Ind. J. Pharm. Edu. Res., 2026; 60(4):1636-1645.
https://www.ijper.org

Original Article

Antipyretic Effect of Geniposide in Dry Yeast-Induced Pyrexia Rats via Intervening the Compositions of Gut Microbiota

Xiao-lin Li[1,2], Sheng-fu Chen[4], Tian-long Liu[2], Ren-jie Wang[1], Rong Wang[2,*], Mao-Xing Li[1,3,5,*]

[1]College of Pharmacy, Gansu University of Chinese Medicine, Lanzhou, CHINA.
[2]Department of Pharmacy, the 940th Hospital of Joint Logistic Support Force, Gansu Plateau Pharmaceutical Technology Center, Lanzhou, CHINA.
[3]Department of Pharmaceutical Sciences, Beijing Institute of Radiation Medicine, Beijing, CHINA.
[4]Department of Pharmacy, Sanya People's Hospital, Sanya, CHINA.
[5]National Key Laboratory of Kidney Diseases, Beijing Institute of Radiation Medicine, Beijing, CHINA.

ABSTRACT

Aim: Geniposide is the main active ingredient in *Gardenia jasminoides* Ellis, but its antipyretic mechanism is still unclear. **Materials and Methods:** To study the change of the gut microbiota in febrile rats with dry yeast-induced and antipyretic of geniposide based on 16SrDNA sequencing, 24 male screening SD rats were randomly and equally divided into normal control group (NG, Sterile water for injection, 10 mL/kg); fever model group (MG, Sterile water for injection, 10 mL/kg); Geniposide group (GG, 100 mg/kg); Aspirin group (AG, 100 mg/kg). The MG, GG, and AG were prepared the fever model with dry yeast. After 5 hr of subcutaneous application with 20% dry yeast suspension (10 mL/kg) to the back neck, the drug was given by intragastric administration. After 5 hr of administration, the colon contents of rats were collected, and the changes in gut microbiota composition and species abundance were detected by 16SrDNA sequencing technology. **Results:** Fever significantly upregulates *Bacillis, Lactobacillales, Lactobacillus, Lachnospiracea incertae sedis, Lactobacillus apodemi* and *Eubacterium uniforme* in rats, whereas fever significantly reduced *Coprobacillus, Prevotellaceae Coprobacillus cateniformis* and *Oscillibacter ruminantium.*, The results were reversed after the administration of geniposide. **Conclusion:** Collectively, our study showed that the compositions of gut microbiota in febrile rats induced by dry yeast were change, and geniposide regulated the gut microbiota in febrile rats.

Keywords: Geniposide, Gut Microbiota, 16S rDNA, Antipyretic.

Correspondence:
Prof. Mao-xing Li
[1]College of Pharmacy, Gansu University of Chinese Medicine, Lanzhou, CHINA.
[2]Department of Pharmaceutical Sciences, Beijing Institute of Radiation Medicine, Beijing, CHINA.
[3]National Key Laboratory of Kidney Diseases, Beijing Institute of Radiation Medicine, Beijing, CHINA.
Email: limaox2020@aliyun.com

Prof. Rong Wang
Department of Pharmacy, the 940th Hospital of Joint Logistic Support Force, Gansu Plateau Pharmaceutical Technology Center, Lanzhou, CHINA.
Email: wangrong-69@163.com

Received: 28-04-2025;
Revised: 09-06-2025;
Accepted: 18-08-2025.

INTRODUCTION

Traditional Chinese Medicine (TCM) has played a unique role in protecting the Chinese people against numerous epidemic diseases. TCM has also developed a characteristic theory that guides TCM in selecting the proper medications for the treatment of various ailments. Two major classes of TCM medications are antipyretics (heat-clearing) and diaphoretics (exterior-releasing), which are frequently utilized to treat a variety of inflammatory and infectious disorders. *Gardenia jasminoides* Ellis, is a popular shrub in the Rubiaceae family.[1] The desiccative ripe fruits of this plant have been applied in China for centuries as one of the first pharmaceutical or food resources. It has a bitter taste and cold nature, primarily affecting the heart, lung, and sanjiao meridians. As a traditional and crucial herbal medicine, *G. jasminoides* is recorded in the Chinese pharmacopeia, with the effects of reducing fever, removing irritability, promoting urination, and detoxifying the blood.[2] Clinically, Gardenia is often combined with other herbs to treat various heat-related illnesses, forming classic prescriptions such as Zhizi Jinhua Tang (Gardenia and Chrysanthemum Decoction), Zhike Zhizi Tang (Gardenia and Bitter Orange Decoction), Zhizi Houpu Tang (Gardenia and Magnolia Bark Decoction), and Zhizi Douchi Tang (Gardenia and Fermented Soybean Decoction).[3] Modern pharmacological research has conducted thorough explorations into the antipyretic effects and mechanisms of Gardenia through various rat fever models and metabolomics studies. Plasma metabolomics research has revealed that Gardenia could develop an antipyretic role by regulating the metabolism of valine, leucine, and isoleucine.[4] In particular, geniposide, a component of the iridoid glycosides, has been identified as the main bioactive constituent responsible for

DOI: 10.5530/ijper.20260479

Gardenia's antipyretic activity; furthermore, anti-inflammatory effects are one of the mechanisms underlying the antipyretic pharmacological actions of geniposide.[5]

For both basic science and clinical practice, the critical role in bridging of gut microbiota in preserving human physiological health and controlling the pharmacological effects of different medications offers a novel perspective. A growing body of research has demonstrated that the manipulation of the gut microbiota is substantially responsible for the *in vivo* efficacy of many medications, including TCM therapies. For instance, the modification of microbiota-gut-brain is a major factor in the antidepressant pleiotropic effects of Zhi Zi Chi decoction;[6] geniposidic acid, which is a bioactive compound isolated from *Gardenia jasminoides* Ellis (*Rubiaceae*), could alleviate gut microbiota dysbiosis in mice with colitis;[7] *Gardeniae* Fructus in ameliorating rheumatoid arthritis dependent on the *Streptococcus, Facklamia, Klebsiella, Enterococcus*, and *Kosakonia* were the critical gut microorganisms;[8] simultaneously, *Gardenia jasminoides* could restoration of the intestinal microbiome composition of antibiotic-associated aggravation of DNCB-induced atopic dermatitis.[9] These researches indicate that the gut microbiota is a decisive mediator of the pharmaco-mechanism of Gardenia, providing a significant development for interpreting the traditional theories of Gardenia.

In recent years, targeted therapy aimed at the intestinal microbiota has become a research focus for cold and bitter herbs and formulations in traditional Chinese medicine.[10-14] Dysbiosis of the gut microbiota is closely associated with febrile conditions in the body. This study used dry yeast to construct the febrile rat model, and then conducted 16S rDNA sequencing analysis to investigate characteristics of gut microbiota in rats after fever. Meanwhile, the effect of geniposide on gut microbiota in febrile rats was studied to elucidate the antipyretic mechanism of geniposide.

MATERIALS AND METHODS

Drugs, Reagents, and Instruments

Geniposide (purity: 98%, Shaanxi Huinengda Biotechnology Co., Ltd., China), dry yeast (Angel yeast Co., Ltd, China) and enteric-coated aspirin (Hunan Xinhui Pharmaceutical Co., Ltd., China) were used in the present study.

MagPure Stool DNA KF Kit B (Shanghai Magen Biotechnology Co., Ltd., China), Qubit™ dsDNA BR Assay Kit (Invitrogen, USA), 2×Phanta Max Master Mix (Nanjing Vazyme Biotechnology Co., Ltd., China); DNA sorting magnetic beads (BGI, China); MiSeq Reagent Kit v3 (600-cycle) (Illumina, USA), DT102 Medical thermometer(Guangzhou Berrcom Medical Device Co., Ltd, China), JXFSTPRP-48 Automatic sample rapid grinder (Shanghai Jingxin Industrial Development Co., Ltd., China), 5355Thermomixer comfort (Eppendorf, Germany); 5417R Centrifuge (Eppendorf, Germany); King Fisher Flex Magnetic Bead extractor (Thermo Fisher, USA); Gemini XPS Microplate Reader (Molecular Devices, USA); PCR instrument (Bio-Rad, USA); Mi Seq sequencing platform (Illumina, USA) were all used in this study.

Preparation and Grouping of Animal Models

The study utilized thirty-two male Sprague-Dawley (SD) rats, around 6-7 weeks of age and 200-220 g in weight, which purchased SPF (Beijing) Biotechnology Co., Ltd., (Animal License Number: SCXK (Beijing) 2019-0010). The animals feeding environment temperature was maintained at 24±1°C and the environment humidity was maintained at 45±15%.

The 24 rats that passed the basic body temperature screening (For 3 days, the rats were tested for adaptive temperature three times a day. Before modeling, the rats were tested for twice prior. Each test was at 1 hr intervals each time. Rats that had two temperature readings that differed by more than 0.5°C and a single temperature exceeding 38°C were discarded)[5] were randomly divided into four groups (n=6): normal control group (NG, Sterile water for injection, 10 mL/kg); fever Model Group (MG, Sterile water for injection, 10 mL/kg); Geniposide group (GG, Geniposide,100 mg/kg); Aspirin group (AG, Aspirin, 100 mg/kg). The MG, GG, and AG group were preparation of fever model with dry yeast (The model preparation method and the temperature measurements were referred to the article of the research group).[5] Following 5 hr of modeling, the medication was administered intragastrically to the rats. Ultimately, the temperature was measured at every point in time, and the maximum temperature rise △t was computed (△t=temperature at every point in time-basal body temperature).[5] There were no dead animals in sight during the modeling phase. The current study's animal experiments were all conducted pursuant to applicable national guidelines and standards for animal ethics and welfare (No. 2021KYLL118, authorized on June 4, 2021).

Animal Sample Materials

After 5 hr of administration, 1-2 mL of colonic contents samples were collected with sterile forceps and stored in sterile cryopreservation tubes, frozen for 30 min with liquid nitrogen, then at -80°C for subsequent DNA extraction and bacterial flora detection.

16S rDNA Sequencing

To configure the PCR reaction system, each qualified genomic DNA sample of 30 ng and corresponding fusion primers were selected. For PCR amplification, PCR reaction parameters were established. The PCR amplification products were purified using DNA sorting magnetic beads and then dissolved in Elution Buffer. Library was constructed, and measured the fragment range and concentration of the library by using a Qubit™ dsDNA BR Assay

Kit. After the library passed quality inspection, 16S rDNA gene V3-V4 sequencing was carried out on an MGISEQ2000 device.

Sequence Analysis

Sequence splicing, filtering, and dechimerization were used to get the optimal sequences following the download of the original sequencing data and the completion of quality control procedures. Unassembleable reads were eliminated. USEARCH (v7.0.1090_i86linux32) was used for the sequence analysis, and sequences with similarity≥97% were classified under the same Operational Taxonomic Units (OTUs). The Green Gene Database was utilized to annotate taxonomic information based on RDP classifier for each representative sequence for each OUT. The results of the clustering were used to run alpha and beta diversity studies. Furthermore, the annotation results might be used to derive categorization information at each level as well as perform a correlation analysis on the variations in sample composition and community structure. The Shenzhen BGI Technology Service Co., Ltd., helped with the sequencing.

Statistical Methods

The temperature results were statistically analyzed using SPSS 22.0 statistical software and expressed as the Mean±SD deviation. R software (v3.4.10) was used to conduct the sequence statistical analysis. $p<0.05$ was defined indicated statistically significant results.

RESULTS

Antipyretic effect of geniposide on dry yeast-induced pyrexia rats

Figure 1a depicts the temperature differences of four groups of rats at different time points. After modeling for 5 hr, the temperature in the rats of MG, GG and AG groups considerably rose ($p<0.01$) compared with the NG. But the temperature of the GG dropped statistically significantly at 3 hr, 4 hr, 5 hr after administration compared with the MG ($p<0.01$, $p<0.05$, $p<0.05$). The AG dropped statistically significantly at 1 hr, 2 hr, 3 hr, 4 hr, 5 hr after administration compared with the MG ($p<0.01$).

OTU Cluster Abundance Analysis

To generate OTU data, six samples were sequenced in each group. The resulting data is displayed in a Flower diagram (Figure 1b). 958 OTUs were identified and 855 of which were detected in all four groups after leveling. As shown in the species cumulative box diagram (Figure 1c) and the rank abundance curves (Figure 1d), with an increase in the number of test samples, the curves gradually tend to be flat, evenly distributed and concentrated, with sufficient sampling, which can be used for subsequent data analysis.

Alpha Diversity Analysis

The rarefaction curves in Figure 1g show a sharp increase and gradually stable, indicating that the sequencing data is reasonable and sufficient to reflect the majority of microbial information in the sample, indicating that the sequencing depth can cover the species composition. This indicated that the intestinal microbial diversity had been fully detected. Based on the alpha diversity indices of the four groups (Figure 1g), there are no significant differences between observed species, chao1, sobs, ace, shannon and simpson of the normal blank group and the fever model group ($p>0.05$). Furthermore, there are also no discernible variations exits between the fever model group and the geniposide group ($p>0.05$).

The beta diversity box diagram based on unweighted_unifrac

Based on the 16S rDNA sequencing results to analyze the bacterial communities in each group beta diversity analysis, beta diversity reflects the similarity in the composition of microbial communities among different groups. This study used the unweighted-unifrac index to measure the coefficient difference between two groups of samples. Figure 1e illustrates that the microbial community structure composition of the FG differs significantly ($p<0.01$) from that of the NG. However, compared with the MG, there is no significant difference in the microbial community structure composition between the GG and the AG.

Partial Least Squares Discrimination Analysis (PLS-DA)

The PLS-DA (Figure 1f) indicates that the horizontal axis represents the key factor for the separation of the MG and the NG. But the GG and the MG were separated in the vertical axis direction; it can be considered that the factor represented by the vertical axis is the main factor causing the division of the two groups.

Analysis of the Structural Composition of Gut Microbiota

We can understand composition status of gut microbiota at the phylum, class, order, family, genus and species levels by histogram (Figure 2).

Analysis of species differences at the phylum level

At the phylum level, Firmicutes(NG: 69.201313%, MG: 60.674374%, GG: 60.199853%, AG: 62.99969%), Bacteroidetes(NG: 24.962665%, MG: 32.811014%, GG: 31.056786%, AG: 27.808374%), Proteobacteria(NG: 2.660313%, MG: 3.266001%, GG: 5.659462%, AG: 3.004115%), Spirochaetes(NG: 0.251189%, MG: 0.474482%, GG: 1.052243%, AG: 1.388414%), Actinobacteria, *Candidatus Saccharibacteria*, Elusimicrobia, Deferribacteres, Tenericutes (NG: 0.000373%, MG:

0.033264%, GG: 0.094327%, AG: 0.029485%) and Fusobacteria were the top ten phyla in the gut microbiota. Compared with the NG, the proportion of Tenericutes of the MG was increased significantly ($p<0.05$). Compared with the MG, the proportion of Tenericutes of the geniposide group was increased.

Analysis of species differences at the class level

Clostridia, Bacteroidia, *Erysipelotrichia, Negativicutes, Deltaproteobacteria, Epsilonproteobacteria, Spirochaetia, Bacilli*, Betaproteobacteria and Actinobacteria were the top ten classes in the gut microbiota. Compared with the NG, the proportion of *Epsilonproteobacteria* ($p<0.05$) and *Bacillis* ($p<0.01$) of the MG was increased significantly, Deltaproteobacteria was reduced. The proportion of *Epsilonproteobacteria* and Deltaproteobacteria ($p<0.01$) in the GG was higher than that of the MG. The proportion of Bacillis in the geniposide group was significantly reduced than that of the MG ($p<0.05$).

Analysis of species differences at the order level

At the order level, *Clostridiales, Bacteroidales, Erysipelotrichales, Selenomonadales, Desulfovibrionales, Campylobacterales, Spirochaetales, Lactobacillales, Burkholderiales* and *Bifidobacteriales* were the top ten order in the gut microbiota. The proportion of *Campylobacterale* ($p<0.05$) and *Lactobacillales* ($p<0.01$) of the MG was higher significantly than that in the NG, the proportion of Deltaproteobacteria of the MG was reduced. The proportion of *Campylobacterale* and *Desulfovibrionales* ($p<0.05$) of the GG was higher than that in the MG, whereas Lactobacillales was significantly lower ($p<0.05$).

Analysis of species differences at the family level

At the family level, *Ruminococcaceae, Lachnospiraceae, Prevotellaceae, Porphyromonadaceae, Eubacteriaceae, Erysipelotrichaceae, Acidaminococcaceae, Rikenellaceae, Desulfovibrionaceae* and *Peptostreptococcaceae* were the top ten family in the gut microbiota. The proportion of *Porphyromonadaceae* ($p<0.05$) in the MG was significantly higher than that of compared to the normal control group, *Desulfovibrionaceae* was reduced. Compared with the MG, the proportion of *Porphyromonadaceae* of the geniposide group was reduced, *Desulfovibrionaceae* ($p<0.05$) was increased significantly.

Analysis of species differences at the genus level

At the genus level, *Prevotella, Clostridium*_XlVa、*Ruminococcus, Barnesiella, Eubacterium, Phascolarctobacterium, Allobaculum, Oscillibacter, Alistipes* and *Pseudoflavonifractor* were the top ten genus in the gut microbiota. Compared to the NG, the MG had a significantly greater proportion of *Barnesiella* (p<0.05). were increased, *Oscillibacter* and *Pseudoflavonifractor* were reduced. Compared with the MG, the proportion of *Barnesiella* (p<0.05) was reduced ,*Oscillibacter* (p<0.01) and *Pseudoflavonifractor* (p<0.05) was increaesd significantly.

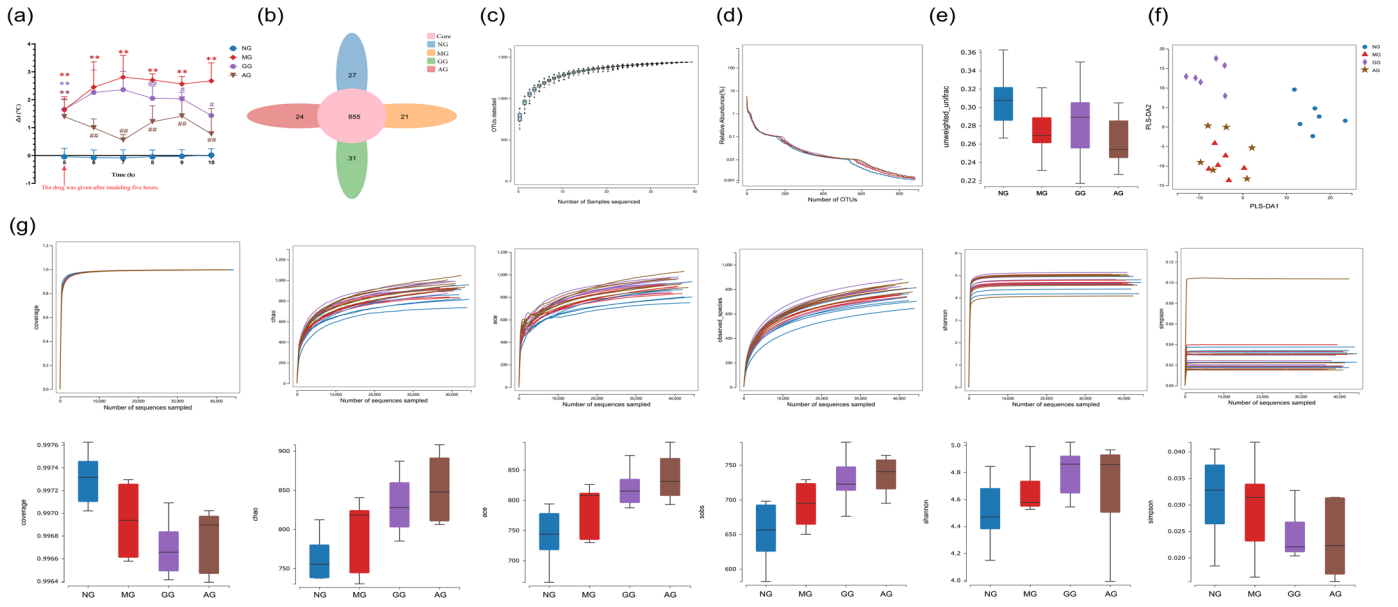

Figure 1: Antipyretic effect of geniposide in dry yeast-induced pyrexia rats and the analysis of alpha diversity and beta diversity. (a) Antipyretic effect of geniposide in dry yeast-induced pyrexia rats. Data are presented as means±SD (n=6 rats per group). (b) The flower diagram of the distribution of OTUs in the four groups. (c) The species cumulative box plot of OTUs. (d) The rank abundance curves of OTUs. (e)The species cumulative box plot of beta diversity. (f)PLA-DA of beta diversity. (g)Rarefaction curve of alpha diversity and the species cumulative box plot of alpha diversity. Note：Compared with normal control group: **p<0.01 and *p<0.05; compared with fever model group: ##p<0.01 and #p<0.05.

Analysis of species differences at the species level

At the species level, *Prevotella copri, Barnesiella intestinihominis, Eubacterium coprostanoligenes, Phascolarctobacterium succinatutens, Allobaculum stercoricanis, Ruminococcus champanellensis, Pseudoflavonifractor capillosus, Flavonifractor plautii, Romboutsia sedimentorum* and *Ruminococcus flavefaciens* were the top ten genus in the gut microbiota. The proportion of dominant genus in each group is shown in. There was no significant difference among the top ten species.

At the species level with low relative abundance (non-top ten species)(Figure 3a), Compared with the NG, the proportion of *Clostridium disporicum, Lactobacillus apodemi, Turicibacter sanguinis, Lactobacillus acidophilus, Coprobacillus cateniformis, Bifidobacterium pseudolongum, Helicobacter ganmani, Clostridium methylpentosum, Eubacterium uniforme, Aerococcus viridans, Anaeroplasma abactoclasticum, Parabacteroides merdae, Parabacteroides distasonis, Enterorhabdus caecimuris* and *Oscillibacter ruminantium* of the MG had significant differences in relative abundance (p<0.05). Compared with the MG, the proportion of *Lactobacillus apodemi, Oscillibacter valericigenes, Eubacterium uniforme, Anaerotruncus colihominis, Clostridium viride, Pseudomonas fragi, Ruminococcus albus, Barnesiella intestinihominis, Pseudoflavonifractor capillosus, Acinetobacter johnsonii, Papillibacter cinnamivorans, Parabacteroides goldsteinii, Coprobacillus cateniformis, Oscillibacter ruminantium, Butyricicoccus pullicaecorum* of the GG had significant differences in relative abundance (p<0.05). The increases and decreases in relative abundance of gut microbiota could be shown in the Figure 3.

Figure 3c showed the UPGMA clustering tree and abundance combination chart for different groups at the species level. The left side shows the results of the UPGMA clustering tree, and the right side shows the species abundance bar diagram. Samples located under the same branch have shorter branch lengths between samples, resulting in more similar species composition and higher similarity between the two samples. The six samples in each group showed a good degree of clustering according to the results.

LEfSe Analysis (the genus level)

LEfSe analysis was to further evaluate variations in community structure among groups and pinpoint the species with significant differences at the genus level. The relative abundance of the six genera at the genus level of the fever model group showed significant differences compared to the NG. The abundances of 27 genera in the geniposide group were clearly different from those observed in the MG (Figure 4a and 4b).

Spearman correlation analysis

Based on the rank sum test, different species at the species level are obtained, and the spearman correlation heat map is drawn by R software. The results showed that after modeling (Figure 4c), *Prevotellamassilia timonensis* had a significant negative correlation with *Ruminococcus flavefaciens*, and a significant positive correlation with *Romboutsia timonensis*. There was a negative correlation between *Phascolarctobacterium succinatutens* and *Flintibacter hominis*, and a significant negative correlation with *Ruminococcus champanellensis. Solibaculum mannosilyticum* had a significant negative correlation with *Segatella hominis* and *Parasutterella excrementihominis*, and a

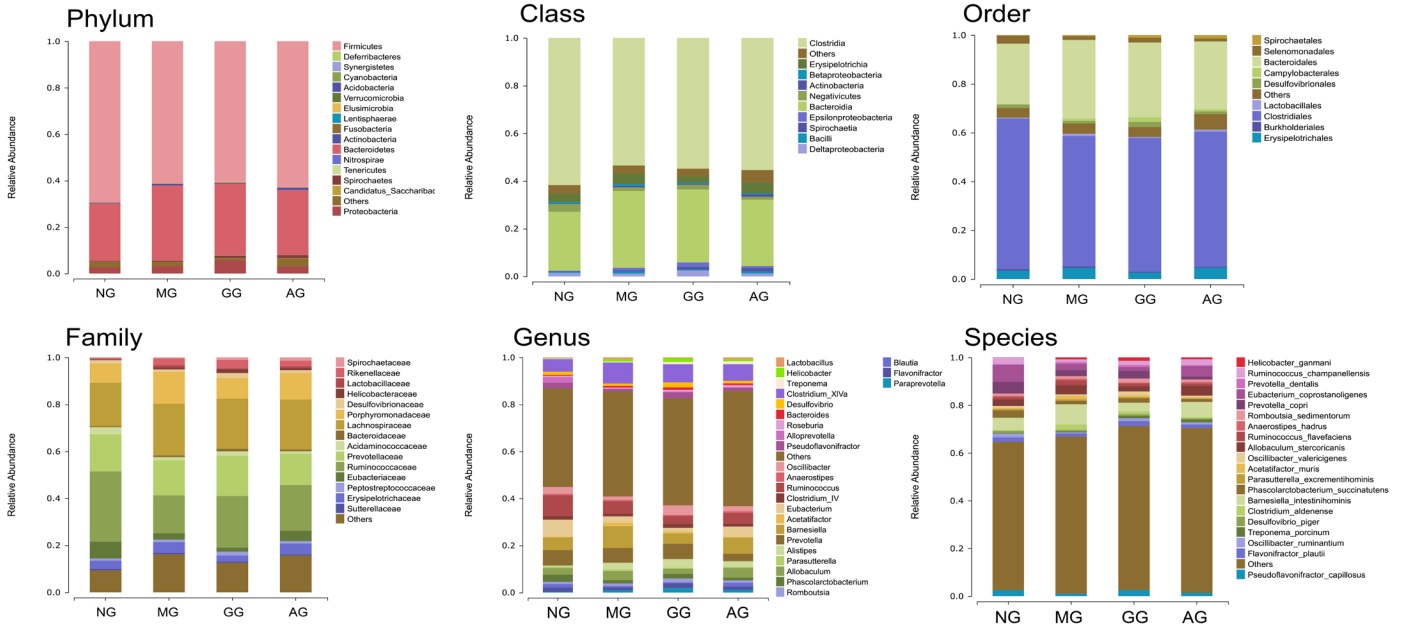

Figure 2: Histogram of species composition at the phylum, class, order, family, genus, and species levels. n=6.

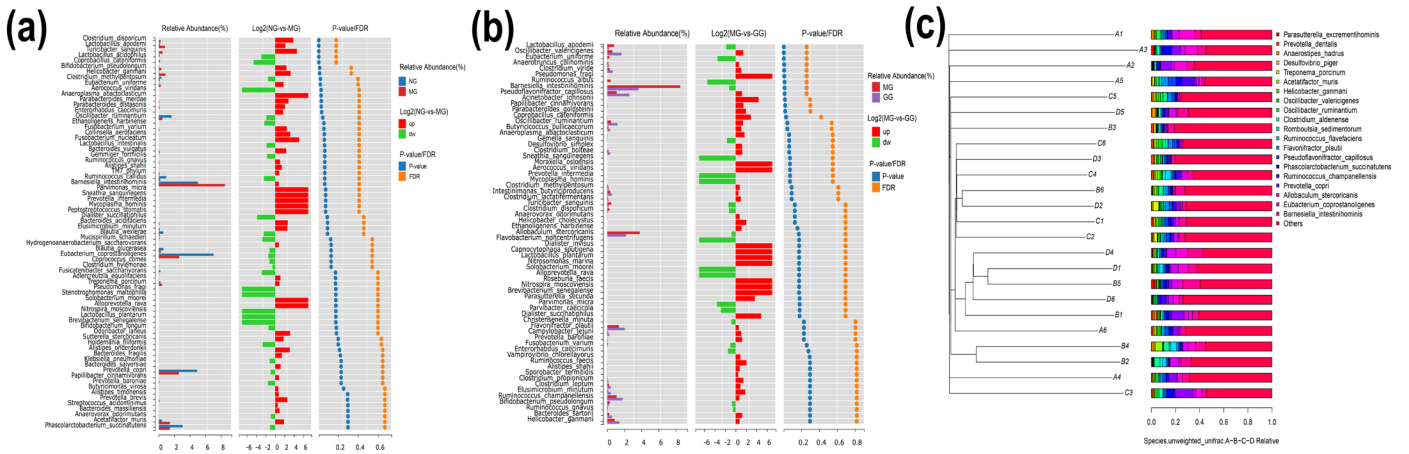

Figure 3: Analysis of species differences and UPGMA taxonomy tree and abundance combination chart at the species level. (a)Analysis of species differences with relatively low relative abundance at the species level between NG and MG. (b)Analysis of species differences with relatively low relative abundance at the species level between MG and GG. (c)UPGMA taxonomy tree and abundance combination chart (the species level). *n*=6. (A) normal control group; (B) fever model group; (C) Geniposide group; (D) Aspirin group.

significant positive correlation with *Flintibacter butyricus* and *Ruminococcus albus*. There was a negative correlation between *Ruminococcus flavefaciens* and *Duncaniella muris*, and a significant positive correlation with *Parasutterella excrementihominis*. There was a positive correlation between *Segatella hominis* and *Flintibacter butyricus. Flintibacter hominis* had a significant negative correlation with *Roseburia hominis*, and a significant positive correlation with *Ruminococcus champanellensis*. There was positive correlation between *Romboutsia timonensis* and *Kineothrix alysoides*.

The results showed that after treatment (Figure 4d), *Segatella copri* had a significant negative correlation with *Flintibacter butyricus* and *Intestinimonas butyriciproducens*. Duncaniella muris had a significant negative correlation with *Acetatifactor muris* and *Faecalibaculum rodentium. Phascolarctobacterium succinatutens* had a significant positive correlation with *Kineothrix alysoides, Romboutsia timonensis, Flintibacter butyricus*, and *Solibaculum mannosilyticum. Ruminococcus flavefaciens* had a significant negative correlation with *Allobaculum stercoricanis*, had a significant positive correlation with *Intestinimonas butyriciproducens*. There was a negative correlation between *Romboutsia timonensis* and *Treponema peruense*. There was a negative correlation between *Flintibacter hominis* and *Parasutterella excrementihominis*, and a significant positive correlation with *Duncaniella dubosii*. There was a negative correlation between *Phocaeicola sartorii* and *Faecalibaculum rodentium*.

PICRUSt analysis

We conducted PICRUSt analysis to investigate the functional profiles of the gut microbiota. A total of 29 pathways, including Carbohydrate metabolism, Environmental information processing, Genetic information processing and others. Secondary bile acid biosynthesis, Biosynthesis of vancomycin group antibiotics, Primary bile acid biosynthesis, Sphingolipid metabolism, Galactose metabolism, RNA transport, Streptomycin biosynthesis, N-Glycan biosynthesis, Staphylococcus aureus infection, Amino sugar and nucleotide sugar metabolism, Endocytosis, Carotenoid biosynthesis, Penicillin and cephalosporin biosynthesis were significantly more abundant in MG than in NG ($p<0.05$), Linoleic acid metabolism and Insulin signaling pathway were significantly lower abundant in MG than in NG ($p<0.05$). Amino sugar and nucleotide sugar metabolism, Fructose and mannose metabolism, Galactose metabolism, Nucleotide excision repair, Mismatch repair, Homologous recombination, Ascorbate and aldarate metabolism, Atrazine degradation, Cell cycle-Caulobacter, Streptomycin biosynthesis, Staphylococcus aureus infection were significantly lower abundant in GG than in MG ($p<0.05$). Cyanoamino acid metabolism, Styrene degradation, Valine, leucine and isoleucine degradation, Butanoate metabolism, Amoebiasis were significantly more abundant in GG than in MG ($p<0.05$).

DISCUSSION

Our experiments used 16S rDNA sequencing to screen the diversity of gut microbiota in 24 samples of rats, and compared the measured samples through alpha diversity analysis, beta diversity analysis, and inter group significance analysis to screen for differential gut microbiota of febrile rats after drug intervention to assess the impact of variation in gut microbiota. Through the analysis of gut microbiota, we found remarkable change in the composition and abundance of gut microbiota between the fever model group and the normal control group, and confirmed the intrinsic connection between fever and gut microbiota. The results of LEfSe analysis in the fever model group showed that the structure of gut microbiota changed after induced by dry yeast, and the gut microbiota of geniposide model

group underwent different structural and compositional changes after administration.

The findings of the alpha diversity analysis demonstrate that the diversity of gut microbiota of rat when treated with drugs remain unchanged. The gut microbiota richness of the fever model prepared by 20% dry yeast and the intervention of geniposide (100 mg/kg) administration after intervention does not differ significantly. The beta diversity analysis results have shown that there are dissimilarities in gut microbiota between different treatment groups, and the inter group difference was larger than the intra group difference.

Compared with that in the normal control group, the relative abundance of *Tenericutes* significantly increased at the phylum level. Compared with that in the normal control group, the relative abundance of *Bacilli, Epsilonproteobacteria, Mollicutes* significantly increased at the class level. Compared with that in the normal control group, the relative abundance of *Lactobacillales, Campylobacterales, Bifidobacteriales, Anaeroplasmatales* significantly increased at the order level. Campylobacteriosis primarily manifests with gastrointestinal symptoms, including diarrhea, abdominal cramping and pain, nausea, and vomiting. Additionally, it may present with fever and fatigue. Compared with that in the normal control group, the relative abundance of *Ruminococcaceae* significantly reduced at the family level. Compared with that in the normal control group, the relative abundance of Porphyromonadaceae, *Rhodospirillaceae, Bifidobacteriaceae, Clostridiaceae, Anaeroplasmataceae, Alcaligenaceae, Helicobacteraceae, Lactobacillaceae* significantly increased at the family level. Please refer to the supplementary materials for specific data. Compared with that in the normal control group, the relative abundance of *Mogibacterium, Alloprevotella, Coprobacillus, Aerococcus* significantly reduced at the genus level. Compared with that in the normal control group, the relative abundance of *Turicibacter, Enterorhabdus, Helicobacter, Anaeroplasma, Lachnospiracea incertae sedis, Olsenella, Parabacteroides, Clostridium sensu stricto, Coprococcus, Alloprevotella, Bifidobacterium, Barnesiella* significantly increased at the genus level. Compared with that in the normal control group, the relative abundance of *Oscillibacter ruminantium, Aerococcus viridans, Lactobacillus acidophilus, Coprobacillus cateniformis* significantly reduced at the species level. Compared with that in the normal control group, the relative abundance of *Clostridium disporicum, Lactobacillus apodemi, Turicibacter*

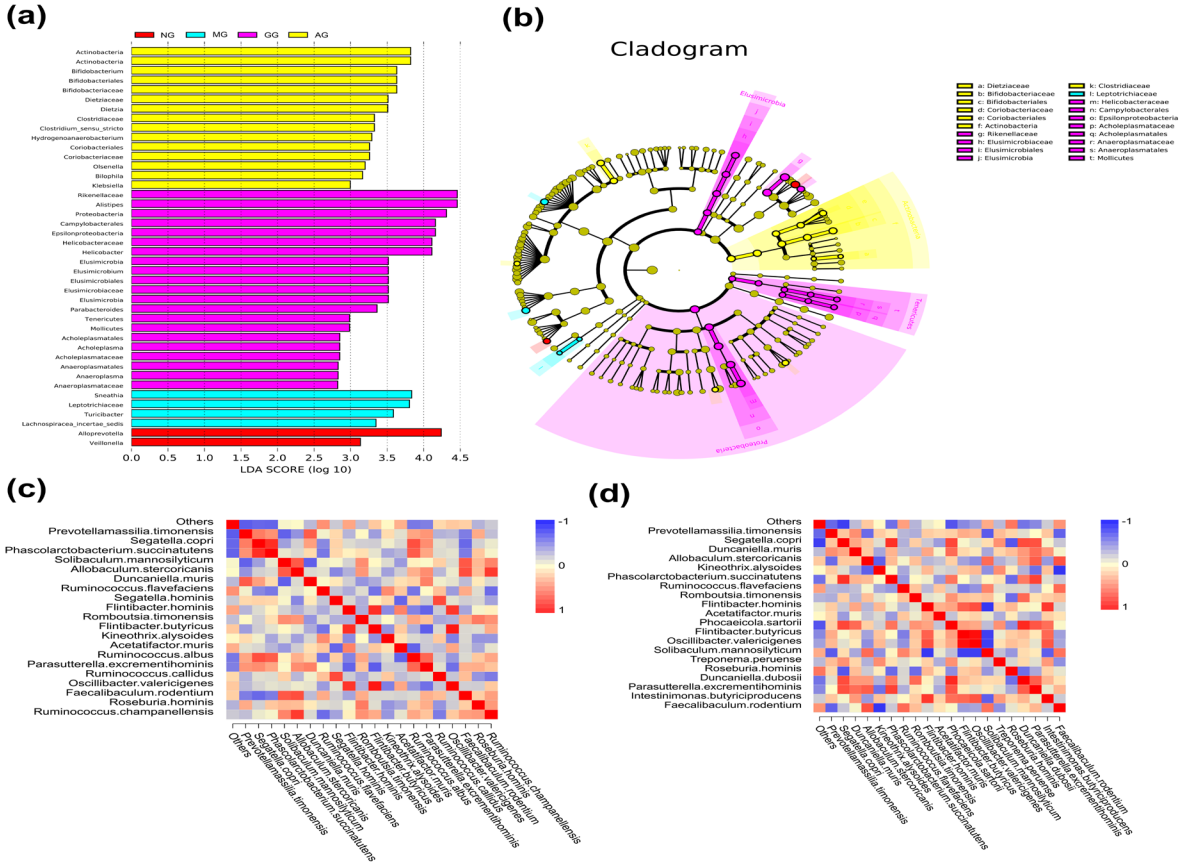

Figure 4: LDA distribution of LEfSe results and the Spearman correlation heatmap. (a)LEfSe results in the genus level and (b)the taxonomic cladogram. The threshold value of the LDA score was set to 2, and an LDA score>2 was considered significant.(c) The Spearman correlation heatmap of relative abundance at the species level between NG and MG; (d) The Spearman correlation heatmap of relative abundance at the species level between MG and GG. *n*=6.

sanguinis, Enterorhabdus caecimuris, Parabacteroides merdae, Anaeroplasma abactoclasticum, Bifidobacterium pseudolongum, Parabacteroides distasonis, Helicobacter ganmani, Eubacterium uniforme significantly increased at the genus level.

Microbiological diversity analyses showed that there were dissimilarities in the gut microbiota community between the model group and the treatment group. Compared with the fever model group, the relative abundance of Deltaproteobacteria significantly increased and the relative abundance of Bacilli significantly reduced at the class level, the relative abundance of *Pseudomonadales* and *Desulfovibrionales* significantly increased at the order level and the relative abundance of *Lactobacillales* significantly reduced at the order level, the relative abundance of *Desulfovibrionaceae, Moraxellaceae, Ruminococcaceae* significantly increased at the family level and the relative abundance of *Lactobacillaceae* significantly reduced at the family level, the relative abundance of *Acinetobacter, Pseudoflavonifractor, Butyricicoccus, Papillibacter, Desulfovibrio, Anaerotruncus, Coprobacillus, Oscillibacter* significantly increased at the genus level and the relative abundance of *Lactobacillus, Barnesiella, Lachnospiracea incertae sedis* significantly reduced at the genus level. Compared with that in the fever model group, the relative abundance of *Acinetobacter johnsonii, Oscillibacter ruminantium, Anaerotruncus colihominis, Coprobacillus cateniformis, Pseudomonas fragi, Clostridium viride, Oscillibacter valericigenes, Butyricicoccus pullicaecorum, Pseudoflavonifractor capillosus, Papillibacter cinnamivorans, Parabacteroides goldsteinii* significantly increased at the species level and the relative abundance of *Ruminococcus albus, Barnesiella intestinihominis*, and *Lactobacillus apodemi* significantly reduced at the species level.

Gut microbiota analysis showed that fever reduced the relative abundance of probiotics, such as *Oscillibacter* (A: 2.91%, B: 1.41%, p=0.173), it plays a crucial role in maintaining intestinal homeostasis, inflammation,[15] and oxidative stress in the human body, can exacerbate DSS-induced ulcerative colitis,[16, 17] type 2 diabetes.[18] Studies have found that the abundance of *Oscillibacter* was positively associated with pathological scores and serum inflammatory cytokine levels, and negatively correlated with the abundances of *Lactobacillus*.[16] Geniposide was increased the relative abundance of *Oscillibacter* (B: 1.41%, C: 4.03%, p=0.005). We discovered that the relative abundance of *Oscillibacter* was significantly reduced in the fever model group at the genus level compare with the normal control group, but the relative abundance of *Oscillibacter* in the geniposide group was significantly increased compare with the fever model group. This is contrary to the outcome of most inflammatory diseases. Research shows that in mice with hepatic encephalopathy, the relative abundance of *Oscillibacter* decreases after treatment.[19] Research has also demonstrated that the abundance of *Oscillibacter* was significantly diminished following anti-inflammatory treatment in rats with neuroinflammation associated with Alzheimer's disease.[20] At the same time, the reduction of *Oscillibacter* contributed to improved cognitive function and enhanced learning and memory capabilities following exercise.[21] The relative abundance of *Lactobacillus* was significantly increased in the fever model group and significantly reduced in the geniposide group (NG: 0.353%, MG: 0.883%, p=0.005; MG: 0.883%, GG: 0.306%, p=0.008). *Lactobacillus* reside in the gastrointestinal tract of humans and play an important role in gastrointestinal disorders,[22] overweight and obesity,[23] mental disorders,[24] inflammatory bowel disease[25] and neurodegenerative disease.[26] The *Lactobacillus* hindered pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α after fever. After administration of geniposide, the rats were still in an inflammatory state and did not show any corrective effect on *Oscillibacter* and *Lactobacillus*. Fever has been shown to influence the gut microbiota across various species, including humans. Alterations in the gut microbiota associated with febrile dependency have been observed in severe cases of COVID-19. Although the body temperature decreased after administration 5 hr, inflammatory reactions still persisted and did not return to normal, resulting in an imbalanced gut microbiota. Meanwhile, this differed slightly from previous inflammatory diseases studies that *Oscillibacter* was increased and *Lactobacillus* was reduced. This discrepancy may arise from the differences in the selection of animal models, the methodologies employed to induce fever and the age of rats. *Oscillibacter* and *Lactobacillus* significantly changed genera may be the signature bacteria of fever.

The gut microbiota of disbalance could be one of the significant contributing element that further deterioration of fever symptoms in various diseases. Figure 4 illustrates how various bacteria levels altered while receiving medication. After analyzing the genus level of each group's microbes using LEfSe, we discovered that the geniposide group had more differential microbes than the others. *Vellionella, Alloprevotella* displayed higher abundance in the normal control group. *Sneathia, Leptotrichiaceae, Turicibacter, Lachnpspiracea incertae sedis* displayed higher abundance in the fever model group. *Rikenellaceae, Alistipe, Proteobacteria*, etc., displayed higher abundance in the geniposide group.

CONCLUSION

Studies have shown that gut microbiota plays an undeniable role in maintaining the stability of the digestive tract and even the internal environment of the body. Therefore, regulating the balance of gut microbiota can achieve the therapeutic effect of treating fever. In the experiment, it was evident that there were changes in the composition of the gut microbiota in the fever model group, indicating dysbiosis of the gut microbiota. Although the administration group showed some improvement in gut microbiota, most of them were not significant. Perhaps due to the small dosage and only one dose, although achieving the therapeutic effect of understanding fever, the body is still in

the fever stage, resulting in no particularly significant difference in gut microbiota between the fever model group and the geniposide group. In the later stage, the fever period of the model will be extended, the number of doses will be increased, and the improvement effect of geniposide on the gut microbiota of febrile organisms will be further studied.

ACKNOWLEDGEMENT

We are grateful to the Shenzhen BGI Technology Service Co., Ltd., (Shenzhen, China). for assisting in sequencing and/or bioinformatics analysis.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

ABBREVIATIONS

NG: Normal control group; **MG:** Fever model group; **GG:** Geniposide group; **AG:** Aspirin group; **TCM:** Traditional Chinese medicine; **OTUs:** The same operational taxonomic units.

FUNDING

This work was supported by the Young Scientists Fund of Gansu Province(25JRRA816,23JRRA1676) and the Gansu Provincial Health Care Research Projects (GSWSKY2022-46).

AUTHOR CONTRIBUTIONS

Xiao-lin Li, Sheng-fu Chen and Tian-long Liu: Editing of manuscript drafts, project planning and oversight. Xiao-lin Li, Tian-long Liu, Renjie Wang, Rong Wang and Mao-xing Li: Conducting experiments, investigation, data collection and analysis. All authors were involved in drafting and revising the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study's animal experiments were all conducted pursuant to applicable national guidelines and standards for animal ethics and welfare (No. 2021KYLL118, authorized on June 4, 2021).

SUMMARY

Fever significantly upregulates *Bacillis, Lactobacillales, Lactobacillus, Lachnospiracea incertae sedis, Lactobacillus apodemi* and *Eubacterium uniforme* in rats, whereas fever significantly reduced *Coprobacillus, Prevotellaceae Coprobacillus cateniformis* and *Oscillibacter ruminantium.*

Geniposide sigificantly upregulates the abundance of *Pseudoflavonifractor*, *Desulfovibrio*, *Anaerotruncus*, etc., in pyretic rats, whereas significantly reduced the abundance of *Lactobacillus*, *Barnesiella*, etc.

REFERENCES

1. Chen L, Li M, Yang Z, Tao W, Wang P, Tian X, *et al*. *Gardenia Jasminoides* Ellis: ethnopharmacology, phytochemistry, and pharmacological and industrial applications of an important traditional Chinese medicine. J Ethnopharmacol. 2020; 257: 112829. doi: 10.1016/j.jep.2020.112829, PMID 32311486.
2. Wang L, Chen S, Liu S, Biu AM, Han Y, Jin X, *et al.* A comprehensive review of ethnopharmacology, chemical constituents, pharmacological effects, pharmacokinetics, toxicology, and quality control of Gardeniae fructus. J Ethnopharmacol. 2024; 320: 117397. doi: 10.1016/j.jep.2023.117397, PMID 37956915.
3. RA, FJ W, XL W and YM Z. The application overview and pharmacological research progress of Gardenia in Chinese and Mongolian medicine. Chin Med Mat. 2021; 43: 459-63.
4. Zhang X, Wang Y, Li S, Dai Y, Li X, Wang Q, *et al.* The potential antipyretic mechanism of Gardeniae Fructus and Its heat-processed products with plasma metabolomics using rats with yeast-induced fever. Front Pharmacol. 2019; 10: 491. doi: 10.3389/fphar.2019.00491, PMID 31143120.
5. Li M, Chen S, Luo K, Li X, Wang R, Yang J, *et al.* Geniposide from Gardeniae Fructus exErts antipyretic effect in febrile rats through modulating the TLR4/NF-κB signaling pathway. J Ethnopharmacol. 2024; 326: 117934. doi: 10.1016/j.jep.2024.117934, PMID 38387681.
6. Tian X, Wang G, Teng F, *et al.* Zhi Zi Chi decoction (Gardenieae fructus and semen Sojae Praeparatum) attenuates anxious depression via modulating microbiota-gut-brain axis in corticosterone combined with chronic restraint stress-induced mice. CNS Neurosci Ther. 2023.
7. Jiang P, Zhang Y, Li X, Chen J. Geniposidic acid attenuates DSS-induced colitis through inhibiting inflammation and regulating gut microbiota. Phytother Res. 2023; 37(8): 3453-66. doi: 10.1002/ptr.7819, PMID 37098758.
8. Tong Y, Xu YD, He J, Gong PY, Hong Y, Guo YJ. Mechanism of Gardeniae Fructus in ameliorating rheumatoid arthritis based on metabolomics and intestinal microbiota. Zhongguo Zhong Yao Za Zhi. 2023; 48(13): 3602-11. doi: 10.19540/j.cnki.cjcmm.20230411.401, PMID 37474993.
9. Kim HI, Hong SH, Lee SY, Ku JM, Kim MJ, Ko SG. Gardenia Jasminoides ameliorates antibiotic-associated aggravation of DNCB-induced atopic dermatitis by restoring the intestinal microbiome profile. Nutrients. 2021; 13(4): 1349. doi: 10.3390/nu13041349, PMID 33919521.
10. Yang X, Dai Y, Ji Z, Zhang X, Fu W, Han C, *et al.* Allium macrostemon Bunge. exerts analgesic activity by inhibiting NaV1.7 channel. J Ethnopharmacol. 2021; 281: 114495. doi: 10.1016/j.jep.2021.114495, PMID 34364968.
11. Gao Y, Liu L, Li C, Liang YT, Lv J, Yang LF, *et al.* Study on the antipyretic and anti-inflammatory mechanism of Shuanghuanglian oral liquid based on gut microbiota-host metabolism. Front Pharmacol. 2022; 13: 843877. doi: 10.3389/fphar.2022.843877, PMID 35837285.
12. Yang Y, Lu W, Zhang X, Wu C. Gut fungi differentially response to the antipyretic (heat-clearing) and diaphoretic (exterior-releasing) traditional Chinese medicines in Coptis chinensis-conditioned gut microbiota. Front Pharmacol. 2022; 13: 1032919. doi: 10.3389/fphar.2022.1032919, PMID 36467054.
13. Ye H. Study on material basis and mechanism of antipyretic effect of gypsum and its compatibility. Changchun University of Chinese medicine, 2020.
14. Xi C. Exploring the mechanism of the antipyretic effect of *Pueraria lobata* and *Pueraria lobata* var. *Thomsonii* in a rat model of wind-cold syndrome based on intestinal flora and thermogenic pathway. Jiangxi University of Chinese Medicine; 2023.
15. Bajaj A, Markandey M, Kedia S, Ahuja V. Gut bacteriome in inflammatory bowel disease: an update on recent advances. Indian J Gastroenterol. 2024; 43(1): 103-11. doi: 10.1007/s12664-024-01541-1, PMID 38374283.
16. Wu M, Li P, An Y, Ren J, Yan D, Cui J, *et al.* Phloretin ameliorates dextran sulfate sodium-induced ulcerative colitis in mice by regulating the gut microbiota. Pharmacol Res. 2019; 150: 104489. doi: 10.1016/j.phrs.2019.104489, PMID 31689519.
17. Jialing L, Yangyang G, Jing Z, Xiaoyi T, Ping W, Liwei S, *et al.* Changes in serum inflammatory cytokine levels and intestinal flora in a self-healing dextran sodium sulfate-induced ulcerative colitis murine model. Life Sci. 2020; 263: 118587. doi: 10.1016/j.lfs.2020.118587, PMID 33065145.
18. Ye J, Meng Q, Jin K, Luo Y, Yue T. Phage cocktail alleviated type 2 diabetes by reshaping gut microbiota and decreasing proinflammatory cytokines. Appl Microbiol Biotechnol. 2024; 108(1): 9. doi: 10.1007/s00253-023-12912-7, PMID 38159123.
19. Yang Z, Liu S, Wei F, Hu J. The effects of Qingchang Ligan formula on hepatic encephalopathy in mouse model: results from gut microbiome-metabolomics analysis. Front Cell Infect Microbiol. 2024; 14: 1381209. doi: 10.3389/fcimb.2024.1381209, PMID 39220284.
20. Wang J, Zhu X, Li Y, Guo W, Li M. Jiedu-Yizhi formula alleviates neuroinflammation in AD rats by modulating the gut microbiota. Evid Based Complement Alternat Med. 2022; 2022: 4023006. doi: 10.1155/2022/4023006, PMID 35958910.
21. Wang G, Zhou HH, Luo L, Qin LQ, Yin J, Yu Z, *et al.* Voluntary wheel running is capable of improving cognitive function only in the young but not the middle-aged male

APPSwe/PS1De9 mice. Neurochem Int. 2021; 145: 105010. doi: 10.1016/j.neuint.2021.105010, PMID 33684544.

22. Yousef M, Rob M, Varghese S, Rao S, Zamir F, Paul P, *et al.* The effect of microbiome therapy on COVID-19-induced gut dysbiosis: A narrative and systematic review. Life Sci. 2024; 342: 122535. doi: 10.1016/j.lfs.2024.122535, PMID 38408636.
23. DiMattia Z, Damani JJ, Van Syoc E, Rogers CJ. Effect of probiotic supplementation on intestinal permeability in overweight and obesity: A systematic review of randomized controlled trials and animal studies. Adv Nutr. 2024; 15(1): 100162. doi: 10.1016/j.advnut.2023.100162, PMID 38072119.
24. Xiong RG, Li J, Cheng J, Zhou DD, Wu SX, Huang SY, *et al.* The role of gut microbiota in anxiety, depression, and other mental disorders as well as the protective effects of dietary components. Nutrients. 2023; 15(14): 3258. doi: 10.3390/nu15143258, PMID 37513676.
25. Li C, Peng K, Xiao S, Long Y, Yu Q. The role of Lactobacillus in inflammatory bowel disease: from actualities to prospects. Cell Death Discov. 2023; 9(1): 361. doi: 10.1038/s41420-023-01666-w, PMID 37773196.
26. Beltrán-Velasco AI, Reiriz M, Uceda S and Echeverry-Alzate V. *Lactiplantibacillus. Lactiplantibacillus* (*Lactobacillus*) plantarum as a Complementary Treatment to Improve Symptomatology in Neurodegenerative Disease: A Systematic Review of Open Access Literature. Int J Mol Sci. 2024; 25(5). doi: 10.3390/ijms25053010.

Cite this article: Li XL, Chen SF, Liu TL, Wang RJ, Wang R, Li MX. Antipyretic Effect of Geniposide in Dry Yeast-Induced Pyrexia Rats via Intervening the Compositions of Gut Microbiota. Indian J of Pharmaceutical Education and Research. 2026;60(4):1636-45.