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RESEARCH ARTICLE

Abrus Cantoniensis Alleviated *Escherichia coli* Induced Intestine Damage in Mice Through Modulating Gut Microbiota

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A B S T R A C T

This study investigated the therapeutic efficacy of *Abrus cantoniensis* on intestine damages in mice caused by *Escherichia coli.* Thirty Kunming mice were randomly assigned to control group (C), infection group (M), and *Abrus cantoniensis* intervention group (J). Animals in group J were orally administered of *Abrus cantoniensis* (100mg/kg/day) for 14 days, while the control and infection groups were given an equivalent volume of normal saline. On day 15, mice in group M and J were intraperitoneally inoculated with *Escherichia coli*. Twenty-four hours later, all mice were euthanized for collection of blood, organ, and intestinal samples. Results found that *Abrus cantoniensis* decreased diarrhea and mortality rates, increased body weight,and decreased liver weights and bacterial loads in intestines. Histopathological analysis of ileum showed that *Abrus cantoniensis* improved intestine villi and crypt structure, and reduced inflammatory cells infiltration. Furthermore, 16S rRNA sequencing of the gut microbial community illustrated that *Abrus cantoniensis* intervention increased the abundance of beneficial bacterial taxa, including *Amulumruptor*, *Eubacterium_F*, *CAG_269*, *Ruminiclostridium_E*, *Faecousia*, *CAG_510*, and *Ruminococcus_E*. These findings indicate that *Abrus cantoniensis* protects against *E. coli*-induced intestinal injury by ameliorating intestinal damage, reducing bacterial burdens, and regulating gut microbiota composition.

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INTRODUCTION

Escherichia coli is a major pathogenic bacterium responsible for foodborne diseases. It poses a significant zoonotic threat and can be transmitted to humans through both direct and indirect routes (Saidenberg *et al*., 2024). Taxonomically, *E. coli* belongs to the family *Enterobacteriaceae* and includes numerous serotypes, some of which are highly pathogenic to both humans and animals (Bartlitz *et al*., 2022; Rehman *et al*., 2022). Contaminated meat, dairy products, vegetables, and drinking water are considered major sources of *E. coli* infection, making it a serious concern for food safety and public health (Rangel *et al*., 2005; Devleesschauwer *et al*., 2019).

Pathogenic *E. coli* strains can attach to and infiltrate intestinal epithelial cells, resulting in breakdown of intestinal barrier integrity and release of different virulence factors such as Shiga toxins and enterotoxins. In addition, the increasing emergence of antibiotic-resistant strains has further complicated the treatment and control of *E. coli* infections (Wang *et al*., 2023). Due to its wide host range, high prevalence, and growing antimicrobial resistance, *E. coli* continues to pose a major global public health challenge (Jiang *et al*., 2022). Therefore, there is an urgent need to explore alternative therapeutic agents for the prevention and management of *E. coli* infections.

The medicinal leguminous plant *Abrus cantoniensis* is well-known for its abundance of bioactive substances,

including as alkaloids, polysaccharides, and flavonoids These phytochemicals have been reported to exhibit anti-inflammatory, antioxidant, and intestinal protective properties, suggesting its potential as a natural therapeutic agent (Yang *et al*., 2014; Qu *et al*., 2022; Wu *et al*., 2022).The water-soluble polysaccharides in *Abrus cantoniensis* can stimulate immune cells, including macrophages and lymphocytes, boost the body's capacity to fend off bacterial intrusion, and simultaneously enhance intestinal flora equilibrium (Qu *et al.*, 2022). Furthermore, *Abrus cantoniensis* coumarin derivatives can reduce oxidative stress and intestinal tissue damage by modulating the mitogen-activated protein kinase (MAPK) signaling pathway (Sun *et al*., 2022). *Abrus cantoniensis's* alkaloid components also show some antibacterial activity, preventing the growth and reproduction of intestinal pathogenic bacteria like *Salmonella spp*. and *Escherichia coli (E. coli)* and directly lowering the invasion of intestinal mucosa by pathogenic bacteria (Yang *et al*., 2014).

As the body's largest digestive organ, the intestine performs a pivotal function in overall health (Zeiser *et al.*, 2022). Currently, over 1,000 gut microbial species have been identified in the human intestine, including prokaryotes and eukaryotes (Rajilić-Stojanović and de Vos, 2014), which exert significant effects on the host's immune function, nutrient absorption, metabolism, and overall wellbeing (Jumpertz *et al.*, 2011; Belkaid and Hand, 2014; Lawrence and Hyde, 2017). Once microbiota dysbiosis occurs, it may induce various diseases (Akagawa and Kaneko, 2022). The gut microbiota is stable under normal physiological settings, but pathological abnormalities cause a number of health issues. (Fassarella *et al.*, 2021). The present study seeks to establish an *Escherichia coli* (*E. coli*)-infected in vivo mouse model to elucidate the therapeutic impacts of *Abrus cantoniensis* on intestinal injury induced by *E. coli* infection. By evaluating indicators such as body weight changes, bacterial loads in various organs, intestinal histopathological examinations, and gut microbiota composition, this study ultimately offers a scientific foundation for developing new approaches for the prophylaxis and therapy of *E. coli*-induced infectious diarrhea. Additionally, it offers a foundation and theoretical backing for the continued use of *Abrus cantoniensis* in the management of piglets' diarrhea caused by Escherichia coli.

MATERIALS AND METHODS

Ethics statement: All animal experiments were approved by Guangxi University's Animal Ethics Committee (Approval No. GXU-2025-317) and carried out in compliance with the regulations for the care and use of laboratory animals.

Preparation of *Abrus cantoniensis* Extract: *Abrus cantoniensis* fresh aerial sections were taken in, cleaned, and allowed to dry in the shade. The plant material was pulverized into a fine powder when it was completely dried. Soxhlet extraction was used to extract one hundred grams of the powder using 1L of a 70% ethanol solution for eight hours. A solid extract was made by concentrating the filtrate under low pressure using a rotary evaporator at 40°C and then drying it in a vacuum oven. Before being given to the mice, the extract was dissolved in normal saline and kept at 4°C.

Animal experiment: Thirty Kunming mice were purchased from Guangxi Medical University's Laboratory Animal Center and bred at Guangxi University's Animal Center. All mice were acclimatized for one week under identical environmental and dietary conditions before initiation of the experiment to minimize baseline physiological and microbiota variations. Following a week of acclimatization, the mice were divided into three groups at random: treatment group (J), infected group (M), and control group (C). Mice in groups C and M were given an equivalent volume of normal saline, while mice in group J were given an oral gavage of *Abrus cantoniensis* at a dose of 100mg/kg body weight from day 0 to day 14 (Xu *et al.,* 2024). Multidrug-resistant *Escherichia coli* (10^9 CFU per mouse; strain PP859186, courtesy of Dr. Kun Li, Nanjing Agricultural University) was injected intraperitoneally into the treatment and infected groups on the 15th day. Control group (C) was not challenged with *E. coli*. Twenty-four hours after infection, all animals were euthanized for collection of blood, intestinal segments, and other organs for subsequent analyses (Fig. 1a).

Histopathology: Ileal tissue segments of approximately 0.5-1cm were extracted from mice in each group and preserved in 4% formaldehyde for 48 hours. Following fixation, the tissues regularly processed and stained with hematoxylin and eosin (H&E) by Wuhan Pinuofei Biotechnology Co., Ltd. (China). An Olympus CX33 microscope (Olympus Corporation, Tokyo, Japan) was used for the histopathological evaluation, which included an assessment of villus structure and tissue damage.

Gut microbiota sequencing: Total microbial DNA was isolated from the rectal specimens of mice assigned to groups J (n=6), M (n=6), and C (n=6) using the Fast DNA Spin Kit for Feces. The purity and concentration of the isolated DNA were determined using the YSNano-100 spectrophotometer and 1.5% agarose gel electrophoresis. The 338F-806R primer pair (F: 5'-ACTCCTACGGGAG GCAGCAG-3'; R: 5'-GGACTACHVGGGTWTCTAT-3') was used to amplify the V3-V4 hypervariable region of the 16S rRNA gene (Xu *et al*., 2025). The Hieff NGS® OnePot II DNA Library Prep Kit for Illumina® was used to create sequencing libraries from the resultant amplicons, after which Bioyi Biotechnology Co., Ltd. performed microbiota sequencing on the Illumina platform.

Bacterial load in intestinal organs: Duodenal, jejunal, ileal, cecal, and colonic tissues were aseptically excised under sterile conditions. Every tissue sample was transferred into an aseptic 4mL centrifugation tube prefilled with 1mL of sterile phosphate-buffered saline and 1mm zirconia grinding beads. Homogenization of the samples was carried out using a bead-beater homogenizer at 4°C and 70Hz for 2minutes. Subsequently, 100µL of the resulting supernatant was subjected to serial dilution in PBS, and 100µL of the optimally diluted bacterial

suspension after dilution was evenly spread across the surface of agar plates. The inoculated agar plates were subjected to incubation at 37°C for 18hours to facilitate bacterial colony enumeration.

Statistical analysis: The statistical analysis was carried out using IBM SPSS version 26.0. ANOVA was used to assess differences between groups, followed by the Student's t-test. Data from the study were given as mean ± SD. Statistical significance was determined at P<0.05.

RESULTS

***Abrus cantoniensis* mitigate diarrhea in mice challenged by *E. coli*:** Group J had a lower incidence of diarrhea and mortality than group M (Fig. 1b). Compared to the control group, mice in group M had varying degrees of body weight reduction (P<0.0001), while *Abrus cantoniensis* increased body weight in group J (P<0.05) (Fig. 1c). Examination of liver, heart, spleen, lungs, and kidneys revealed that group M had significantly greater liver weights than groups C and T (P<0.05) (Fig. 1d).

Bacterial burden analysis revealed that group M had significantly higher bacterial loads in the ileum (P<0.01), cecum (P<0.05), and colon (P<0.05), whereas *Abrus cantoniensis* significantly reduced bacterial loads in the ileum (P<0.05), cecum (P<0.05), and colon (P<0.05) (Fig 1e). There was no substantial difference in bacteria burdens between the duodenum and jejunum.

Intestinal histopathological analysis: Histopathological analysis of ileum showed that *E. coli* damaged the integrity of intestinal villi, changed the morphology of crypt, and caused infiltration of inflammatory cells in group M, while mice supplemented with *Abrus cantoniensis* presented ameliorative intestine villi and crypt, and decreased inflammatory cells in group J (Fig. 2).

Analysis of intestinal bacterial diversity: To evaluate variations in microbial community diversity across different cohorts, targeted alpha diversity analyses were implemented (Fig. 3a). The Good's coverage indices across the three groups ranged from 99.3 to 99.8%, suggesting that the sequencing data were sufficient to represent the complete bacterial community structure. Regarding the α-diversity indices (which reflect species richness), the highest values were observed in the M

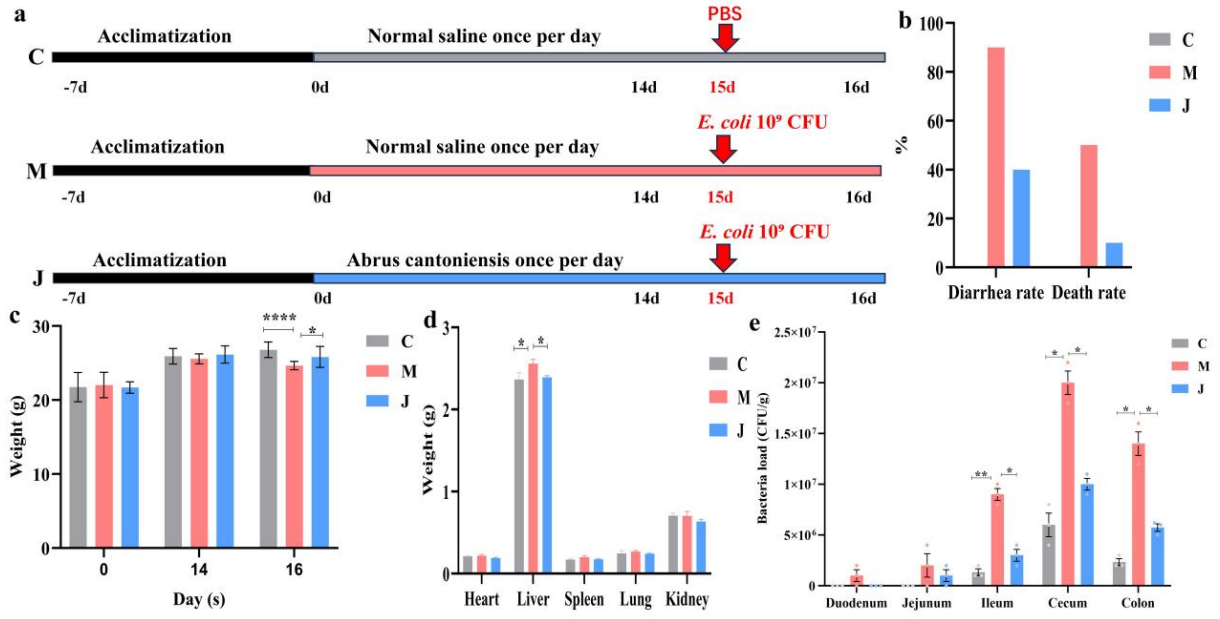

Fig. 1: The influence of *Abrus cantoniensis* on *Escherichia coli* infected mice. a: Experimental design, b: Diarrhea rate and mortality rate of mice, c: Body weight of animals, d: Organ weight of animals, e: Bacterial loads of mice. Data are presented as mean ± SEM (n=10). ****P<0.0001, *P<0.05.

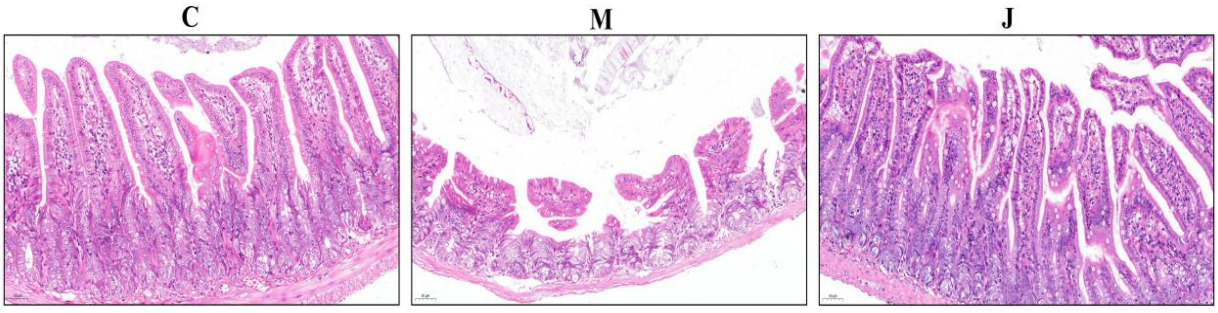

Fig. 2: Pathological analysis of ileum of mice in different groups via H&E staining.

group, followed by the J group, with the lowest values detected in the C group. The Shannon index, an indicator that accounts for both species' richness and evenness, was similarly the highest in the C group, followed sequentially by the J group and the M group.

With increasing sequencing reads, both the rarefaction curves (Fig. 3b) and rank-abundance curves (Fig. 3c) gradually approached a plateau, suggesting that the sequencing depth was adequate to represent the microbial community structure and diversity. These findings indicate that the current sequencing data were sufficient for downstream analysis.

Beta diversity was further evaluated using Principal Coordinate Analysis (PCoA) based on Bray–Curtis distance and Non-metric Multidimensional Scaling (NMDS) based on the Jaccard index. The PCoA plot showed clear separation between the *E. coli*-challenged model group and the control and treatment groups ($P<0.01$), indicating that *E. coli* infection significantly altered the intestinal microbial composition. Similarly, NMDS analysis produced a stress value of <0.2, confirming that the analysis reliably reflected differences among samples (Fig. 4a, 4b). Overall, these results suggest that *E. coli* infection markedly affected the composition and structure of intestinal microbiota in mice.

Analysis of intestinal bacterial community composition: At the phylum level, Bacteroidota (67.72%), Firmicutes_A (20.31%), and Firmicutes_D (10.41%) constituted the primary phyla within group C, Bacteroidota (74.38%), Verrucomicrobiota (12.28%), and Firmicutes_D (5.11%) were mainly found in group M, while Bacteroidota (70.94%), Firmicutes_A (15.01%), and Firmicutes_D (10.73%) were the dominating phyla in group J (Fig. 5b). At the class level, Bacteroidia (67.72%), Clostridia_258483 (20.30%), and Bacilli (10.41%) were the primary classes in group C, Bacteroidia (74.38%), Verrucomicrobiae (12.28%), and Bacilli (5.11%) were the primary classes in group M, while Bacteroidia (70.94%), Clostridia_258483 (15.00%), and Bacilli (10.73%) constituted the primary classes within group J (Fig. 5c). At the order level, Bacteroidales (67.66%), Lachnospirales (15.62%), and Lactobacillales (9.64%) were the main orders in group C, Bacteroidales (74.37%), Verrucomicrobiales (12.29%), and Lactobacillales (2.66%) were mainly detected in group M, while Bacteroidales (71.05%), Lachnospirales (7.81%), and Oscillospirales (5.56%) were the dominating orders in group J (Fig. 5d). At the family level, Muribaculaceae (62.74%), Lachnospiraceae (15.72%), and Lactobacillaceae (9.66%) were the primary families in group C, Muribaculaceae (62.76%), Akkermansiaceae (12.39%), and Bacteroidaceae (9.03%) were the main families in group M, while Muribaculaceae (67.61%), Lachnospiraceae (7.81%), and Erysipelotrichaceae (4.85%) were the primary families in group J (Figure 5e).At the genus level, *Paramuribaculum* (17.03%), *Duncaniella* (12.76%), and *UBA7173* (10.74%) were the stable genera in group C, *Paramuribaculum* (18.80%), *Akkermansia* (14.65%), and *Duncaniella* (10.58%) were the primary genera in group M, while *Paramuribaculum* (24.33%), *Amulumruptor* (12.29%), and *UBA7173* (8.35%) were mainly examined in group J (Fig. 5f).Additionally, the three cohorts shared a common set of 371 ASVs among their respective microbial communities. (Figure 5a).

Multiple t-tests were conducted to quantify variations in bacterial community composition among groups at both the phylum and genus taxonomic levels. At the phylum level, the proportions of both Verrucomicrobiota ($P<0.05$) and Proteobacteria ($P<0.05$) exhibited statistical significance, with their abundances in the model group being significantly higher than those in the control group (Fig. 6a). At the genus level, compared with the control group, the model group exhibited significantly increased abundances of *Akkermansia*, *Alloprevotella*, *Turicimonas*, *Parasutterella*, *CAG_1031*, and *RUG13077* ($P<0.05$), while the abundances of *Lactobacillus*, *CAG_269*,

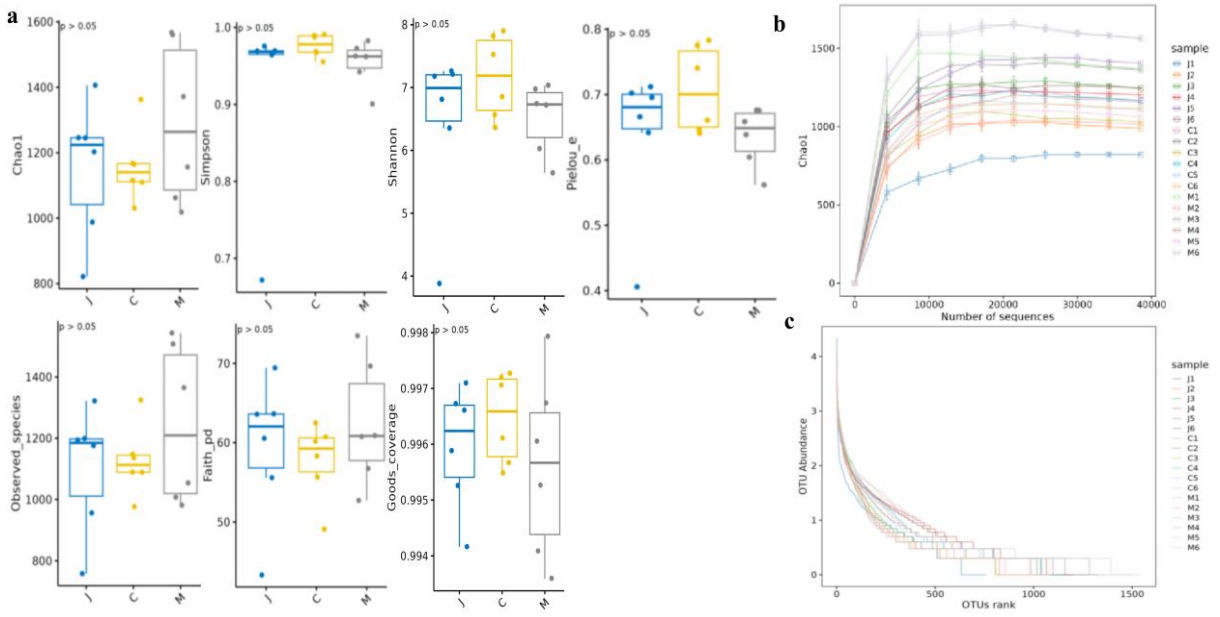

Fig. 3: Alpha diversity analysis of mice. a: Diversity indexes, b: Rarefaction curve; c: Rank abundance curve.

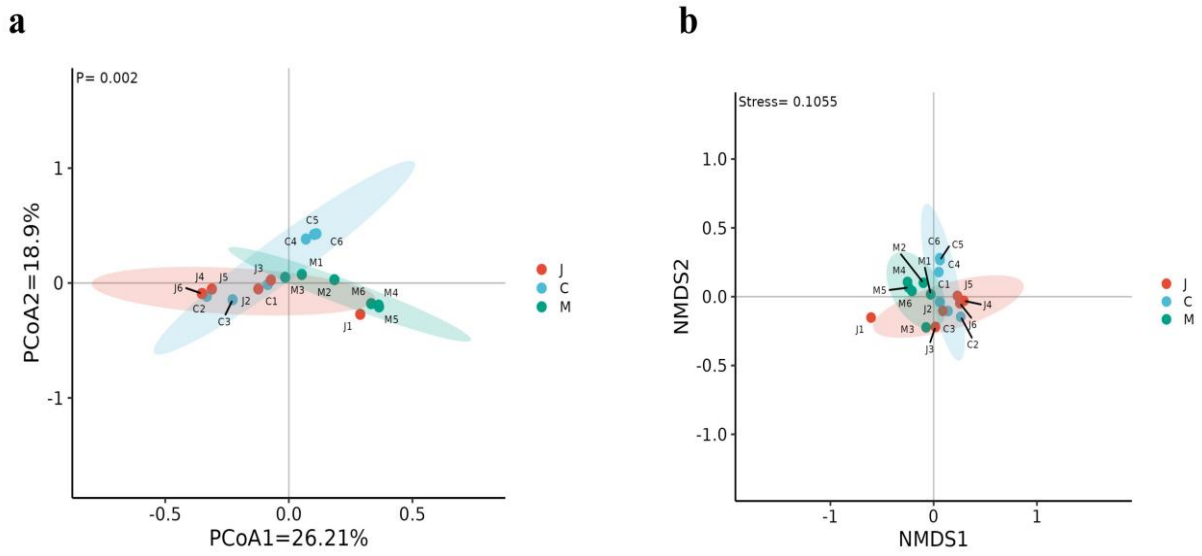

Fig. 4: Beta diversity analysis of mice. a: Principal coordinate analysis (PCoA), b: Non-metric multidimensional scaling (NMDS).

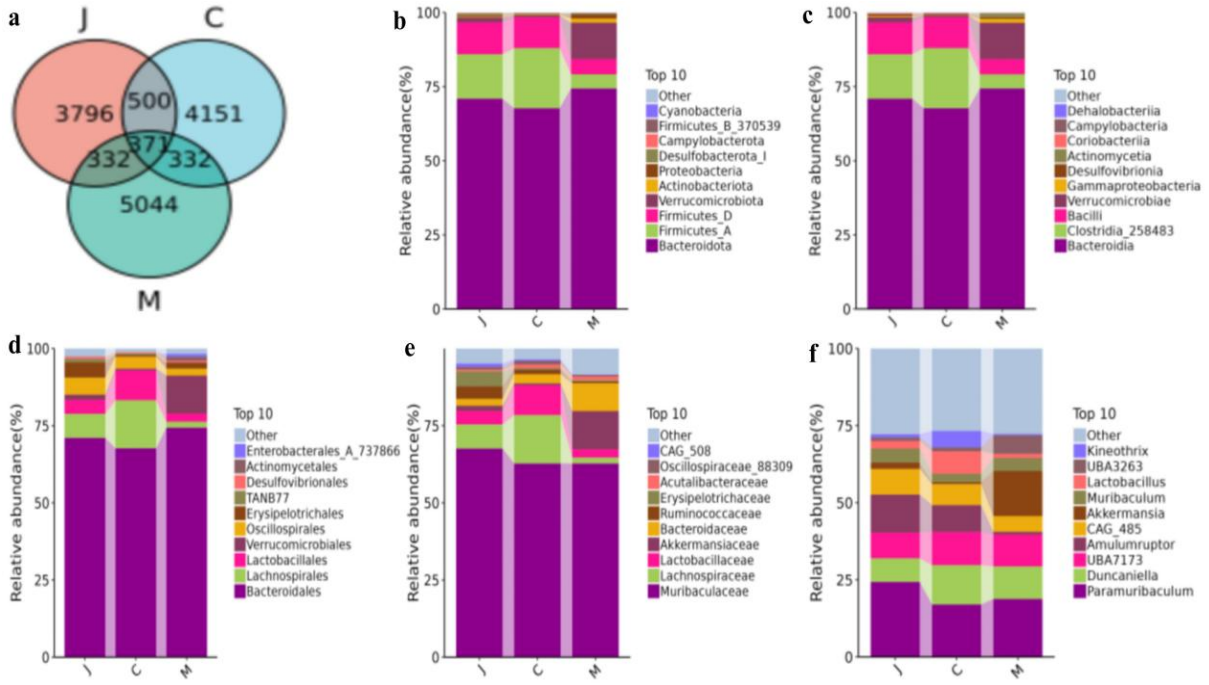

Fig. 5: Venn diagram and intestinal microbiota of mice in different taxa. a: Venn diagram, b: Phylum; c: Class, d: Order, e: Family, f: Genus.

Tidianibacter, Eubacterium_G, and *Dwaynesavagella* were significantly decreased (P<0.05). In comparison to the treatment group, the model group showed significantly elevated abundances of *Alloprevotella*, *Parabacteroides_B_862066*, *Limivicinus*, *RUG13077*, and *Anaerofustis* (P<0.05), whereas the abundances of *Amulumruptor*, *Eubacterium_F*, *CAG_269*, *Ruminiclostridium_E*, *Scybalousia*, *Faecousia*, *CAG_632*, *CAG_510*, and *Ruminococcus_E* were significantly reduced (P<0.05). Relative to the control group, the treatment group displayed significantly increased abundances of *CAG_273*, *CAG_632*, *Limivicinus*, *CAG_510*, and *Choladousla* (P<0.05), while the abundances of *Duncaniella*, *CAG_873*, *Eubacterium_G*, and *Dwaynesavagella* were significantly lowered (P<0.05) (Fig. 6b).

To visualize the marker species and their intergroup relationships, we employed LDA-derived histograms to characterize variations in microbial community composition. In contrast, the hierarchical distribution of these taxa was depicted through taxonomic lineage maps. When analyzed at the genus level, the control cohort exhibited the characteristic feature of significant genera such as *Lactobacillus* and *CAG_873*, while the model group featured *UBA3263*, *Parabacteroides_B_862066*, and *Klebsiella_724518* as key discriminative species. In the treatment group, *Amulumruptor*, *Turicibacter*, and *UMGSI994* served as unique marker taxa (Fig. 7).

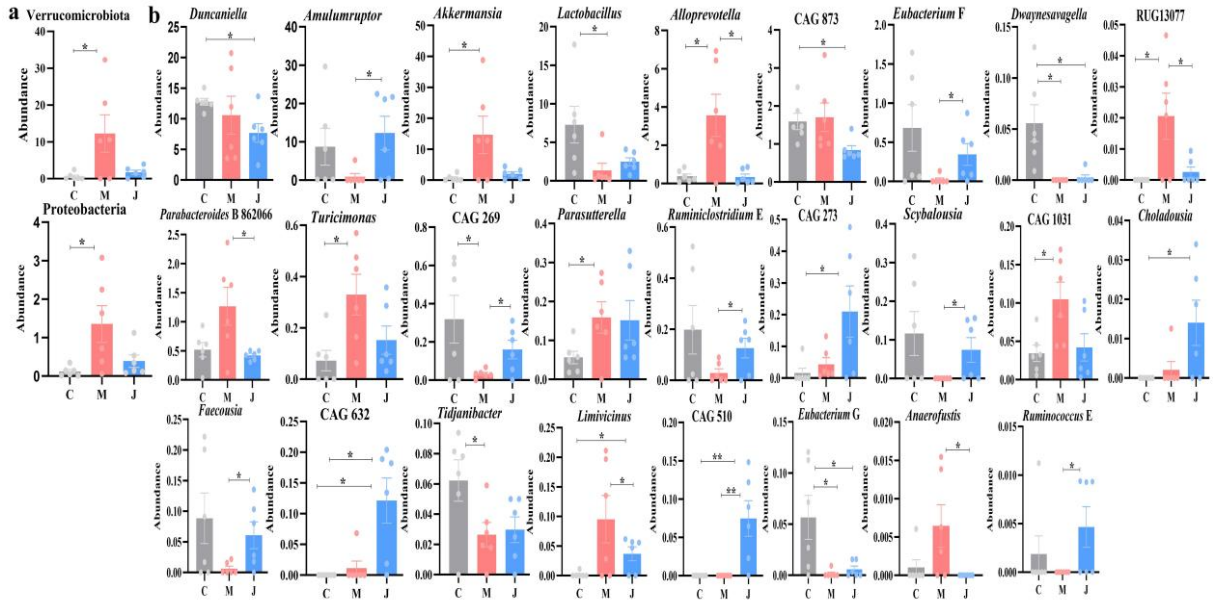

Fig. 6: Revealing different bacteria in different mice groups. a: Phylum, b: Genus. Data are presented as mean ± SEM (n= 6), **P<0.01, *P<0.05.

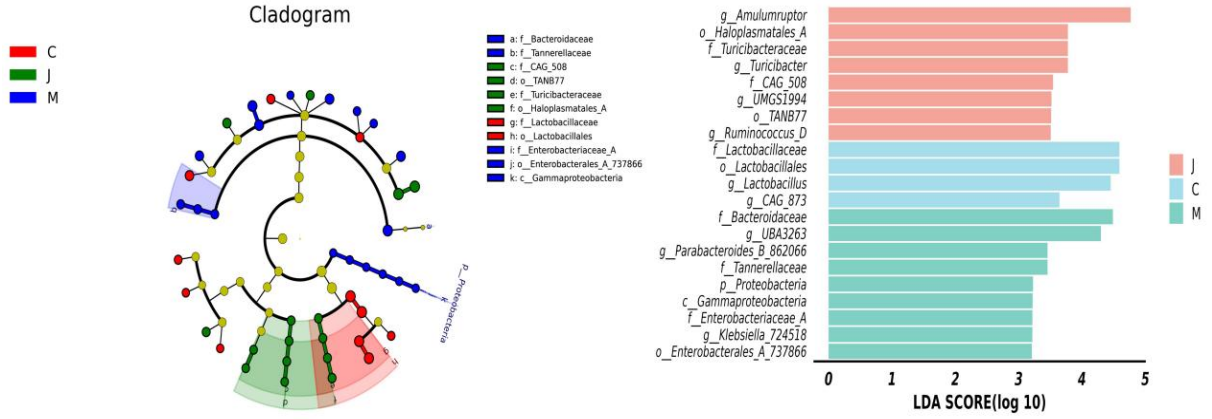

Fig. 7: Linear discriminant analysis effect size (LEfSe) and LDA scores for intestinal bacterial microbiota.

DISCUSSION

Escherichia coli (*E. coli*) commonly contaminates food of animal origin, fresh produce, and drinking water, posing a serious public health concern worldwide (Fegan and Gobius, 2013; Alhadlaq *et al*., 2024). Antibiotics remain the main treatment option for *E. coli* infections; however, their excessive and inappropriate use has contributed to the emergence of multidrug-resistant strains. In addition, antibiotic therapy may disrupt intestinal barrier integrity and lead to gut microbiota imbalance (Feng *et al*., 2019). Therefore, there is increasing interest in identifying alternative therapeutic strategies, particularly those derived from medicinal plants. *Abrus cantoniensis*, a traditional Chinese medicinal herb, has shown potential in protecting intestinal health by inhibiting pathogenic bacteria and reducing intestinal damage caused by infections (Wang *et al*., 2022; Lin *et al*., 2025). Previous studies have also reported that total flavonoids extracted from *Abrus cantoniensis* can inhibit the growth and adhesion of pathogenic *E. coli* (Sun *et al*., 2022).

In the present study, a mouse model of *E. coli*-induced enteritis was established to evaluate the protective effects of *Abrus cantoniensis* and explore its possible mechanisms. The results showed that *Abrus cantoniensis* significantly reduced diarrhea and mortality in infected mice. *E. coli* infection also caused a marked decrease in body weight, which is consistent with earlier findings (Ledwaba *et al*., 2020). Furthermore, bacterial load analysis revealed significantly higher intestinal colonization in the infected model group compared with the control group. Treatment with *Abrus cantoniensis* improved body weight and reduced bacterial loads in infected mice (Fig. 1). Histopathological examination showed severe intestinal damage in *E. coli*-infected mice, including villus destruction, distorted crypt architecture, and inflammatory cell infiltration. However, these pathological changes were notably improved following treatment with *Abrus cantoniensis* (Fig. 2).

Analysis of gut microbiota using 16S rRNA sequencing showed that the relative abundance of Verrucomicrobiota and Proteobacteria increased significantly in the infected model group at the phylum level. An increase in Proteobacteria is often associated with intestinal dysbiosis and inflammatory conditions (Shin *et al*., 2015; Rizzatti *et al*., 2017). At the genus level, *Abrus cantoniensis* treatment significantly increased the abundance of *Amulumruptor* compared with the infected group. This increase suggests a potential role of *Abrus cantoniensis* in restoring microbial balance and improving intestinal microecological stability following *E. coli* infection. Additionally, the abundance of *Eubacterium_F* was markedly elevated in the treatment group. This genus is generally considered beneficial and is associated with intestinal health and metabolic balance (Louis *et al*., 2014; Mukherjee *et al*., 2020). Similarly, the relative abundances of *RuminiclostridiumE*, *Faecousia*, *CAG_510*, and *Ruminococcus_E* were also significantly increased. These genera are commonly regarded as beneficial microbes and are linked to improved intestinal health. These bacteria are known to contribute to intestinal health by producing short-chain fatty acids (SCFAs), enhancing intestinal barrier integrity, and suppressing inflammatory responses. For instance, Ruminococcus and Ruminiclostridium species are involved in fiber fermentation and SCFA production, which play important roles in maintaining gut homeostasis and reducing intestinal inflammation (Wang *et al*., 2020; Li *et al*., 2021; Li *et al*., 2025).

The therapeutic effects observed in the present study are comparable to those reported for other natural interventions used against E. coli-induced intestinal injury. Previous studies have shown that probiotics such as Lactobacillus spp. and plant-derived compounds including flavonoids and polysaccharides can reduce intestinal inflammation, restore microbial balance, and improve intestinal morphology in infected animals (Davoodabadi *et al*., 2015; Qassadi *et al*., 2023). Similar to these reports, *Abrus cantoniensis* treatment in the current study decreased bacterial loads, improved intestinal villus structure, and increased the abundance of beneficial microbiota. These findings suggest that *Abrus cantoniensis* exhibits therapeutic potential comparable to other natural agents and may serve as an alternative strategy for managing E. coli-associated intestinal disorders.

Overall, the findings of this study suggest that *Abrus cantoniensis* can alleviate *E. coli*-induced intestinal damage by reducing bacterial colonization, improving intestinal morphology, and restoring gut microbiota balance. The increase in beneficial bacterial populations further supports its protective role in maintaining intestinal health. These results highlight the potential of *Abrus cantoniensis* as a promising alternative therapeutic approach for the management of *E. coli* infections.

Although this study demonstrates therapeutic efficacy, further investigations involving molecular pathways such as inflammatory signaling, intestinal barrier proteins, and immune regulation are necessary to fully elucidate the underlying mechanisms.

Conclusions: This study revealed that *Abrus cantoniensis* reduces intestinal injury, lowers bacterial loads, and modulates gut microbiota, providing evidence for its potential therapeutic application.

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Authors contribution: Conceptualization of the idea was made by QZ, JB, and XW, and methodology was designed by QZ, JB, and XW. The investigation was conducted and original draft was prepared by YG, YZ, MI, YW, and WS, while review and editing were done by MI, YW, and WS. Visualization by done by YH, QZ and JW, while the study was supervised by YH, QZ, WS and JW.

Data availability statement: The sequencing data from this investigation has been deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA1377910.

Competing interests: There is no competing interest.

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