Applied Microbiology and Biotechnology
https://doi.org/10.1007/s00253-023-12763-2

APPLIED MICROBIAL AND CELL PHYSIOLOGY

Crotonis Fructus-induced gut microbiota and serum metabolic disorders in rats

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Received: 27 April 2023 / Revised: 15 August 2023 / Accepted: 30 August 2023

Abstract

Crotonis Fructus (CF), a poisonous traditional laxative, has been used to treat constipation, edema, ascites, and inflammation for more than 2000 years. However, CF possesses toxicity and its toxic mechanism is still unclear. Thus, this research explored the deleterious impacts and underlying mechanisms of CF by evaluating alterations in gut microbiota composition and metabolites. High-throughput sequencing was employed on the 16S rDNA gene to explore the intestinal flora. The untargeted metabolomics method was utilized for evaluating serum metabolomics analysis. The results showed that CF could induce obvious hepatic and gastrointestinal damage by histopathologic morphology of the liver, stomach, duodenum, and colon. According to 16S rDNA sequencing, CF can cause gut microbiota disturbance in rats, and the abundance of opportunistic pathogens such as *Clostridia_UCG_014_unclassified* increased significantly, while the levels of beneficial bacterial *Lactobacillus* remarkably declined after CF treatment. Additionally, metabolomics analysis demonstrated that CF may induce toxicity by disrupting the glycerophospholipid metabolism pathway and metabolites such as phosphatidylcholine and phosphatidylethanolamine. Moreover, a correlation study revealed the link between intestinal flora, serum metabolites, and toxicity-related biochemical markers. The results provide a new idea for the research and clinical application of toxic traditional medicine.

Key points

- *Crotonis Fructus could affect the gut flora and serum metabolic disruption in SD rats.*
- *Crotonis Fructus could promote the proliferation of harmful bacteria and inhibit beneficial bacteria.*
- *Glycerophospholipid metabolism was disturbed by Crotonis Fructus.*

Keywords Crotonis Fructus · Toxicity · Gut microbiota · Metabolites

Introduction

Crotonis Fructus (CF), the fruits of *Croton tiglium* L., belongs to the family *Euphorbiaceae* and named as Ba-Dou in China. CF has been historically used in the treatment of constipation, ascites, dysuria, and inflammation for more than 2000 years in Asia (Song et al. 2017). Modern pharmacological studies have shown that CF possessed good effects on lung cancer (Niu et al. 2020), colon cancer (Aboulthana et al. 2022), skin fungal infection (Lin et al. 2016), tuberculosis (Zhao et al. 2016), and leukemia (Kupchan et al. 1976). However, CF could cause gastrointestinal injury (Liu et al. 2017) and inflammation (Dzietko et al. 2015), and the underlying mechanism is still unknown.

Due to the multi-component and multi-target characteristics of traditional Chinese medicine (TCM), it is difficult to reflect the overall effect by studying only a few components

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Published online: 15 September 2023

(Xiao et al. 2022). Metabolomics is a systematic qualitative and quantitative analytical technique that enables the comprehensive evaluation of metabolites and provides information on drug toxicity and physiological and pathological changes (Zhao et al. 2014). Studies have shown that metabolomics has been used to reveal the molecular biomarkers of drug enterotoxicity (Yang et al. 2016), cardiotoxicity (Su et al. 2016; Zhang et al. 2022), hepatotoxicity (Cao et al. 2020; Luo et al. 2021), lung toxicity (Ji et al. 2021), and nephrotoxicity (Xie et al. 2020).

In addition, intestinal microbiota has been increasingly recognized to be closely related to many diseases and drug toxicity, which is considered to be a crucial hidden "organ" due to its great influence on the metabolism, physiology, and immune function (Bäckhed et al. 2005; Duan et al. 2019). Studies have revealed the correlation between intestinal flora and gastrointestinal disease (Cai et al. 2021), chronic kidney disease (Li et al. 2019), diabetes (Gurung et al. 2020), liver disease (Kong et al. 2021), obesity (Ley et al. 2006), and cancer (Deng et al. 2021). Moreover, it has been reported that gut microbiota could play a significant role in hepatotoxicity by promoting an inflammatory response and oxidative stress (Gong et al. 2021). *Euphorbia pekinensis* diterpenoids can cause severe intestinal mucosal damage by affecting the gut microbiota (Wang et al. 2021b). Besides, when processed CF was used in combination with jujube, the toxicity was significantly reduced and the imbalance of intestinal flora was restored (Yue et al. 2019; Feng et al. 2021). These results indicate that the toxicity of CF may be related to intestinal flora and metabolites. However, little is known about the metabolic changes in the gut microbiome that are associated with CF-altered gut microbiota.

Therefore, 16S rDNA sequencing and ultra-high performance liquid chromatography-quadrupole time-of-fight mass spectrometry (UPLC-Q-TOF–MS) were used to investigate the toxic effects of CF on gut microbiota and serum metabolomics for the first time in this study, and histopathology and biochemical indicators were also evaluated, which may provide a new idea for the research and clinical application of toxic traditional medicine.

Materials and methods

Chemicals

Acetonitrile was bought from Thermo Fisher (MA, USA). Formic acid was bought from Macklin (Shanghai, China). Ultra-pure water was bought from Guangzhou Watsons Food and Beverage Co., Ltd. (Guangzhou, China). CMC-Na was purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). CF were purchased from Anguo medicinal materials market (An Guo, China), and identified by Jing Hu (the associate professor of Tianjin University of TCM).

Preparation of CF samples

CF was pulverized and filtered through a 50 mesh sieve. 0.5% CMC-Na was used to make 105 and 525 mg/kg CF solutions, which were equal to 10 and 50 times of the clinical dose, respectively.

Animals and treatment

Male SD rats (180 ± 20 g) were bought from Beijing Vital River Laboratory Animal Technology and maintained with 24 ± 2 °C, 60 ± 5% relative humidity, keeping a 12 h light/dark cycle. The experiments were approved by the Animal Ethics Committee of Tianjin University of TCM, which according to the Experimental Animal Care and Use Handbook (National Research Council of the United States, 1996).

The rats were assigned to three groups at random: the normal group (N), the low-dose CF group (CL), and the high-dose CF group (CH). The CL and CH groups were given CF at a dose of 105 and 525 mg/kg, respectively, and the N group was given the same dose of CMC-Na. In all cases, the administration was done using oral gavage, and the rats were dosed continuously for a duration of 21 days. The body weight and feed consumption were recorded daily.

Sample collection and preparation

Fecal samples were obtained on the 21st day, and blood samples were collected from the abdominal aorta. After, the rats were given 10% chloral hydrate. Then, the blood samples were left undisturbed for 30 min before being centrifuged for 15 min at 3000 rpm. The samples were frozen and stored at − 80 °C. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) kits from Nanjing Jiancheng Institute of Biological Engineering, and interleukin-1β (IL-1β), superoxide dismutase (SOD), diamine oxidase (DAO), tumor necrosis factor-α (TNF-α), and malondialdehyde (MDA) ELISA kits from Quanzhou Ruixin Biotechnology were used for analysis. The liver, duodenum, colon, and stomach tissues were preserved with 4% neutral paraformaldehyde.

Sequencing analysis of gut microbiota

DNA from the fecal samples was extracted by CTAB and sequenced by LC-Bio biotechnology platform. The extracted DNA was detected by UV–vis spectrophotometer. The 16S rDNA was sequenced using the primer pairs 341F (5′-CCTACG GGNGGNGGCWGCAG-3′) and 805R (5′-GACTHVGGTATC TAATCC-3′), which were labeled with specific barcodes and universal sequencing primers at their 5′ ends. The amplified

samples were purified using AMPure XT beads (Beckman Coulter Genomics, USA) and quantified with a Qubit instrument (Ni et al. 2023). The PCR products were sequenced and analyzed by Agilent 2100 biological analyzer and Illumina platform (Cheng et al. 2022a). The diversity of α and β was analyzed by principal component analysis (PCA), principal coordinate analysis (PCoA), and non-metric multi-dimensional scale analysis (NMDS).

Serum metabolomics analysis

Serum samples were pretreated with acetonitrile and detected by UPLC-Q-TOF/MS. The data were processed by MassLynx 4.2 (Waters, Milford, USA). The data were imported into Progensis QI and SIMCA-P 14.1 software for analysis. Principal component analysis (PCA) and orthogonal projection discriminant analysis (OPLS-DA) were utilized for data processing and dimensionality reduction. Those with variable importance in the projection (VIP) > 1 are considered to be the differential metabolites. Potential biomarkers were processed by HMDB (http://www.hmdb.ca/), MassBank (http://www.massbank.jp/), and MetabaAnalyst5.0 (http://www.metaboanalyst.ca/) for further analysis.

Histological analysis

The liver, stomach, duodenum, and colon tissues were dehydrated, embedded in paraffin, sliced into 4 μm and stained with hematoxylin and eosin (H&E). The morphological characteristics were observed by vertical optical microscope as previously reported (Zhuang et al. 2021).

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 9.0 (CA, USA). The experimental data was presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to compare the differences between groups, and a P-value < 0.05 was considered statistically significant.

Results

Effects of CF on body weight and food consumption in rats

During the administration, the body weight and food consumption in rats were weighed every day. The results showed that there was no significant difference between the CF groups and N group in body weight and food consumption changes (Fig. 1).

Biochemical indexes and histopathological analysis

Biochemical indexes and histopathological analysis of the liver, duodenum, colon, and stomach were investigated. Compared with the N group, the serum levels of TNF-α, MDA, DAO, IL-1β, ALT, and AST in the CF groups were significantly increased, while the levels of SOD were dramatically reduced (Fig. 2A–G). In addition, histopathological results showed that there were a large number of inflammatory cell infiltration and necrosis in the liver, stomach, duodenum, and colon in CF-treated rats (Fig. 3). Therefore, rats given CF showed obvious liver and gastrointestinal damage.

Effects of CF on the gut microbiota

The fecal samples were analyzed by 16Sr DNA sequencing. Venn's diagram showed that the total number of amplified sequence variation (ASV) in the N, CL, and CH group was 1693, 1609, and 1756, respectively. Among them, there were 641 ASVs overlaps both in the N and CL group, 626 ASVs both in the N and CH group, and 489 ASVs overlapping in all groups (Fig. 4A). The results of Chao1 and Shannon showed that α diversity between N and CF groups did not display significant distinction (Fig. 4B, C). However, PCA, PCoA, and NMDS analyses showed that there was a significant difference in the number of ASV between N and CF groups (Fig. 4D),

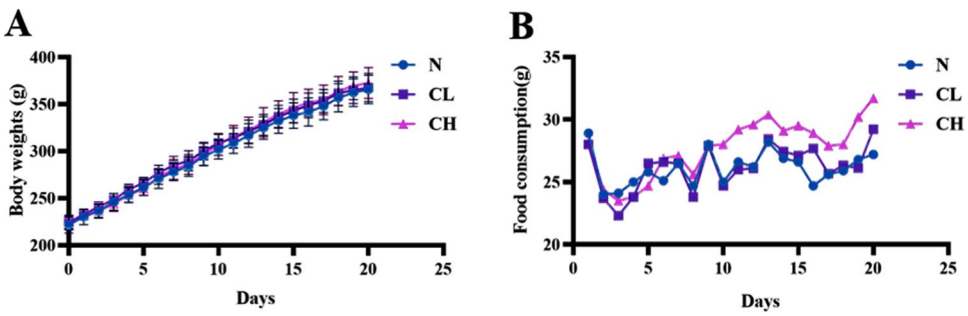

Fig. 1 Effect of CF on body weight (**A**) and food consumption (**B**) in rats

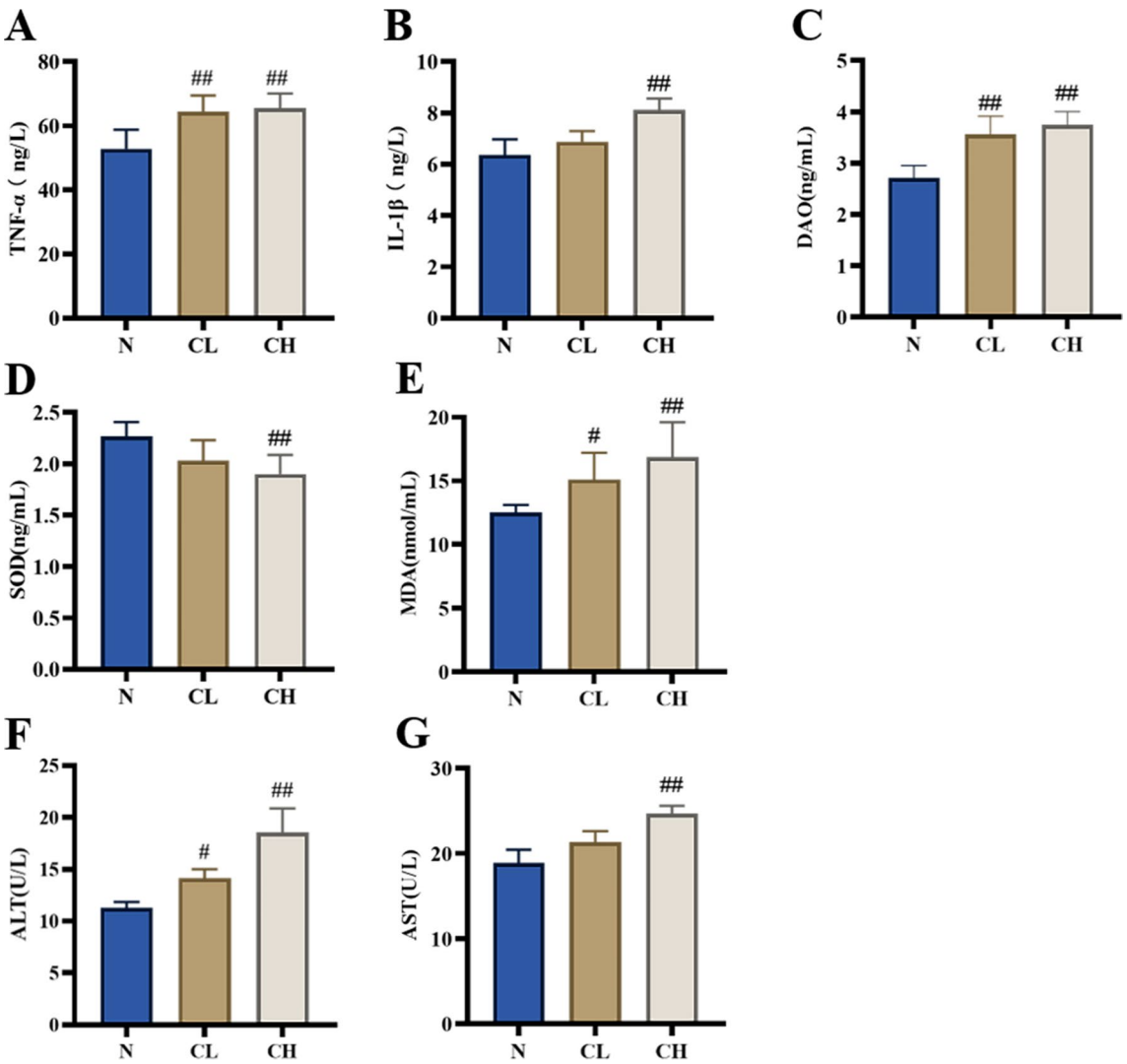

Fig. 2 Effect of CF on biochemical indexes in rats. TNF-α (**A**), IL-1β (**B**), DAO (**C**), SOD (**D**), MDA (**E**), ALT (**F**), and AST (**G**) in each group. Means ± SEM was used to express the results of data statistics. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. normal group

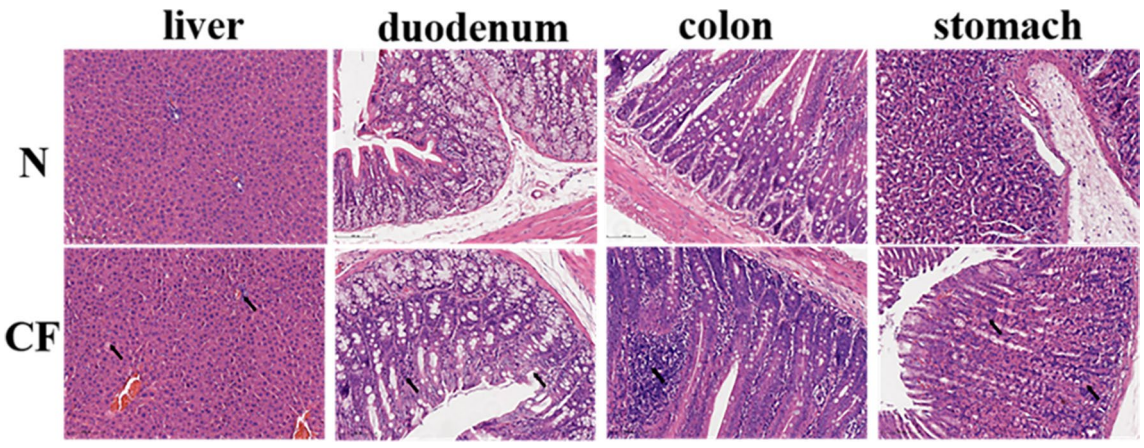

Fig. 3 Effect of CF on histopathological images of the liver, stomach, duodenum, and colon

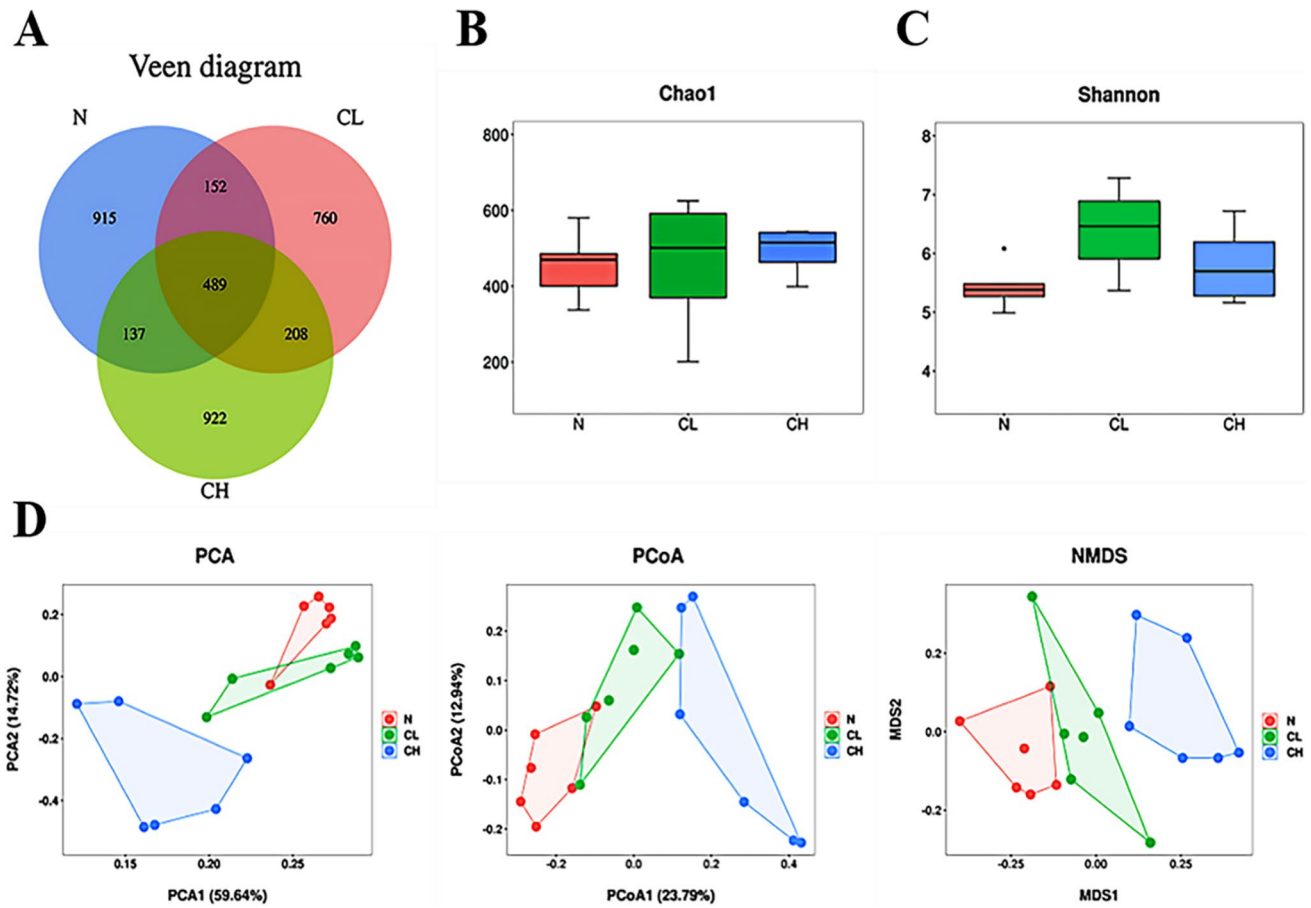

Fig. 4 Effect of CF on the diversity of intestinal microorganisms. ASV Venn analysis (**A**), Chao1 (**B**), Shannon (**C**), and PCA, PCoA, and NMDS (**D**). Means ± SEM was used to express the results of data statistics. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. normal group

which suggesting that the toxicity of CF could induce significant disturbance of intestinal flora in rats.

To identify the main taxonomic groups regulated by CF, we analyzed the relative abundance of gut bacterial taxonomic category between the CF groups and N group. At the phylum level (Fig. 5A), 19 phyla of intestinal flora were identified and the dominant gut bacteria were *Firmicutes* and *Bacteroidetes*. The sum of the relative abundance of *Firmicutes* and *Bacteroidetes* in the N, CL, and CH groups was 93.35%, 95.33%, and 95.55%, respectively. Thus, CF-induced toxicity had little influence on the abundance of these two main phyla.

At the family level (Fig. 5B), the relative abundance of *Lactobacillaceae* and *Bacteroidaceae* in the CH group (accounting for 23.87% and 0.09%, Fig. 5D) was obviously decreased ($P < 0.01$ and $P < 0.05$, respectively) compared with the N group (accounting for 47.75% and 1.07%). At the genus level (Fig. 5C), *Lactobacillus* and *Bacteroides* were markedly decreased by CF ($P < 0.01$ and $P < 0.05$, respectively, Fig. 5E), which accounted for the changes in *Lactobacillaceae* and *Bacteroidaceae*. Moreover, the abundance of *Oscillospiraceae_NK4A214_group* and *Oscillospiraceae_UCG-005* were markedly increased after CF administration, which accounted for the changes in *Oscillospiraceae* (Fig. 5D). In addition, *Clostridia_UCG-014* and *Eubacterium_coprostanoligenes_group* were significantly increased in the CF groups compared with the N group (Fig. 5E).

Furthermore, a linear discriminant analysis effect size (LEfSe) was used to pinpoint bacterial species' biomarkers related to the toxicity of CF. We found that there were significant differences in the composition of intestinal flora between the CH and N group, and 21 different bacteria (LDA scores > 3.5) were identified. Seven bacteria were found in the N group, while 14 bacteria were found in the CH group (Fig. 6A). Notably, we found that the CF treatment significantly increased the relative abundance of *c__Clostridia*, which was mainly due to the enrichment of *Clostridia_UCG-014*, *Oscillospiraceae_UCG-005* and *Eubacterium_coprostanoligenes_group* (Fig. 6B), which may play a role in the toxic effect of CF to some extent.

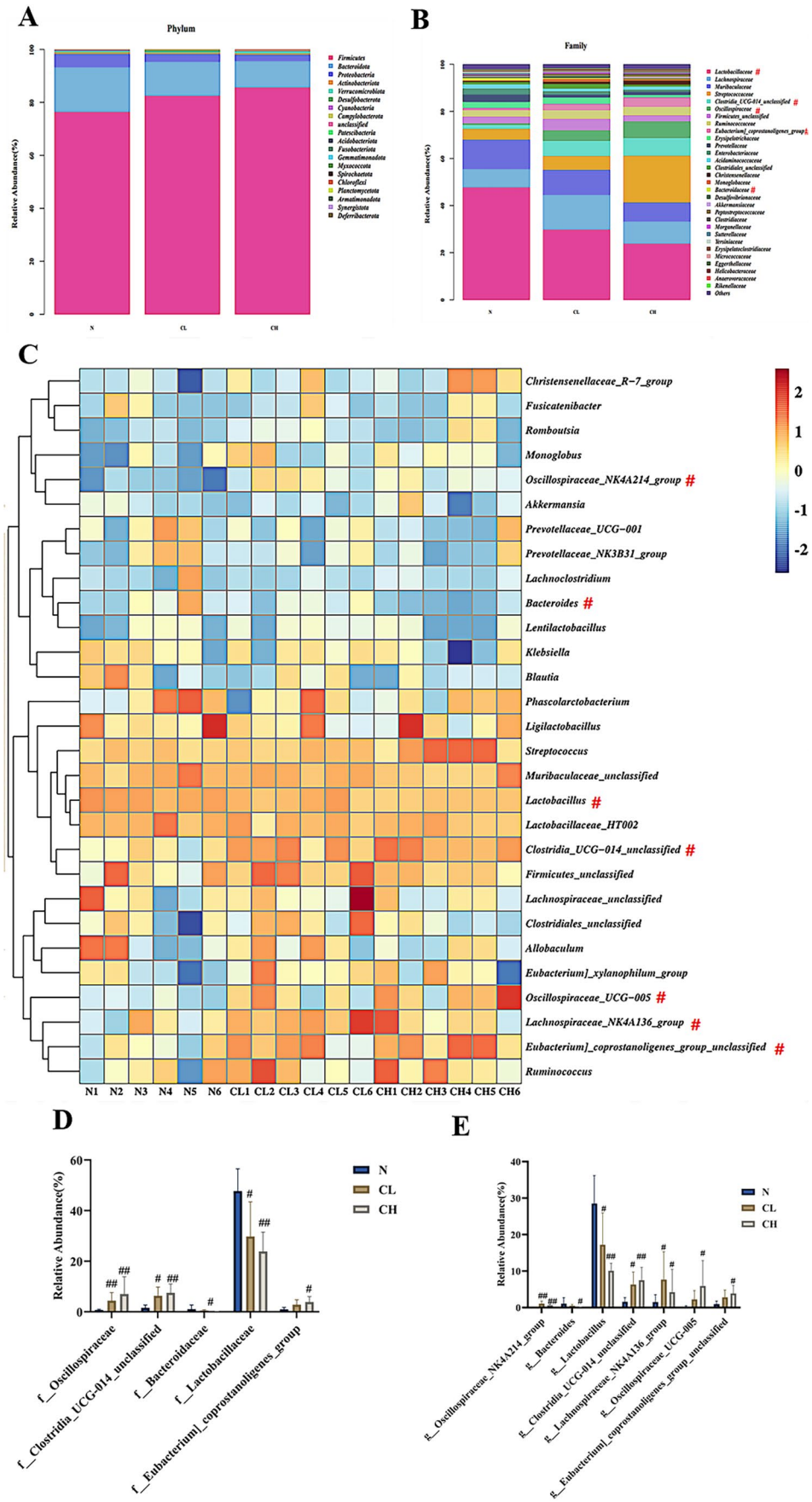

Fig.5 Effect of CF on the relative abundance of intestinal microbiota. Phyla (**A**), family (**B**), genus (**C**), specific bacteria at the family (**D**) and genus levels (**E**). Means ± SEM was used to express the results of data statistics. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. normal

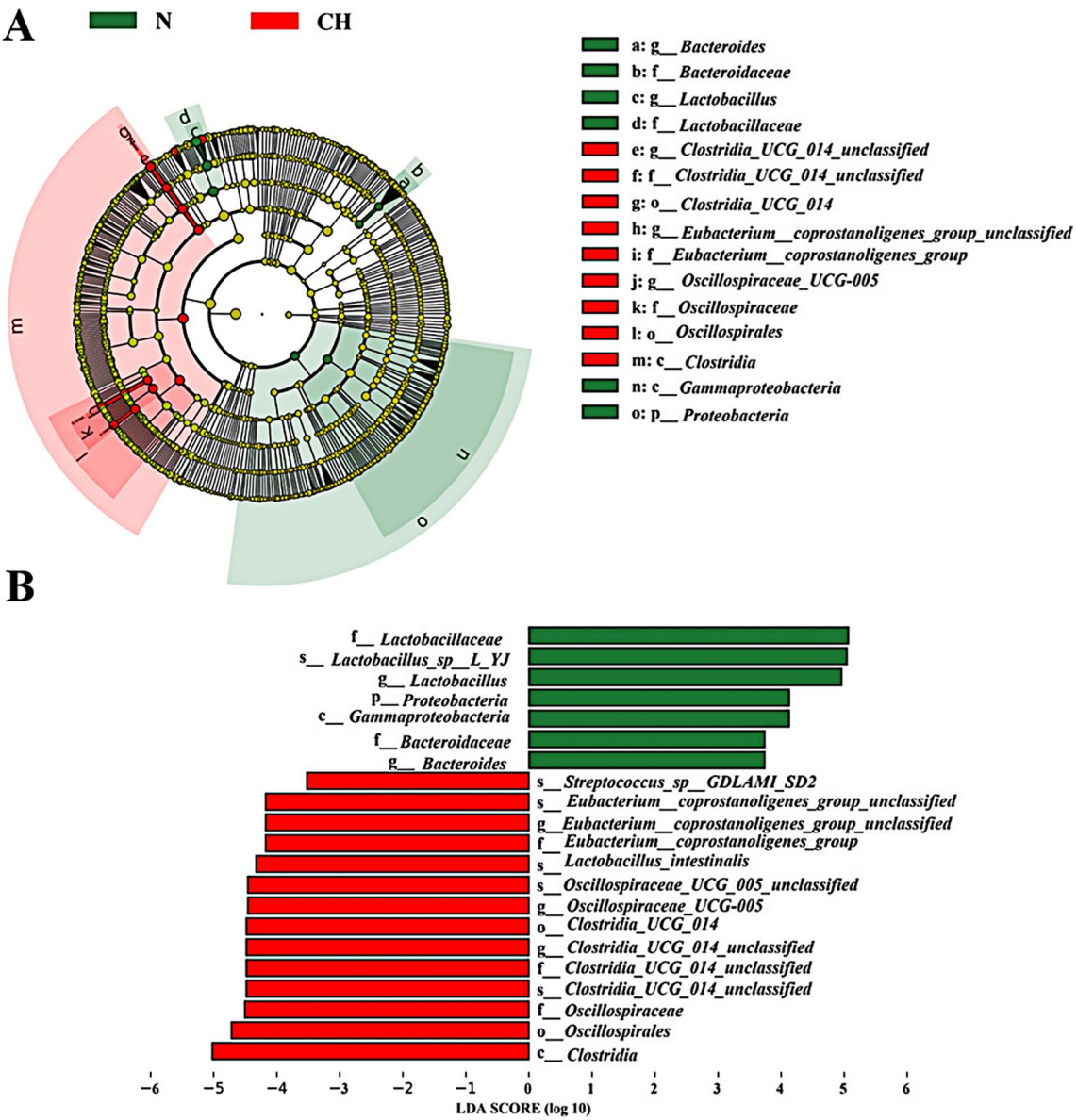

Fig. 6 LEfSe cladogram (**A**) and LDA scores of the important genus (**B**)

Effects of CF on the serum metabolites

The serum metabolites were investigated by UPLC-Q-TOF–MS, and the data were processed for multivariate statistical analysis. The PCA results showed that each point represented a serum sample, and the points in different groups tended to gather together. The N and CF samples can be clearly distinguished, indicating that there was a significant metabolic difference after CF treatment (Fig. 7A).

The OPLS-DA analysis was used to identify metabolites with significant changes, and the findings revealed that each group of samples was split into discrete areas (Fig. 7B). The results showed that the N group was modeled with the CL and CH group, respectively (OPLS-DA-CL: $R^2X = 0.608$, $R^2Y = 0.961$, $Q^2 = 0.862$; OPLS-DA-CH: $R^2X = 0.705$, $R^2Y = 0.996$, $Q^2 = 0.989$). The scatter diagram revealed that the metabolic spectrum of the CL and CH group could be completely separated from that of the N group (Fig. 7C, D). The metabolites were identified by load S diagram and VIP value, and those with VIP > 1 and $P < 0.05$ were considered to be potential biomarkers.

As a result, 9 potential biomarkers were identified, which is shown in supplementary Tab. S1. Compared with the N group, the levels of PE (20:0/24:1(15Z)), PC (14:0/16:0), PE (22:2(13Z,16Z)/P-18:0), N-glycoloylganglioside GM2, docosadienoate (22:2n6), L-cysteinylglycine disulfide, GlcCer(d18:1/24:1(15Z)), and glyoxylic acid metabolites in the CH group were significantly decreased. However, the

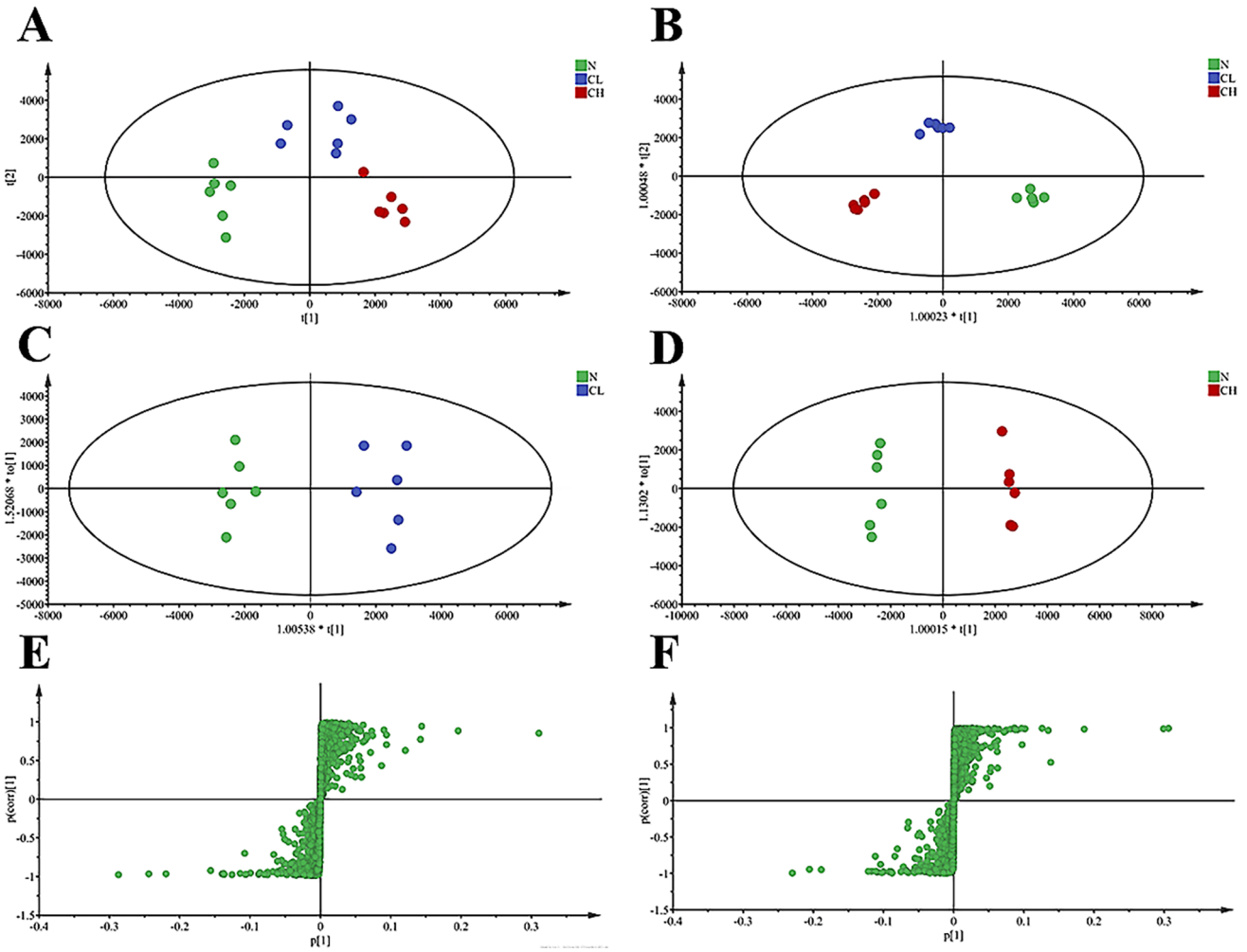

Fig. 7 Multivariate statistical analysis and metabolic profiling. The PCA (**A**) and OPLS-DA (**B**) scores of the N, CL, and CH group. Score scatter plots, permutation tests, and S-plots are produced by the OPLS-DA analysis between the N group and CL group (**C**). Score scatter plots, permutation tests, and S-plots are produced by the OPLS-DA analysis between the N group and CH group (**D**)

levels of S-hydroxymethyl glutathione were increased significantly. The changes of serum metabolites were studied by thermographic analysis (Fig. 8).

The metabolic pathways of biomarkers were analyzed, and 9 metabolic pathways were screened. Glycerol phospholipid metabolism and acetaldehyde and dicarboxylic acid metabolic pathways are considered to be closely related to CF-induced metabolic disorders ($P < 0.05$, impact > 0.1) (Fig. 9).

Correlation among intestinal microflora, serum metabolites, and biochemical indexes

To further investigate the involvement of intestinal microflora in the toxic effects of CF, we correlated the differential bacteria with various biochemical indexes and serum metabonomics by Spearman correlation analysis (Cheng et al. 2022b). The results showed that *Lactobacillus* and *Bacteroides* were negatively related to most liver and intestinal injury indices, while *c__Clostridia* (*Oscillospiraceae_UCG-005*, *Oscillospiraceae_NK4A214_group*, and *Clostridia_UCG-014*) was positively correlated with the toxic biochemical indices. Besides, *Oscillospiraceae_UCG-005* and *Clostridia_UCG-014* were negatively correlated with differential metabolites such as PE (20:0/24:1(15Z)), PC (14:0/16:0), glyoxylic acid, N-glycoloylganglioside GM2, docosadienoate (22:2n6), and L-cysteinylglycine disulfide (Fig. 10A). These metabolites were also negatively related with biochemical indices such as TNF-α, IL-1β, DAO, MDA, AST, and ALT (Fig. 10B).

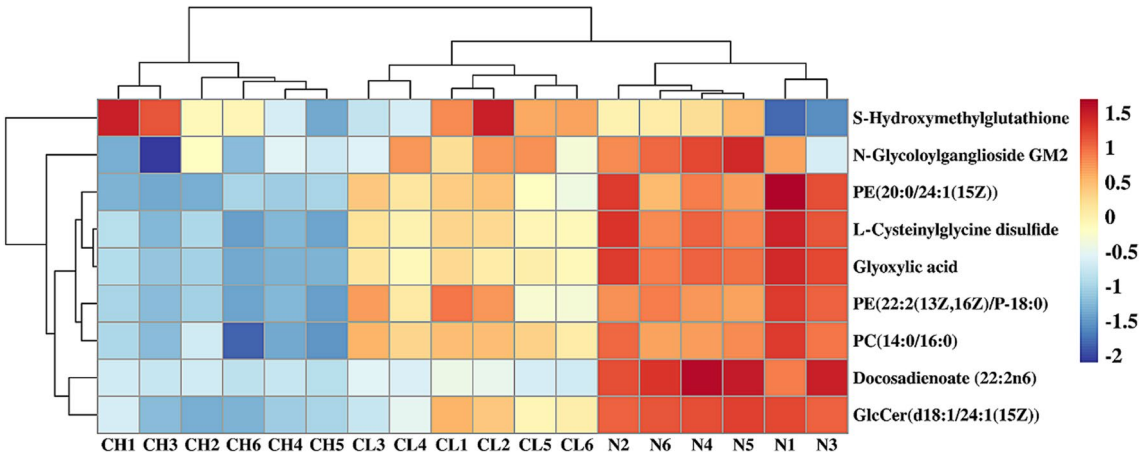

Fig. 8 Heatmap analysis of 9 biomarkers of CF toxicity

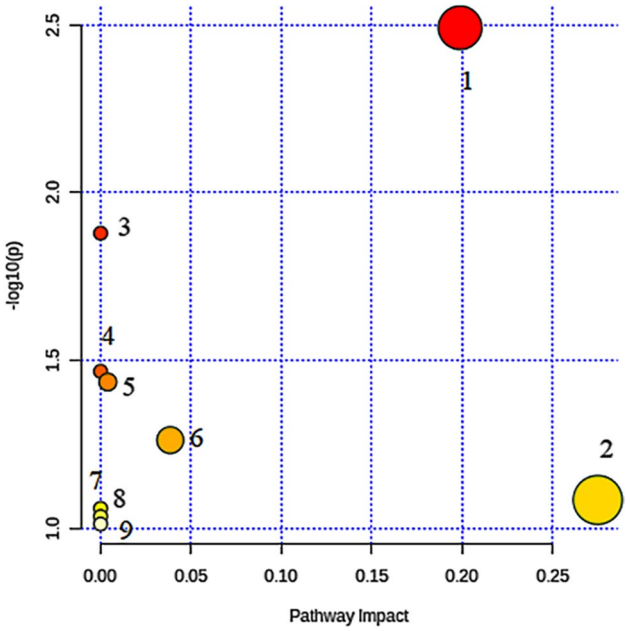

Fig. 9 Metabolic pathways of potential biomarkers. 1 glycerophospholipid metabolism; 2 glyoxylate and dicarboxylate metabolism; 3 linoleic acid metabolism; 4 alpha-linolenic acid metabolism; 5 glycosylphosphatidylinositol (GPI)-anchor biosynthesis; 6 sphingolipid metabolism; 7 glycine, serine, and threonine metabolism; 8 arachidonic acid metabolism; 9 arginine and proline metabolism

Discussion

CF has been employed to treat various gastrointestinal diseases for over two millennia in TCM. Previous studies have shown that CF has acute toxicity. However, the long-term toxicity and toxicity mechanism are still not clear. Studies have shown that the efficacy and toxicity of drugs are closely related to intestinal flora (Feng et al. 2021). However, the correlation between CF toxicity and intestinal flora and metabolism has not been reported. Therefore, in this study, the relationship between the toxicity of CF and intestinal microflora and serum metabolism was investigated.

In the preliminary experiment, several doses of CF were given by intragastric administration in rats, and we found that there was no significant difference in the biochemical indexes, intestinal microbiota, and metabolites between the low dose of CF groups (31.5 and 52.5 mg/kg, equivalent to 3 and 5 times of the clinical dose) and N group. According to the results of previous experiments, subacute toxicity in rats was carried out by intragastric administration of CF (105 and 525 mg/kg) for 3 weeks. Histopathological analysis showed that CF had obvious hepatotoxicity and gastrointestinal damage in rats. This was further verified by the significantly increase of serum AST and ALT levels, which are important biomarkers for clinical diagnosis and evaluation of liver disease (Zhao et al. 2017). Oxidative damage is a significant contributing factor to organ damage, which usually evaluated by MDA and SOD (Lou et al. 2018). MDA can cause secondary cell membrane damage and is the most deeply studied lipid peroxidation product (Ammar et al. 2022), while SOD is an antioxidant enzyme that protects cells (V et al. 2022). Compared with the N group, the levels of MDA in the CH group were significantly increased, while the levels of SOD were significantly decreased, suggesting CF could induce oxidative damage in rats. TNF-α and IL-1β are important inflammatory cytokines related to immune regulation (Herman Mahečić et al. 2020; Jaremek and Nieradko-Iwanicka 2020). The serum levels of TNF-α and IL-1β in the CH group were significantly increased, which may lead to severe inflammation and tissue injury (Zhang et al. 2014; Shan et al. 2018). Moreover, DAO is a highly active intracellular enzyme in the small intestine and can be released into the peripheral bloodstream when enterocytes are damaged (Schnedl and Enko 2021; Ji et al.

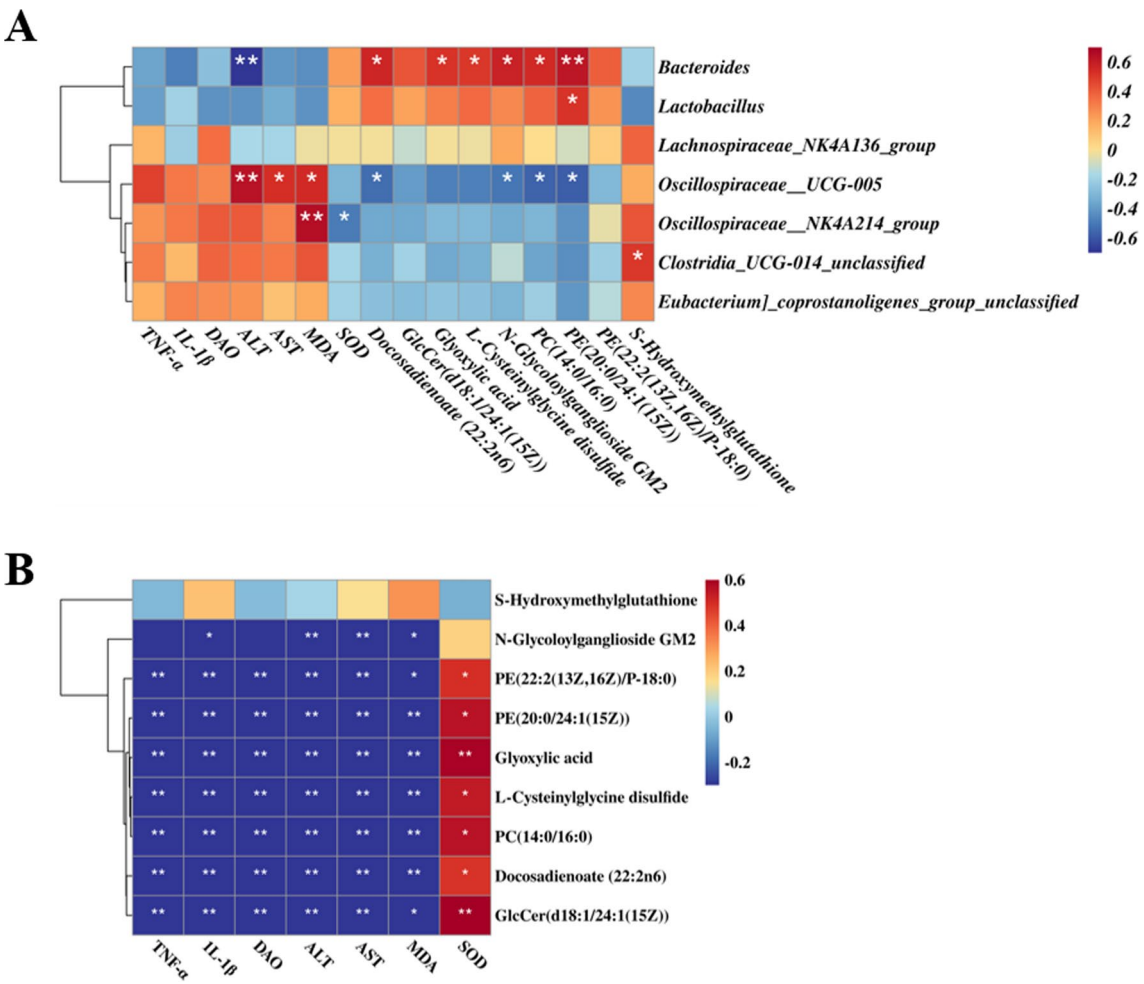

Fig. 10 Heatmaps showing correlations between differential gut bacteria and serum metabolites and biochemical indices in rats. Analysis of the relationships between intestinal flora and markers associated to CF toxicity. **A** Correlations between gut bacteria and serum metabolites and biochemical indices. **B** Correlations between serum metabolites and biochemical indices. $*P<0.05$ and $**P<0.01$ indicate an obvious correlation between two parameters

2022). The levels of DAO in the CH group were also significantly increased, which may be related to the gastrointestinal damage of CF.

Accumulating evidence revealed that the changes in gut microbiota are associated with gastrointestinal injury pathogenesis (Yang et al. 2022). 16S rDNA is currently the most commonly used method in bacterial systematics research and the bacterial hypervariable regions V3 and V4 are considered to be optimal regions for analyzing the bacterial diversity in various environments (Claesson et al. 2010; Logue et al.2016). Thus, 16S rDNA high-throughput sequencing was utilized to determine the diversity and variation in gut microbial composition and differences in dominant bacteria before and after CF treatment. The β diversity analysis of the structure and composition of intestinal flora showed that the CF groups and N group could be significantly separated, which proved that the toxicity of CF could cause intestinal flora disorder in rats.

According to the results of gut microbiota, the dysbiosis in gut microbiota including the decrease in beneficial bacteria and increase in harmful bacteria levels was prospective related to CF-induced liver and gastrointestinal injury. For example, the relative abundance of *Lactobacillus* and *Bacteroides* was not only decreased significantly after CF treatment for 3 weeks, but was also obviously associated with biochemical indices. A serious of previous research results demonstrated the rationality of our findings. Studies have found that *Lactobacillus* could produce antimicrobial chemicals, support intestinal cell development, and maintain intestinal mucosal barrier function (Zhang et al. 2020). *Bacteroides* also plays an important role in improving inflammation and immunodeficiency diseases (Liu et al. 2022). Previous research results confirmed that the decrease in the relative abundance of *Lactobacillus* and *Bacteroides* may induce inflammation and gastrointestinal injury (Li et al.

2022). Interestingly, our findings were consistent with the results of relationship between the probiotic and intestinal toxicity of other Chinese medicinal herbs such as *Kansui Radix* (Jiang et al. 2018) and *Euphorbiae Semen* (Wei et al. 2022). Thus, the dynamic balance of gut microbiota might play a vital role in the toxicity of TCM.

Moreover, CF-induced gut microbiota dysbiosis with an obvious increase in the abundance of *Clostridia_UCG-014*, which is a conditional pathogen related to many diseases (Wells and Wilkins 1996). Additionally, the relative abundance of *Eubacterium_coprostanoligenes_group* and *Oscillospiraceae* was also increased in CF groups, which are considered to be related to liver damage (Wang et al. 2021a) and liver diseases (Low et al. 2022; Zhao et al. 2022). LEfSe analysis also suggested that s_*Clostridia_UCG-014*, *s_Eubacterium_coprostanoligenes_group*, and *s_Oscillospiraceae_UCG-005* were the dominant bacteria that changed significantly after CF treatment. The spearman correlation analysis also suggested that *Oscillospiraceae_UCG-005*, *Oscillospiraceae_NK4A214_ group*, and *Clostridia_UCG-014* were positively correlated with the toxic indices. Thus, the enrichment of *Clostridia_UCG-014*, *Oscillospiraceae_UCG-005*, and *Eubacterium_coprostanoligenes_group* which accounted for the changes in *c__Clostridia* might play a vital role in the toxicity of CF.

Metabonomics is an effective means to study the toxicity and mechanism of traditional medicine through the changes of endogenous metabolism (Duan et al. 2018). We used non-targeted serum metabonomics to analyze the toxicity of CF, and 9 endogenous metabolites and 9 metabolic pathways were detected. Among them, glycerophospholipid metabolism and glyoxylate and dicarboxylate metabolism may be closely related to the toxicity of CF. As an important part of cell membrane, glycerol phospholipids can affect cancer, immunity, and inflammation by regulating signal pathways (Wang et al. 2021c; Wu et al. 2022). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are phospholipids, which are the basic building components of the lipid bilayer membrane and help to repair damaged cell membranes (Patel and Witt. 2017). They function as signaling molecules, sending messages between cells and controlling inflammation (Wu et al. 2016). Compared to the N group, the levels of PE(20:0/24:1(15Z)), PE(22:2(13Z,16Z)/P-18:0), and PC(14:0/16:0) were significantly down-regulated in the CH group. The dynamic balance of PC and PE is strictly maintained by hydrolysis and synthesis to ensure a consistent quantity of phospholipids in the cell membrane (Xu et al. 2021). It has been found that the decrease in PC and PE levels may have an effect on cellular signal transmission and inflammatory responses, potentially leading to the development of inflammatory diseases (van der Veen et al. 2017; Choi et al. 2022). Besides, PE (20:0/24:1(15Z)), PE (22:2(13Z,16Z)/P-18:0), and PC (14:0/16:0) were negative correlated with TNF-α, IL-1β, *Oscillospiraceae_UCG-005*, and *Clostridia_UCG-014*. These findings are consistent with the previous results and further support the correlation between phospholipids and inflammatory markers. Previous studies have revealed that the gut microbiota has a role in regulating host metabolism and the response to lipids. However, the mechanisms underlying the effect of microbiota that influence metabolism are not yet fully understood (Kayser et al. 2019; Ko et al. 2020).

In summary, the experiment showed that CF could cause obvious liver and gastrointestinal injury in rats, and its toxicity may be related to the disturbance of intestinal flora and serum metabolism. This study is one of the few that includes the gut microbiota and metabolites for the toxicity of CF and provides a new idea for the research and clinical application of toxic TCM. However, its molecular biological effects and underlying mechanism remain unclear. Microbial markers-metabolites-toxicity interaction are much more complex, and further studies aimed at revealing the mechanism are necessary.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00253-023-12763-2.

Author contribution JLW carried out the experiments, analyzed the data, and wrote the paper. ZFJ and WP helped with performing experiments and analyzing data. JH wrote a thorough assessment of the text and designed the study. All authors read and approved the manuscript.

Funding Financial support for this study was provided by the National Natural Science Foundation of China (No. 81873083, 82374137).

Data availability This article, as well as its supplementary information files, contains all of the data that was created or analyzed during the study.

Declarations

Ethical approval The care and use of animals in this study complied with all applicable international, national, and institutional guidelines.

Conflict of interest The authors declare no competing interests.

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