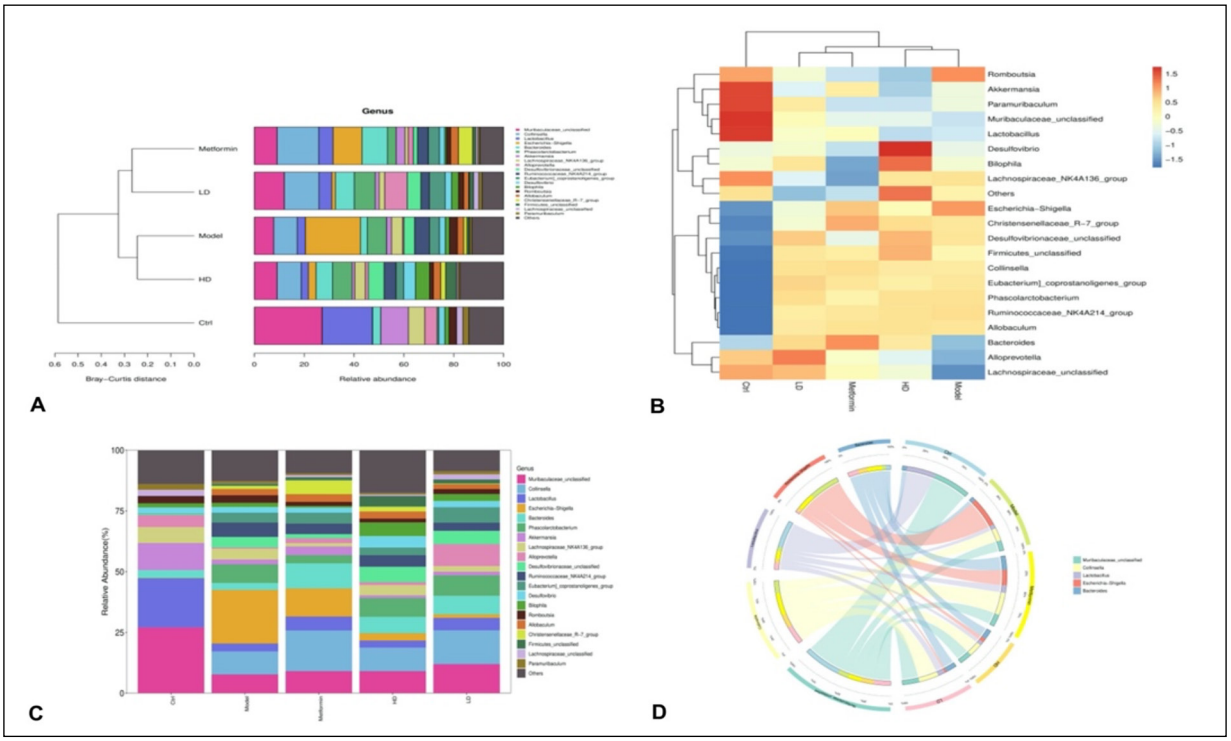

Figure 5. Analysis of the composition of genus level flora. **A**, Cluster map of colony changes in each group at the genus level; **B**, Heat map of colony changes in each group at the genus level; **C**, Histogram of colony changes in each group at the genus level; **D**, Group Circos diagrams at the genus level.

Discriminant Analysis of LEFSE Multilevel Species Differences

The model group was compared to the normal control group, the normal control group screened out 40 biomarkers and the model group screened out 96 biomarkers; the top five biomarkers in the normal control group were: *proteobacteria, enterobacteriaceae, Proteus, Enterobacter* and uncategorized *Shigella Escherichia coli*; the top five biomarkers in the model group were: *bacteroideae, bacillus, Bacteroides, Moxiella,* uncategorized *Moxiella*. The model group was compared with the high-dose Chinese medicine group, the model group screened out 40 biomarkers and the high-dose Chinese medicine group screened out 15 biomarkers; the top five biomarkers in the model group were: *Proteus, enterobacteriaceae, enterobacteria, Escherichia shigella* and uncategorized *Escherichia shigella*; the top five biomarkers in the high-dose Chinese medicine group were: *g clostridia, s clostridia, f clostridia, RA90 staphylococcus* and *g Bacillus piliformis* (as shown in Figure 6).

Discussion

According to IDF data, there are about 537 million diabetic patients in the world at present, most of whom are T2DM patients. T2DM is one of the main chronic diseases affecting human health. Intestinal flora has been proved to be involved in the occurrence and progress of T2DM mellitus. The disorder of intestinal flora can lead to the abnormal function of intestinal mucosa and the increase of intestinal permeability. As a result, many harmful metabolites cannot be excreted, but enter the systemic circulation, causing a series of inflammation in the body, and ultimately damaging the function of islet cells[7-10]. Some people have proved that it is one of the mechanisms of lowering blood sugar to correct the disorder of intestinal flora by adjusting intestinal flora. Traditional Chinese medicine is an oral medicine, which is bound to be absorbed by the intestinal tract and affect the intestinal flora. *Potentilla discolor and Euonymus alatus* are used for treating diabetes. Our study observed the changes of intestinal flora in T2DM rats through concentrated extract in combination with *Potentilla discolor-Euonymus alatus*, thus further revealing the hypoglycemic mechanism of *Potentilla discolor-Euonymus alatus* for T2DM.

According to this study, at the class level, the intestinal flora classes of rats in each group included *Bacteroides, Clostridium, Coriobacteriaceae, proteobacteria, Bacillus, Gamma Proteus, Firmicuteria, Verrucomicrobia, erysipelothrix, Firmicutes, Actinomycetes* and *Campylobacter*. Among them, the *γ-proteobacteria* in the model group was 22.30%, significantly higher than that in the model group ($p < 0.05$). After drug intervention, the *γ-proteobacteria* level decreased significantly ($p<0.05$), with 11.97% in the metformin group, 3.24% in the high-dose Chinese herbs group and 1.72% in the low dose group. The rate of *erysipelothrix* in the model group was 4.73%, which was significantly higher than that in the normal control group ($p<0.05$). After intervention, it was 4.68% in the metformin group, 3.93% in the high-dose Chinese herbs group and 2.92% in the low-dose Chinese herbs group. *Actinobacillus* in the model group was 2.30%, significantly higher than that in the normal control group ($p<0.05$). After intervention, it was 0.01% in the metformin group, 0.00% in the high-dose Chinese medicine group and 0.00% in the low-dose Chinese medicine group. All of them were significantly lower than those in the model group ($p<0.05$). This indicated that *Potentilla discolor-Euonymus alatus* herb pair could regulate the class-level flora of intestinal floras in T2DM rats. The presence of *γ-proteobacteria* in low-fat diet is low, while that in high-fat diet is high. Therefore, adjusting the abundance of intestinal flora of *γ-proteobacteria* is an important way to effectively prevent and control glucose and lipid metabolism; *erysipelothrix* has been detected in the urine of diabetic patients, which may be due to the low immunity of diabetic patients. As a result, the *erysipelothrix* enters the body and causes some symptoms; therefore, *erysipelothrix* is also an important indicator of diabetes. *Actinobacillus* is closely related to infectious diseases, such as endocarditis, meningitis, osteomyelitis, etc. The increase of blood sugar in diabetic patients damages the immune system, thus increasing the abundance of *Actinobacillus*.

In this study, the intestinal floras of rats in each group included *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia* and *Epsilonbacteraeota*. The *Firmicutes/Bacteroides* was 0.88 in the control group and 3.40 in the model group. After the intervention of metformin and traditional Chinese medicine, the *Firmicutes/Bacteroides* decreased significantly ($p<0.05$), reaching 1.71 in the metformin group,

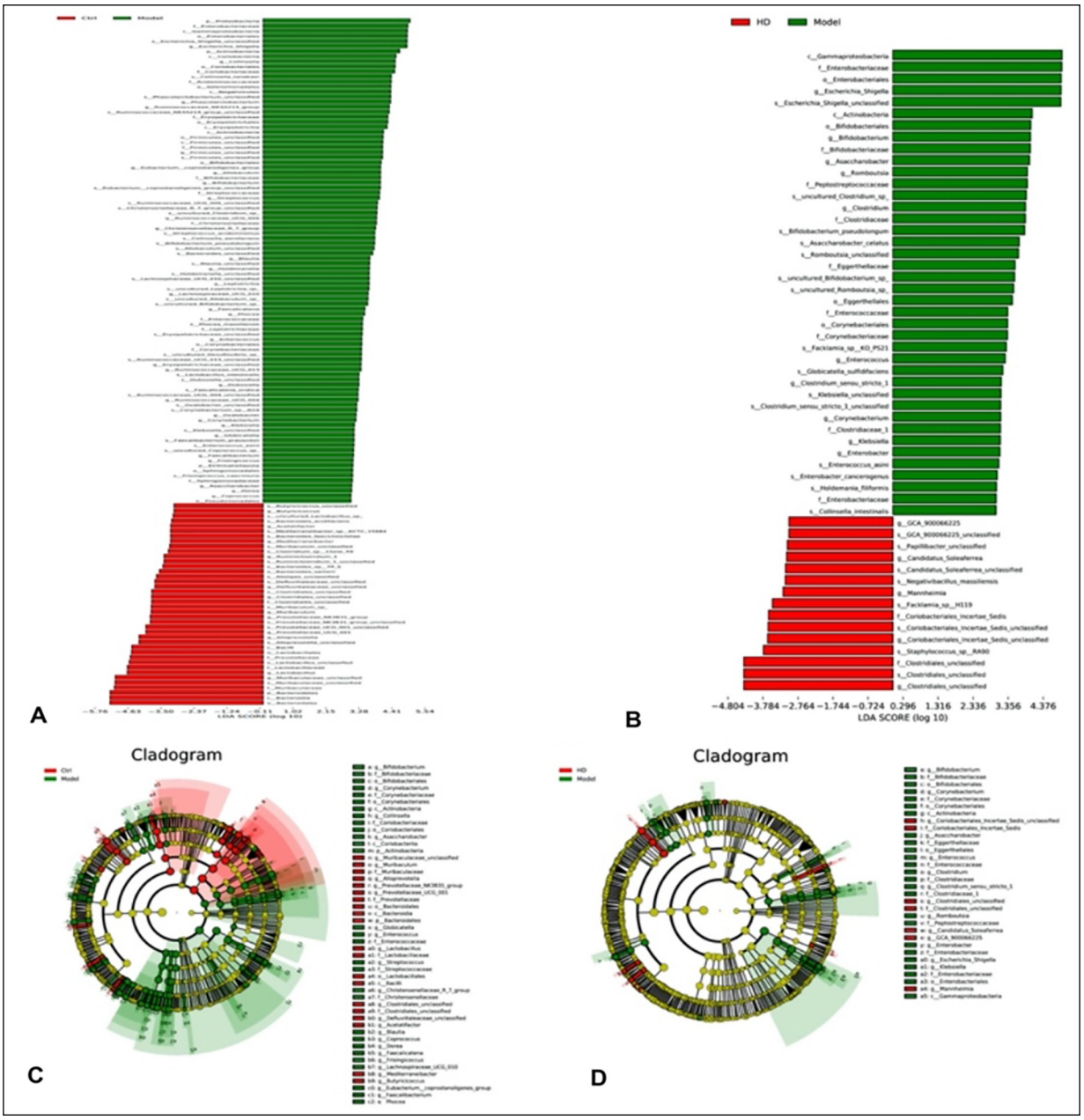

Figure 6. Discriminant analysis of LEFSE multilevel species differences. **A**, Histogram of distribution of LDA values in normal control group and model group by LEfSe analysis; **B**, Histogram of distribution of LDA value of traditional Chinese medicine high-dose group and model group by LEfSe analysis; **C**, Clade diagram of LEfSe analysis in normal control group and model group; **D**, Clade diagram of LEfSe analysis of traditional Chinese medicine high-dose group and model group.

2.74 in the high-dose Chinese herbs group and 1.34 in the low-dose Chinese herbs group. Studies[11,12] have shown that the number of *Firmicutes* in the intestinal tract of obese diabetic mice increased, while the number of *Bacteroidetes* and *Firmicutes/Bacteroides* decreased. *Firmicutes*, known as obese bacteria, can increase the accumulation of sugar and fat in the body and gain weight by improving the utilization rate of food. However, *Bacteroidetes* is called "lean bacteria". The increase of blood sugar in diabetes causes the abundance of *Bacteroides* to decrease but causes the abundance of *Firmicutes* to increase. So, the ratio of *Firmicutes/Bacteroidetes* in diabetic intestinal flora will increase. The same is true of this study. After drug intervention, the abundance of *Bacteroides* to *Bacteroides* increased, thus reducing the ratio of *Firmicutes/Bacteroides*.

Therefore, *Potentilla discolor-Euonymus alatus* medicine can regulate blood sugar in T2DM rats. At the phylum level, the hypoglycemic effect may be realized by increasing the abundance of *Bacteroidetes*.

In our study, the intestinal floras of rats in each group includes: the *uncategorized genus Murmurus*, *Corisella, Lactobacillus, Shigella Escherichia coli, Bacteroides, Vibrio phasianus, Akkermansia, Lachnospiraceae NK4A136_group, Heteroplasma, Desulfovibrio, Rumatococcaceae NK4A214 group, Eubacterium, Desulfovibrio, Biliophilus, Romebuthiella., Heterobacterium, Crestanaceae R-7 group, Firmicutes, spirillum, Allantozoon parapsilosis*, and other genera. The relative abundance of *Lactobacillus* and *Akkermansia* decreased by 3.28% and 1.99% in the model group ($p<0.05$). The relative abundance of *Shigella Escherichia coli* and *Vibrio phaseus* increased by 22.08% and 7.67% ($p<0.05$). After the intervention of metformin and traditional Chinese medicine ($p<0.05$), they were all improved to a certain extent. It belongs to *Gram-positive bacteria* and is one of the normal intestinal flora. *Lactobacillus* is very important to maintain the normal intestinal balance of the body and is of great significance to intestinal health. It can play a probiotic role to increase the number of beneficial flora in the intestinal tract and adjust the structure of flora, thus enhancing the body's disease resistance. *Lactobacillus* can produce short-chain fatty acids, such as lactic acid, to lower the pH value in the intestinal tract and produce antibacterial property, thus inhibiting the growth and reproduction of harmful colonies. It can maintain the intestinal barrier and keep the integrity of intestinal epithelial cells[13,14]. *Akkermansia* is also a normal bacterium in the intestinal tract of the organism, and it is a *Gram-negative anaerobic bacterium*. Some scholars have found that taking *Akkermansia* orally can improve the symptoms of mice with abnormal glucose and lipid metabolism. *Akkermansia* is also an important factor in maintaining the regulation of intestinal barrier, and it is very important to the homeostasis of the organism. *Akkermansia* can reduce the level of inflammatory factors in diabetic patients and improve the inflammatory state of the body, especially the inflammatory factors caused by the increase of blood sugar. It can improve the glucose tolerance of diabetic mice and promote the expression of insulin secretion genes. Our study indicated that the levels of *Akkermansia* in the model group was the lowest, but it was improved after drug intervention. Therefore, increasing the abundance of *Akkermansia* in intestinal tract is probably one of the mechanisms of hypoglycemic effect of *Potentilla discolor-Euonymus alatus*.

Conclusions

Potentilla discolor-Euonymus Alatus could improve blood glucose levels, the composition and structure of intestinal flora in T2DM rats and regulate the diversity of intestinal flora. The ratio of *Firmicutes/Bacteroidetes* was adjusted, mainly to increase the number of *Bacteroides*; the flora related to intestinal barrier was also adjusted, mainly to increase the number of *lactobacillus* and *Akkermansia* bacteria.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and publication of this article.

Funding

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Ethics Approval

All experiments conform to the ethical requirements of animal experiments. The study was reviewed and approved by the Experimental Animal Ethics Committee of the First Affiliated Hospital of Guangdong Pharmaceutical University.

Informed Consent

Our experiments did not involve clinical trials and had no informed consent form to sign.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

Shaoying He, Xinmei Qiu participated in animal modeling and statistical analysis. Yeqing Wang,Zhiqing Su, Biyu Zhang; Zheng Wen,Yifu Yang and Bingfeng Xing participated in drafting the manuscript.Min Hong participated in statistical analysis. Rui Liao conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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