

Effect of *Angelica sinensis* /Danggui Wuji Granule on Intestinal Flora in Mice with Aplastic Anemia

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Research Article

Keywords: Gut microbiota, Danggui Wuji Granule, *Angelica sinensis*, Aplastic anaemia, 16S rDNA

Posted Date: March 4th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3996187/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background *Angelica sinensis* (AS) is a traditional Chinese medical and has a good therapeutic effect on Aplastic anaemia. Danggui Wuji Granule(DGWJ) is a health product with the main effect of treating Aplastic anaemia. It mainly composed of *Codonopsis Radix* extracts, *Ligustrum lucidum* extract, *Radix paeoniae Alba* extract, *Angelica* extract, *lycium barbarum* extract, *Jujube extract*, *Pericarpium Citri Reticulate* Extracts, *Gelatinum Asini*, *Black-bone silky fowloligopeptides*, Ferrous Gluconate.

Methods The animal model of Aplastic anaemia in mice was established by cyclophosphamide method. Mice were randomly divided into control group, model group, AS water extract group and DGWJ granulehigh, medium and low dose groups according to body weight. The blood routine, thymus and spleen indexes of mice were measured by pharmacodynamics, and the intestinal flora of mice was identified by 16 S rDNA sequencing technology. Pseudo-sterile mice were prepared by gavage with compound antibiotics, and the bacterial liquid was prepared from the feces of mice in the treatment group with Aplastic anaemia with obviousearly curative effect. The pseudo-sterile mice were given with bacterial liquid, then CTX was injected intraperitoneally last week to establish a Aplastic anaemia model. The blood routine of mice was measured by pharmacodynamics, and the contents of mice cecum were analyzed by 16 S rDNA sequencing.

Results AS and DGWJ granule can significantly improve the blood routine indexes and anemia symptomscaused by Aplastic anemia model; According to the data of intestinal flora, at the level of genus, Kruskal-Wallis rank sum test was used to evaluate the significance of species richness differences among the four groups,among which *Candidatus_Saccharimonas*, *Lachnospiraceae*, *Enterorhabdus*, *Alloprevotella* and *Turicibacter*had significant differences. The data of fecal microbiota transplantation showed that the low dose group of FMT DGWJ Granule improved the changes of blood routine indexes caused by Aplastic anemia. At the level of genus, the Kruskal-Wallis rank sum test showed that there were significant differences among the four groupssuch as *Lactobacillus*, *Clostridia*, *Lachnospiraceae*and *Colidextribacter*. Correlation thermography analysis shows that *Lactobacillus* had a negative correlation with WBC, RBC, HGB and HCT. *unclassified_f__Lachnospiraceae*, *norank_f__Ruminococcaceae*, *norank_f__Oscillospiraceae*, *GCA-900066575*, *Colidextribacter*, *Blautia* are positively correlated with RBC,HGB and HCT.

Conclusion AS water extract and DGWJ have therapeutic effects on Aplastic anaemia model mice caused by cyclophosphamide, which can improve blood routine, thymus index and spleen index of Aplastic anaemia mice, and are verified by fecal microbiota transplantation experiment. The mechanism of AS water extract and DGWJ in treating Aplastic anaemia may be through increasing the relative abundance of Bacteroides such as Bacteroidota, Patescibacteria, *Muribaculacea*, *Alistipes* and *Alloprevotella* in the intestine of mice with Aplastic anaemia.

Introduction

Angelicae Sinensis(AS) Radix is derived from the dried roots of *Angelica sinensis* (Oliv.) Diels, an Umbelliferae plant^[5]. Mainly grows in China, Gansu, Sichuan, Yunnan and other regions. It was first described in Shen Nong's Classic of Materia Medica during the Han Dynasty. The function of this plant, which is both a traditional medicine and food homologous plant, is to promote blood circulation, moisten intestines, regulate menstruation, and alleviate pain..

In recent years, more than 70 kinds of chemical constituents have been isolated from *Angelica sinensis*, mainly including polysaccharides, volatile oils and flavonoids^[2, 23, 15]. Therefore, Enhancing immunity, anti-tumor activity, anti-oxidation activity, anti-radiation activity, enriching blood, protecting liver, and others are among its pharmacological activities^[10, 21].

Cyclophosphamide is an antitumor drug, also known as p-[1,1,2-bis(< unk>-chloroethyl)]-1-oxygen-3-nitrogen-2-phosphazene-p-oxide.. Clinically, it is often used to treat malignant lymphoma, multiple myeloma, breast cancer and other diseases. Moreover, it is an immunosuppressant, resulting in leukopenia and decreased immune function in the blood.

Angelica polysaccharide in *angelica sinensis* has been shown to have a significant impact on the number of red blood cells, white blood cells, hemoglobin, and nucleated cells in bone marrow, as demonstrated by studies^[25]. This experiment used cyclophosphamide to prepare a mouse model of Aplastic anemia, and the pharmacodynamics and intestinal flora correlation of *Angelica sinensis* and DGWJ granules on Aplastic anemia mice were studied. An experiment was conducted using fecal bacteria transplantation to determine if taking blood-enriching drugs can treat Aplastic anemia by improving intestinal flora.

Methods and Materials

Extraction of *Angelica sinensis* and DGWJ granules

Preparation of *Angelica sinensis* aqueous extract: Weigh 200 g of *Angelica sinensis* decoction pieces, put them into a round-bottomed flask, add 10 times of purified water, heat them in an electric heating jacket, keep them slightly boiling for 120 min, reflux extract them twice, filter them, combine the filtrates, concentrate them in a water bath (80°C) to obtain an aqueous extract with a crude drug mass concentration of 0.2 g/mL, and store it at 4°C.

Preparation of high, medium and low doses of Danggui Wuji Granules: Weigh 26.4 g, 9.9 g and 6.6 g Danggui Wuji Granules respectively, add purified water and grind them to 40 mL, so that the final concentrations are 0.66 g/mL, 0.33 g/mL and 0.165 g/mL.

Animal grouping, modeling and drug administration

Seventy-two SPF ICR mice, half male and half female, weighing 18–22 g were selecte. Adaptation period is 1–2 days. They were randomly divided into control group, model group, *Angelica sinensis* water extract

group (4.5 g/kg) and Danggui Wuji granule high, medium and low dose groups (3.3 g/kg d, 6.6 g/kg d), with 12 rats in each group.

After 3 weeks of administration, the model was established, and cyclophosphamide (40 mg/kg) was injected intraperitoneally every day for 5 days, with no restriction on diet and free drinking water. Normal mice were injected intraperitoneally with normal saline every day.

The recommended dosage of Danggui Wuji Granule is 20 g/d, and the adult dosage is 60 kg, that is, the adult dosage is 330 mg/kg d, and the low, medium and high doses of mice are 10 times, 20 times and 30 times of the adult dosage, that is, 3.3, 6.6 and 9.9 g/kg d; The recommended daily dosage of *Angelica sinensis* for adults is 9 g (the recommended dosage in Pharmacopoeia 2020 is 6–12 g), and the dosage of mice is 30 times that of adults, which is 4.5 g/kg body weight.

Observation of mice signs

Observe the physique, coat color, paw color, mental state, and activity of mice in each group. One hour after the last administration, all mice took out their eyeballs to take blood, put them in EDTAK2 anticoagulation tubes. After blood collection, the spleens were dissected to remove the excess fat tissue, and the analytical balance was quickly used to accurately weigh. Next, we calculated the organ index [organ index = organ mass (g)/body weight (g)], and compared the spleen and thymus index of mice in each group. All mice were weighed every other week, recorded and compared.

Diversity analysis of intestinal flora in mice based on 16S rDNA high-throughput sequencing

Sample collection

After taking blood, the mice were killed by neck removal, dissected, and the contents of ileocecum and colon of mice were put in a sterile RNase-free EP tube, placed in a liquid nitrogen tank, and then stored at -80°C.

Total DNA extraction and detection

DNA extraction of intestinal contents was carried out according to the instructions of DNA extraction kit. DNA integrity was detected by 1% agarose gel electrophoresis (5 V/cm, 20 min), and the total DNA concentration and purity were determined by NanoDrop 2000.

PCR amplification, PCR product identification, purification and quantification, library construction, and L Lumina sequencing

Bioinformatics analysis

Fastp^[3](<https://github.com/OpenGene/fastp>, version 0.20.0) software was used for quality control of the original sequencing sequence, and Flash^[16] (<http://www.cbcb.umd.edu/software/flash>, version 1.2.7) software was used for splicing. Using UPARSE^[8] software (<http://drive5.com/uparse/>, version 7.1), according to the similarity of ninety-seven percent^[20]. Using RDP classifier^[22] (<http://rdp.cme.msu.edu/>, version 2.2) to annotate each sequence. The classification units with significant differences in abundance were screened at the genus level by LEfSe method, and the discriminant criteria were: Kruskal-Wallis test screening value $\alpha < 0.05$, paired Wilcoxon test screening value $\alpha < 0.05$, and significant discriminant value LDA > 2.

Fecal collection

One day after the end of modeling, the feces of mice in control group, model group, Angelica sinensis water extract group and low dose DGWJ Granule group were collected by stress defecation method.

Bacterial liquid preparation

Take 6 faeces from each group, add 5ml of sterile physiological saline (faeces weight: sterile physiological saline volume = 1:5) to 1g of faeces, vortex until they are evenly mixed, filter through a sieve with a pore size of 0.20mm, take the filtrate, centrifuge at 6000 r/min at 4°C for 5min, remove the sediment, and resuspend in 10ml of sterile physiological saline (faeces weight: sterile physiological saline volume = 1:10). Store at -20°C for later use

Construction of Pseudo-sterile Mouse Model

ICR mice, female, weighing 18-20g, were weighed and randomly divided into control group (10 mice) and pseudo-sterile mouse model group (40 mice). Except the control group, the mice in the model group were given ampicillin 0.39 g/kg, neomycin sulfate 0.26 g/kg, metronidazole 0.195 g/kg and vancomycin 0.26 g/kg(20 mL/kg b.w)^[11, 13] daily, for 7 days. On the 7th day, fresh feces of pseudosterile mice and normal mice were collected in sterile enzyme-free EP tubes. Direct smear examination of feces was used. Every 0.05 g of feces was added with 50 µl of sterile physiological saline, and after standing for 5 min, it was homogenized until the feces liquid was uniform, dropped on the glass slide, smeared evenly, and fixed by external flame baking with alcohol lamp. After primary dyeing, mordant dyeing, decolorization and secondary dyeing, the smear was observed under a microscope (400 times)

Grouping, modeling and administration of pseudosterile mice

Forty pseudo-sterile mice were randomly divided into control fecal bacteria transplantation group (FMT control group), model fecal bacteria transplantation group (FMT model group), fecal bacteria transplantation group of Angelica sinensis water extract (FMT Angelica sinensis water extract group) and low-dose fecal bacteria transplantation group of DGWJ Granule (FMT DGWJ Granule low-dose group), with 10 females in each group. Except for the normal fecal bacteria transplantation group, the other groups of pseudo-sterile mice were injected with cyclophosphamide (40 mg/kg) every day for 5 days

from the day of modeling, with no restriction on diet and free drinking water. At the same time, each group was given the corresponding fecal bacterial solution, and each mouse was given 200 μ L bacterial solution by gavage for 14 days.

Observation of pseudo-sterile mice signs

One hour after the last administration, all the mice removed their eyeballs to take blood, put them in EDTAK 2 anticoagulant tubes, and detected RBC, WBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT in the whole blood of mice by automatic flow cytometry.

Diversity analysis of intestinal flora in mice based on 16S rDNA high-throughput sequencing

This Methods as above.

Data processing

SPSS statistical software was used to analyze the data, and the measurement data were expressed by the mean standard deviation (SD). The data that met the normal distribution and the homogeneity of variance were compared and analyzed among groups, and One-way ANOVA was used to analyze the data with significant differences, Student's t test was used, and Kruskal-Wallis test was used to test the data that did not meet the requirements, with a difference of $P < 0.05$.

Materials

Angelica sinensis, anguo city Changda Chinese Herbal Pieces Co., Ltd., batch number: 2102003, Danggui Wuji Granule, Wanmei (Guangdong) Commodity Co., Ltd., batch number: 20190626. CTX(SIGMA, USA), DNA extraction kit(Omega Bio-tek, USA), Agarose(biowest, ES), AxyPrep DNA Gel Extraction Kit(Axygen, USA), FastPfu Polymerase(TransGen, CHN), Database-building kit(Bioo Scientific, USA), Sequencing kit(Illumina, USA). Vancomycin, Ampicillin, Neomycinsulphate, Metronidazole(Solarbio, CHN), Enhanced gram staining solution(LEAGENE, CHN). **Animals**

ICR mice, SPF grade, weighing 18–22 g, were provided by Beijing Weitong Lihua Experimental Animal Technology Co, Ltd., and the animal production license number was SCXK (Beijing) 2016-0006. It is kept in the animal room of Institute of Traditional Chinese Medicine, Chinese Academy of Traditional Chinese Medicine, in a barrier environment. The animal license number is SYXK (Beijing) 2019-0003. The animal experiment involved in this paper was approved by the Animal Ethics Committee of Institute of Traditional Chinese Medicine, Chinese Academy of Traditional Chinese Medicine, with the approval number of 2020B174.

Results

General observation of mice

Compared with the normal mice, the mice in the model group showed mental fatigue, decreased activity, hunched back, drowsiness, closed eyes, sparse and dull fur, loose stools and weak constitution. Compared with the model group, the mice in each administration group have improved, among which the mice in the water extract group of *Angelica sinensis* and the low-dose group of DGWJ Granule are in better condition.

Test of blood routine in mice

The blood routine results of mice in each group are shown in Fig.1A,1B,1C,1D. The experimental results showed that, compared with the control group, The contents of RBC, WBC, HGB and HCT in the model group decreased significantly ($P < 0.01$), which indicated that the model was successful. The contents of RBC, WBC, HGB and HCT in the model group decreased significantly ($P < 0.01$), which indicated that the model was successful. Compared with the model group, each dosage group of *Angelica sinensis* water extract and DGWJ Granule can significantly improve the decrease of RBC and WBC caused by modeling ($P < 0.05$ or $P < 0.01$), and can also improve the decrease of HGB and HCT caused by modeling to some extent, but the difference is not statistically significant.

Tests of thymus index and spleen index in mice

The results of thymus index and spleen index in each group are shown in Fig.1E,1F. It showed that compared with the control

group, the thymus index and spleen index in the model group were significantly decreased ($P < 0.01$ or $P < 0.05$). Compared with the model group, the spleen index of Danggui water extract group and Danggui Wuji granule groups increased in different degrees.

High-throughput sequencing of 16S Rdna

Species composition analysis

Venn diagram was used to count the common and unique species in each group. The results showed that there were 84 species in the four groups at the genus level, including 3 and 5 unique species in the control group and the blood deficiency model group, and 3 unique species in the *Angelica sinensis* water extract group and the DGWJ Granule low-dose group. See Fig.1G1.

Difference test between groups of diversity index

The indexes reflecting Community richness are: Sobs, Chao, ACE The indexes reflecting Community diversity are Shannon and Simpson. At the OTU classification level, compared with the control group, the richness and diversity of intestinal flora in the model group of Aplastic anaemia decreased ($P < 0.05$). Compared with the Aplastic anaemia model group, the richness and diversity of Danggui Wuji Granule low-dose group and Danggui water extract group increased ($P < 0.05$). It is suggested that Aplastic

anaemia can change the richness and diversity of intestinal community, and *Angelica sinensis* may treat Aplastic anaemia by restoring the richness and diversity of intestinal flora. See Fig.1H,1I,1J,1K and1L.

Principal component analysis

According to PCoA analysis, at the genus level, $P=0.012<0.05$, $R=0.1833$. $P<0.05$ means that the reliability of this test is high, and a larger R means that the grouping has a high degree of explanation for the difference, that is, the grouping has a great influence on the difference. The results showed that there were significant differences in intestinal microbial community between control group and Aplastic anaemia model group, Danggui Wuji granule low dose group and Danggui water extract group. The specific results are shown in Fig.1M.

Community composition analysis (Bar diagram)

Through python-2.7 software analysis, we can get the community structure composition of different groups at the genus level. At the phylum level, compared with the control group, the abundance of Bacteroidota, Patescibacteriaia, Actinobactota and Desulfobacterota in the blood deficiency model group decreased, while the abundance of Firmicutes increased. Compared with the model group of Aplastic anaemia, the abundance of Bacteroidota and Patescibacteriaia in the water extract group of *Angelica sinensis* and the low dose group of DGWJ granules increased.

At the genus level, compared with the control group, the abundance of *Muribaculaceae*, *Clostridia*, *Candidatus_Saccharimonas* and *Lachnospiraceae* in the Aplastic anaemia model group decreased. Compared with the model group of Aplastic anaemia, the abundance of *Muribactaceae*, *Clostridia*, *Candidatus_Saccharimonas* and *Lachnospiraceae* in the water extract group of *Angelica sinensis* increased, and the abundance of *Muribactaceae* and *Candidatus_Saccharimonas* in the low dose group of DGWJ Granules increased. See Fig.1N,1O.

Correlation thermography analysis

Analysis of the correlation between microbial species and environmental factors by using heatmap. Taking WBC, RBC, HGB and HCT as environmental factors, the results showed that *Lactobacillus* had a negative correlation with WBC, RBC, HGB and HCT. *unclassified_f__Lachnospiraceae*, *norank_f__Ruminococcaceae*, *norank_f__Oscillospiraceae*, *GCA-900066575*, *Colidextribacter*, *Blautia* are positively correlated with RBC, HGB and HCT. *Alloprevotella*, *Prevotellaceae_UCG-001* and *Romboutsia* were positively correlated with WBC, *Monoglobus*, *Roseburia* and *norank_f__Lachnospiraceae* were negatively correlated with WBC. See Fig.1P.

Analysis of Species Difference —— Multi-group Comparison

Using R-3.3.1 (stat) software, the obtained community composition abundance data were evaluated by Kruskal-Wallis rank sum test, and the species information with significant differences among groups was obtained. At the phylum level, there are significant differences among Patescibacteriaia, Proteobacteria

and Cyanobacteria. At the genus level, there are significant differences among *Candidatus_Saccharimonas*, *Lachnospiraceae*, *Enterorhabdus*, *Alloprevotella* and *Turicibacter*. See Fig.3A,3B.

Pseudo-sterile mouse verification

One week after antibiotic administration, fresh feces of normal mice and pseudo-sterile mice were taken for fecal smear and Gram staining respectively. The results showed that the number of microorganisms in feces of mice treated with antibiotics decreased obviously, but intestinal microorganisms could not be completely eliminated. See Fig.2A,2B.

Test of blood routine in pseudo-sterile mice

The results showed that the contents of RBC, WBC, HGB and HCT in the FMT model group were significantly decreased compared with those in the control FMT group ($P < 0.01$), which indicated that the model was successful. Compared with the FMT model group, the contents of RBC, WBC, HGB and HCT in the FMT Danggui Water Extract Group and the FMT Danggui Wuji Granule low-dose group showed an upward trend, and the difference of RBC content in the FMT Danggui Wuji Granule low-dose group was significant ($P < 0.05$). See Fig.2C,2D,2E,2F.

16S rDNA high-throughput sequencing analysis of mice transplanted with FMT.

Species composition analysis of FMT

Venn diagram was used to count the common and unique species in each group. The results showed that there were 99 species in FMT control group, FMT model group, FMT Angelica sinensis water extract group and FMT Danggui Wuji granule low-dose group, including 2 unique species in FMT control group and FMT model group, 1 unique species in FMT Angelica sinensis water extract group and 0 unique species in low-dose +FMT group respectively. See Fig.1G2.

Difference test between groups of diversity index of FMT

The results of diversity index difference test showed that the richness and diversity of intestinal flora in FMT model group decreased compared with that in FMT control group at OTU level, but the results were not significant. Compared with the FMT model group, there was no significant difference in the richness and diversity of intestinal flora between the water extract of Angelica sinensis +FMT group and the low dose group of FMT Danggui Wuji Granule, and the results of the low dose group of FMT Danggui Wuji Granule showed significant difference ($P < 0.05$). It is suggested that fecal bacteria transplantation can change the richness and diversity of intestinal community. See Fig.2G,2H,2I,2J,2K.

Principal component analysis of FMT

According to PCoA analysis, at the generic level, the $P = 0.001 < 0.05$, $R = 0.1916$ between FMT control group and FMT model group, Angelica sinensis water extract +FMT group and FMT Danggui Wuji granule

low dose group. $P < 0.05$ indicates that the reliability of this test is high, and a larger R indicates that the grouping has a high degree of explanation for the difference, that is, the grouping has a great influence on the difference. The results show that there are significant differences in intestinal microbial communities between FMT control group and FMT model group, Angelica sinensis water extract +FMT group and FMT Danggui Wuji Granule low dose group. See Fig.2L.

Community composition analysis (Bar diagram) of FMT

Through python-2.7 software analysis, the composition of community structure of different groups at the level of phylum and genus can be obtained. At the phylum level, compared with the FMT control group, the abundance of Bacteroidota and Patescibactriaia in the FMT model group decreased; compared with the FMT model group, the abundance of Actinobactota in the Angelica sinensis water extract +FMT group increased; the abundance of Bacteroidota in the low-dose FMT Danggui Wuji Granule group increased; and the abundance of Patescibactriaia in the Angelica sinensis water extract +FMT group and the low-dose FMT Danggui Wuji Granule group increased.

At the genus level, compared with the FMT control group, the abundance of *Muribaculacea* and *Clostridia* in the FMT model group decreased; Compared with the FMT model group, the abundance of *Alloprevotella* in the water extract of Angelica sinensis +FMT group increased, and the abundance of *Bacteroides* and *Alloprevotella* in the low dose group of FMT Danggui Wuji Granule increased. See Fig.2M,2N.

Analysis of Species Difference — Multi-group Comparison of FMT

Using R-3.3.1 (stat) software, the results of Kruskal-Wallis rank sum test show that there are significant differences between Actinobacteriota and Verrucomicrobiota at the phylum level. At the genus level, there are significant differences in the genera of *Lactobacillus*, *Clostridia*, *Lachnospiraceae*, and *Colidextribacter*. See Fig.3C,3D.

Discussion

Cyclophosphamide is an anti-tumor drug, also known as P-[N,N- bis (β -chloroethyl)]-1- oxygen - 3- nitrogen - 2- phosphazene -P- oxide. It is often used to treat malignant lymphoma, multiple myeloma, breast cancer and other diseases in clinic^[6], and it is also an immunosuppressant, so it often causes leukopenia in blood and immune function^[24]. Angelica sinensis is the dried root of *Angelica sinensis*(Oliv.)Diels of Umbelliferae. Danggui Wuji Granule is a health care product with the main effect of treating Aplastic anaemia, which has the effect of improving nutritional anemia.

In this experiment, cyclophosphamide was used to establish a blood deficiency model in mice. The results of peripheral hemogram showed that cyclophosphamide had a significant effect on red blood cells, white blood cells, HGB and HCT in peripheral hemogram of mice, which was significantly lower than those in control group, and significantly affected thymus index and spleen index of mice. Thymus is an

important lymphoid organ in vivo, and its function is closely related to immunity. It mainly produces T lymphocytes and produces and secretes thymosin and hormones. Spleen is the main hematopoietic organ of the body, and it is also an important lymphoid organ. Its main functions are to store blood, increasing blood volume, producing lymphocytes and make immunoglobulins. In the theory of traditional Chinese medicine, the spleen is the source of qi and blood biochemistry, and Jing Yue Quan Shu Xue says, "It comes from the source and is born in the spleen". The experimental results in this chapter show that compared with the control group, the thymus index and spleen index of the model group decreased, indicating that cyclophosphamide mainly acts on the immune and hematopoietic organs of the body, which leads to the decrease of RBC, WBC, HGB, HCT and other indexes in the peripheral blood picture. Compared with the model group, the RBC and WBC in each administration group showed an upward trend in different degrees, and the RBC and WBC of mice in the water extract group of *Angelica sinensis* and low-dose DGWJ Granule group were significantly increased ($P < 0.01$), indicating that *Angelica sinensis* and DGWJ Granule have certain therapeutic effects on Aplastic anaemia.

By detecting and comparing the intestinal flora of mice in control group, model group, *Angelica sinensis* water extract group and low dose DGWJ granule group, it was found that compared with the control group, the abundance of *Bacteroides*, *Patescibacteria*, *Actinobacillus* and *Desulfobacterium* decreased in model group, while the abundance of *Firmicutes* increased. At the level of genus, the abundance of *Muribaculaceae*, *Clostridium*, *Candidatus_Saccharimonas* and *Lachnospiraceae* in the model group decreased. Compared with the model group, at the level of genus, the abundance of *Muribaculaceae*, *Clostridium*, *Candidatus_Saccharimonas* and *Lachnospiraceae* in the water extract group of *Angelica sinensis* increased, while the abundance of *Muribaculaceae* and *Candidatus_Saccharimonas* in the low dose group of DGWJ Granules increased. Among them, members of *Muribaculaceae* are the main users of mucin monosaccharides, and a symbiotic bacterial community (including 5 strains) that can utilize sialic acid and n- acetylglucosamine can be constructed, which can effectively inhibit the growth of *Clostridium difficile* in vitro and prevent *Clostridium difficile* from colonization in mice treated with antibiotics^[18]. In the model group, the relative abundance of the genus decreased, but compared with the model group, the relative abundance of the genus increased in the water extract group of *Angelica sinensis* and the low dose group of DGWJ granules, indicating that the blood-enriching drugs may achieve the effect of treating Aplastic anaemia by regulating the abundance of *Muribaculaceae* bacteria in mice. *Lactobacillus* is a kind of common probiotics^[19]. The experimental results show that the relative abundance of *Lactobacillus* in the intestinal tract of the model group is higher than that of the control group, and the relative abundance of *Lactobacillus* in the intestinal tract of mice is decreased after giving low-dose *angelica sinensis* water extract group and DGWJ granule. Through literature study, it was found that the relative abundance of *Lactobacillus* increased when the iron content in iron deficiency anemia experiment^[14, 17, 18] was low, which indicated that the increase of relative abundance of *Lactobacillus* in the intestinal flora of experimental animals was a normal phenomenon under the condition of blood deficiency, which was consistent with previous studies.

The human intestine has the richest and most diverse microbial community in all body parts, and it is considered as the main part of the interaction between the host immune system and microorganisms, partly because of its size and complexity of microbial flora and the existence of special lymphatic structure in mucosa^[9]. At present, the attempt to manipulate intestinal microbial composition by fecal bacteria therapy has now become the focus of a large number of clinical case reports, and has achieved exciting results^[1].

• In order to verify whether taking blood-enriching drugs can treat the TCM syndrome of Aplastic anaemia through intestinal flora, pseudo-sterile mice were prepared by fecal bacteria transplantation experiment and compound antibiotic gavage. After the successful preparation of fecal smear, the feces of mice in the treatment group with Aplastic anaemia with remarkable curative effect in the early stage were used as raw materials to prepare bacterial liquid, which was used to ravage pseudo-sterile mice, and CTX was injected intraperitoneally in the last week to establish the Aplastic anaemia model. The results showed that the levels of red blood cells, white blood cells, HGB and HCT in the FMT model group were significantly lower than those in FMT control group ($P < 0.05$), suggesting that the model of Aplastic anaemia was successfully prepared. Compared with the FMT model group, the levels of red blood cells, white blood cells, HGB and HCT in the FMT Angelica sinensis water extract group increased, while the levels of red blood cells, HGB and HCT in the low-dose FMT DGWJ Granule group increased, indicating that the bacterial liquid prepared from the feces of mice in the Aplastic anaemia treatment group had therapeutic effect on mice with Aplastic anaemia, further verified that blood-enriching drugs could play a role in treating Aplastic anaemia by adjusting intestinal flora. 16S rDNA diversity analysis of intestinal flora showed that the composition and structure of intestinal flora in different FMT groups were inconsistent, and the abundance of *Muribaculacea* and *Clostridia* decreased in FMT model group. It is found that ginseng polysaccharide (GP) can increase the abundance of *Muribaculacea* to achieve the anti-tumor effect, indicating that this genus may be related to the aging mechanism, and may also be related to Aplastic anaemia^[12]. *Clostridia* is considered as an intestinal pathogen harmful to health^[7], and the abundance of *Clostridia* in FMT model group decreased, indicating that this genus may be the pathogen of Aplastic anaemia. Compared with FMT model group, the abundance of *Beppvonia* in FMT Danggui water extract group and the low dose FMT Danggui Wuji granule group increased. It was found that *Alloprevotella* was the main dominant microorganism in HFD (high-fat diet) and also a common beneficial bacterium^[4], which indicated that FMT Danggui water extract group and FMT Danggui Wuji Granule low-dose group could treat Aplastic anaemia by increasing the abundance of *Alloprevotella*.

Declarations

Authors' contributions

Zongyuan Li: Writing - original draft, Formal analysis, Investigation. **Shisheng Chen:** Conceptualization, Formal analysis, Investigation, Writing - review & editing. **Jianhui Sun:** Conceptualization, Investigation, Writing - review & editing. **Xiaomin Li:** Formal analysis, Writing - review & editing. **Liyu Hao:** Conceptualization, Methodology, Writing - review & editing. **Zeyue Yu:** Conceptualization, Formal analysis,

Writing - review & editing. **Jianliang Li**: Conceptualization, Formal analysis, Writing - review & editing. **Yurong Deng**: Conceptualization, Writing - review & editing. **Hanhui Huang**: Conceptualization, Writing - review & editing. **Hairu Huo**: Conceptualization, Writing - review & editing, Funding acquisition, Project administration, Supervision. **Hongmei Li**: Conceptualization, Funding acquisition, Project administration, Supervision.

Ethical approval and consent to participate All animals are provided by Beijing Weitong Lihua Experimental Animal Science and Technology Co., Ltd., with the animal production license number of SCXK (Beijing) 2016-0006. It is kept in the animal room of Institute of Traditional Chinese Medicine, Chinese Academy of Traditional Chinese Medicine, and is in a barrier. The animal license number is SYXK (Beijing) 2019-0003. The animal experiment involved in this paper was approved by the Animal Ethics Committee of Institute of Traditional Chinese Medicine, Chinese Academy of Traditional Chinese Medicine with the approval number of 2020B174.

Consent for publication All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work.

Funding This work was supported by the Scientific and technological innovation project of China Academy of Chinese Medical Sciences(Grant No. CI2021B015), and The Fundamental Research Funds for the Central Public Welfare Research Institutes (Grant No. ZZ15-WT-04, ZZ15-WT-08). All the authors of the manuscript are immensely grateful to their respective universities and institutes for their technical assistance and valuable support in the completion of this research project.

Conflict of interest No competing financial interests exist.

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Figures

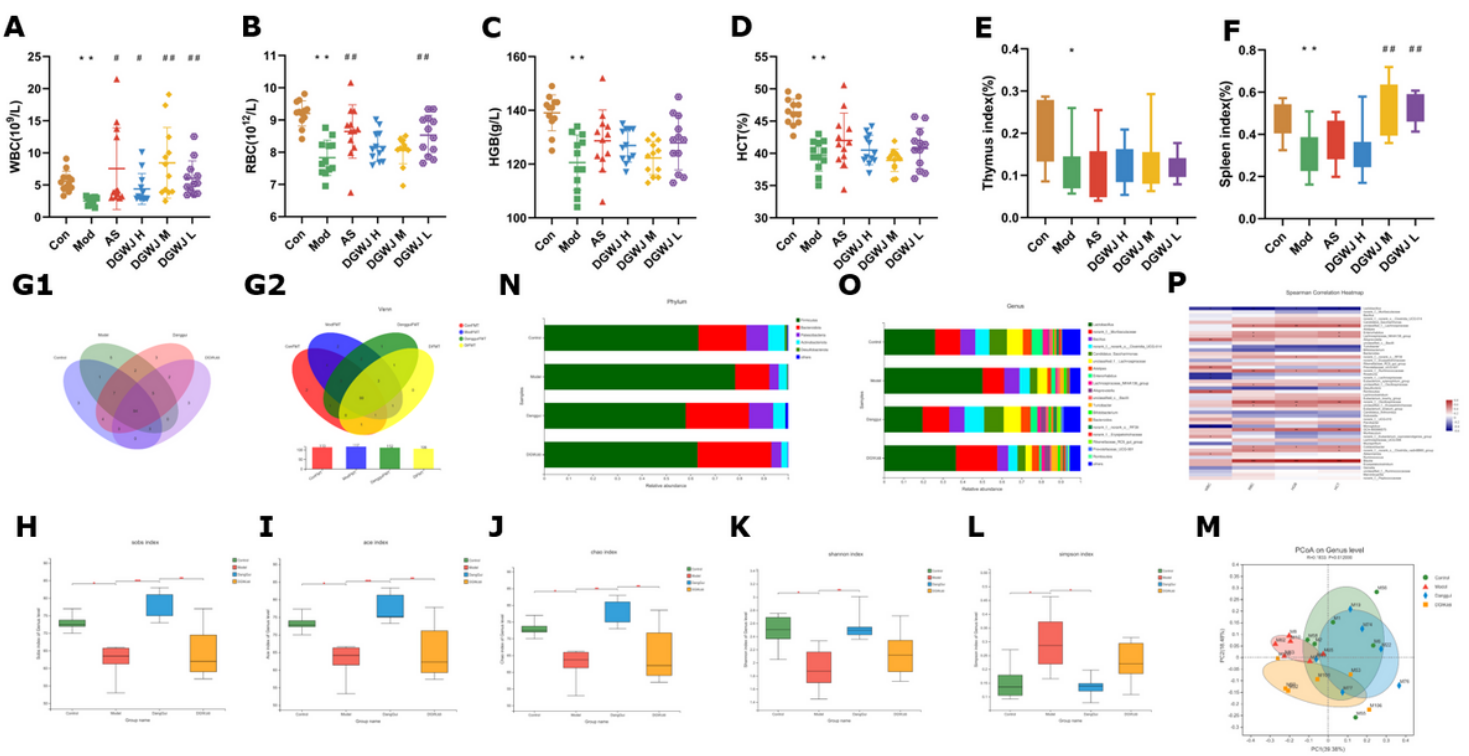


Figure 1

WBC level in each group(A); RBC level in each group(B); HGB level in each group(C); HCT level in each group(D); Thymus index level in each group(E); Spleen index level in each group(F); Venn diagram at genus level(G1); Venn diagram at genus level of FMT(G2); Sobs Index in each group(H); ACE Index in each group(I); Chao Index in each group(J); Shannon Index in each group(K); Simpson Index in each group(L); PCoA plot (Genus levels)(M); Species distribution of gut microbiota in each group of mice at the

phylum level(N); Species distribution of the intestinal flora of mice in each group at the genus level(O); Heatmap of environmental factors(P).

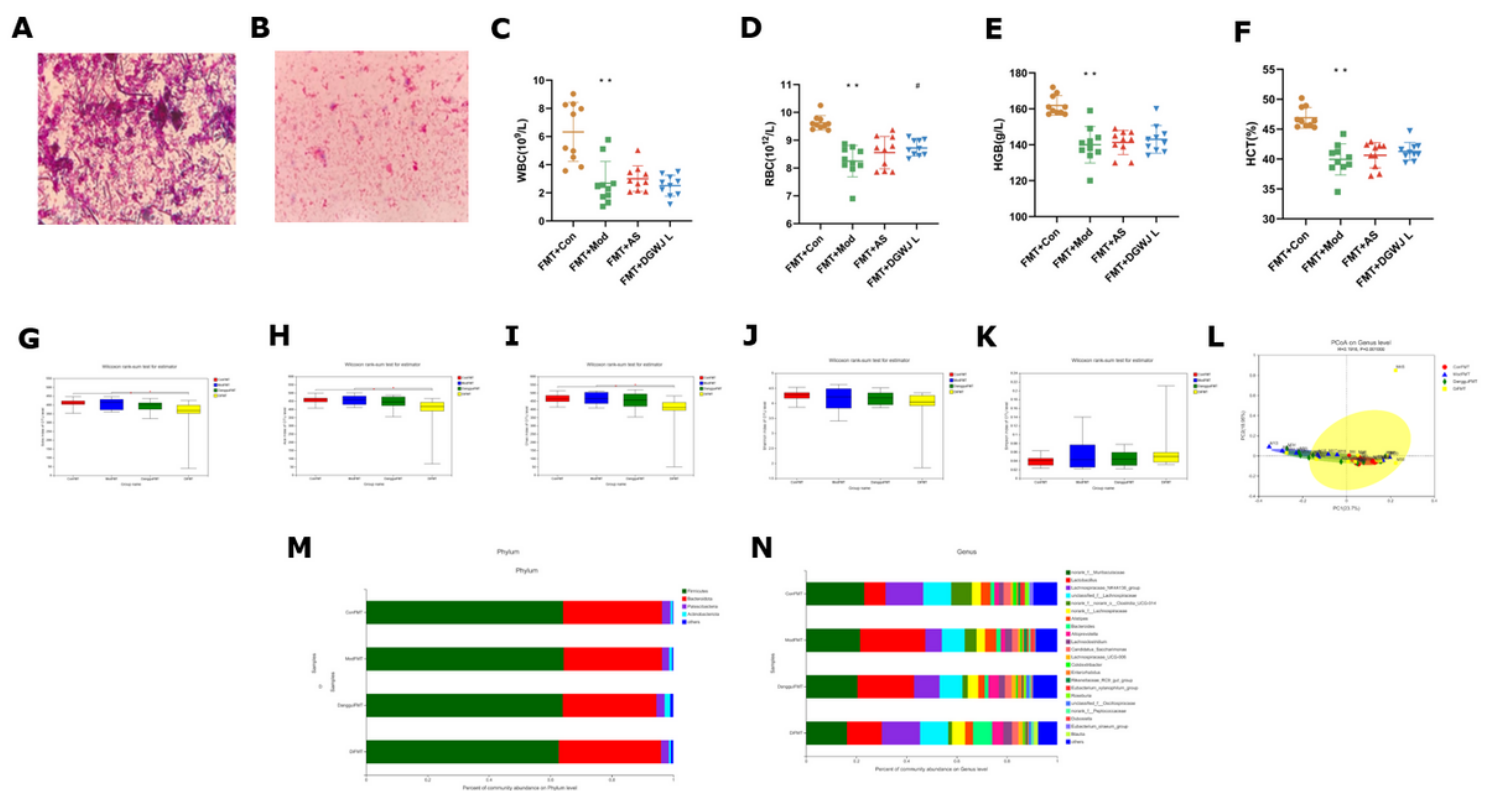
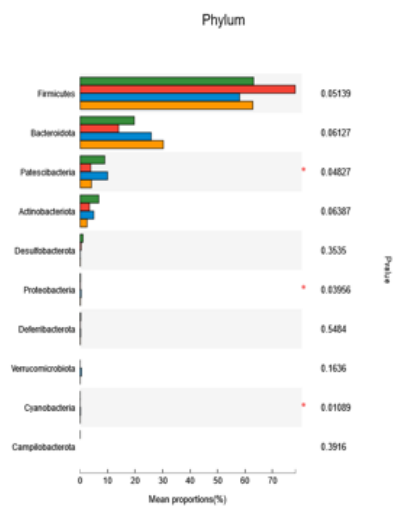


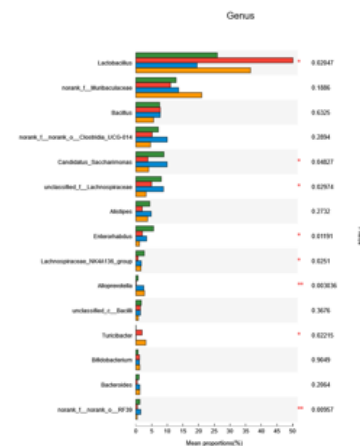
Figure 2

Gram stain of mouse feces in control group(A); Gram stain of mouse feces after antibiotic intervention(B); WBC level in each group of FMT(C); RBC level in each group of FMT(D); HGB level in each group of FMT(E); HCT level in each group of FMT(F); Sobs Index in each group of FMT (G); ACE Index in each group of FMT (H); Chao Index in each group of FMT (I); Shannon Index in each group of FMT (J); Simpson Index in each group of FMT (K); PCoA plot of FMT(Genus levels)(L); Species distribution of gut microbiota in each group of mice at the phylum level(M); Species distribution of the intestinal flora of mice in each group at the genus level(N).

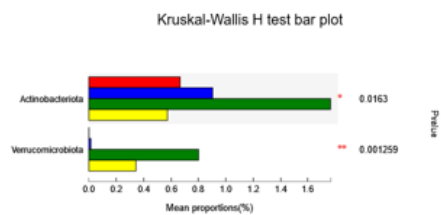
A



B



C



D

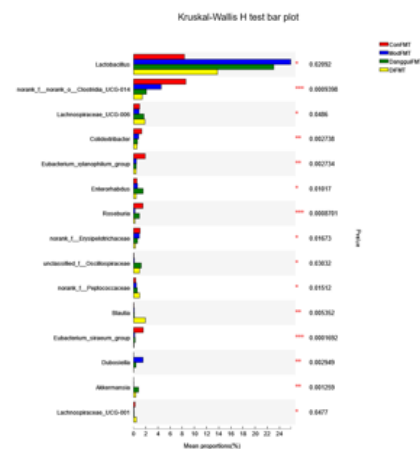


Figure 3

Comparison chart of multiple groups at Phylum level(A); Comparison of multiple groups at the Genus level(B); Comparison chart of multiple groups at Phylum level of FMT(C); Comparison of multiple groups at the Genus level of FMT(D).