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RESEARCH ARTICLE

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Colchicine disrupts bile acid metabolic homeostasis by affecting the enterohepatic circulation in mice

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Abstract

Although the medicinal properties of colchicine (COL) have been widely known for centuries, its toxicity has been the subject of controversy. The narrow therapeutic window causes COL to induce gastrointestinal adverse effects even when taken at recommended doses, mainly manifested as nausea, vomiting, and diarrhea. However, the mechanism of COL-induced gastrointestinal toxic reactions remains obscure. In the present study, the mice were dosed with COL (2.5 mg/kg b.w./day) for a week to explore the effect of COL on bile acid metabolism and the mechanism of COL-induced diarrhea. The results showed that COL treatment affected liver biochemistry in mice, resulting in a significant down-regulation of the mRNA expression levels of bile acid biosynthesis regulators *Cyp7a1*, *Cyp8b1*, *Cyp7b1*, and *Cyp27a1* in liver tissues. The mRNA expression levels of bile acid transporters *Ntcp*, *Oatp1*, *Mrp2*, *Ibabp*, *Mrp3*, *Osta*, and *Ostb* in liver and ileum tissues were also significantly down-regulated. In addition, COL treatment significantly inhibited the mRNA expression levels of *Fxr* and its downstream target genes *Shp*, *Lrh1*, and *Fgf15* in liver and ileum tissues, affecting the feedback regulation of bile acid biosynthesis. More importantly, the inhibition of COL on bile acid transporters in ileal and hepatic tissues affected bile acid recycling in the ileum as well as their reuptake in the liver, leading to a significantly increased accumulation of bile acids in the colon, which may be an important cause of diarrhea. In conclusion, our study revealed that COL treatment affected bile acid biosynthesis and enterohepatic circulation, thereby disrupting bile acid metabolic homeostasis in mice.

KEYWORDS
bile acids, colchicine, enterohepatic recirculation, metabolomics, toxicity

1 | INTRODUCTION

Colchicine (COL) is an ancient anti-inflammatory drug widely used in the treatment of gout, as well as in the treatment of familial Mediterranean fever, Behcet's disease, cardiovascular diseases, and inflammatory diseases (Deftereos et al., 2022; Imazio, 2015; Leung et al., 2015). The pharmacological action of COL is primarily to inhibit the onset and amplification of the inflammatory response in humans by affecting inflammatory cells and mediators of inflammatory cell activation, which is inextricably linked to the unique role of COL in cellular mitosis (Huber et al., 2023; Leung et al., 2015). COL can bind to free intracellular tubulin dimers to form tubulin dimers-COL complexes, which can be added to the growing ends of microtubules, poisoning microtubule dynamics, interfering with microtubule elongation,

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and disrupting the mitotic spindle, ultimately preventing cell division (Dasgeb et al., 2018; Hastie, 1991). However, compared with the clearer pharmacological action, the toxicological mechanisms of action of COL in the body are relatively lacking, especially the common gastrointestinal adverse reactions (nausea, vomiting, and diarrhea) after COL administration have been the focus of controversy (Slobodnick et al., 2015). Although these adverse effects will resolve with the cessation of COL, it can also lead to a very unpleasant experience for patients, and even more torturous for those who need to take COL for a long time. In addition, accidental ingestion of COL-containing plants or overdose of COL is extremely dangerous; if there are no effective treatments following poisoning episodes, multiple organ failure and even death could result (Cozza et al., 2021). Therefore, an in-depth study of the toxicological properties of COL in vivo has become more necessary and urgent, which will be conducive to the further promotion and safe application of COL in clinical practice.

COL is a tricyclic lipopolysaccharide alkaloid that is rapidly absorbed in the jejunum and ileum after oral administration and undergoes extensive first-pass metabolism in the liver (Cocco et al., 2010). Next, cytochrome P3A4 (CYP3A4) and P-glycoprotein in enterocytes and hepatocytes further metabolize COL to 2- and 3-demethylcolchicine, which is finally excreted via the kidneys (20%–40%) and bile (60%–80%) (Imazio & Nidorf, 2021; Niel & Scherrmann, 2006). Bile is secreted by the liver, and its main component is bile acids, which are the end products of cholesterol metabolism in the body (Joyce & Gahan, 2016). They are synthesized by cholesterol 7α-hydroxylase (Cyp7a1) in the classical pathway of hepatocytes or sterol 27-hydroxylase (Cyp27a1) in the alternative pathway of extrahepatic tissues and can then be secreted into the gallbladder for concentration and storage after binding with glycine, taurine, or sulfuric acid (Cai et al., 2022; Russell, 2009). After food intake in human, the gallbladder is stimulated by the enteric hormone cholecystokinin, bile acids into the duodenum which then undergo enterohepatic recirculation. Approximately 95% of the bile acids are reabsorbed in the ileum, whereas the remaining 5% of primary bile acids escape reabsorption in the distal ileum and enter the colon (Ridlon et al., 2006). The microbiota system in the gut is a powerful "organ" with the genetic potential to carry out thousands of chemical reactions that modify bile acids into countless forms, which greatly increases the diversity and biological function of bile acids (Guzior & Quinn, 2021; Santacroce et al., 2021). On the other hand, it has been reported that gut microbiota can affect the expression of Cyp7a1, oxysterol 7α-hydroxylase (Cyp7b1), and Cyp27a1 in mice (Cai et al., 2022). Cyp7a1 is a rate-limiting enzyme for bile acid biosynthesis, and its expression level directly determines the size of bile acid pool (Sayin et al., 2013). In addition, perturbations of gut microbiota can strongly affect bile acid metabolism, especially the inability of the host to metabolize some primary bile acids, resulting in accumulation of primary bile acids and reduction of secondary bile acids (Jiang et al., 2022). Abnormal changes of bile acids in the gut can induce diarrhea in the host (Alemi et al., 2013; Bunnett, 2014). Furthermore, a large number of studies have shown that drug treatment can significantly change the host's intestinal microbiota (Forslund et al., 2015; Freedberg et al., 2015; Javdan et al., 2020; Vich Vila et al., 2020), and our previous study also confirmed that COL treatment altered the composition of intestinal microbiota in mice and reduced its diversity (Shi et al., 2020). Given that COL is excreted mainly through bile, it is still unknown whether the changes in gut microbiota induced by COL treatment affect bile acid biosynthesis and metabolism. Therefore, it is necessary to reveal the effects of COL treatment on the metabolic pathway of bile acid in the host.

In this study, we systematically studied the effects of COL on bile acid biosynthesis, transport, feedback regulation, and metabolism using a variety of research strategies. We aimed to explore the potential mechanisms of gastrointestinal adverse reactions (diarrhea) caused by COL from the perspective of bile acid metabolism regulation, which will further enhance people's understanding of the toxicity characteristics of COL.

2 | MATERIALS AND METHODS

2.1 | Mouse husbandry and treatment

Male Kunming mice (5 weeks old, 22–25 g) were bred and sourced from the Experimental Animal Center of Lanzhou University (Gansu, China). The mice were housed in autoclaved conventional cages (three animals per cage) and acclimatized for a week at 22 ± 2°C, 50%–60% relative humidity, and a 12-h day/night rhythm. All animals were allowed ad libitum access to sterilized rodent maintenance chow and deionized water. After adaptation, 12 mice were randomly allocated into two groups. The mice in the first group were given 0.9% normal saline by gavage, the second group received COL daily (2.5 mg/kg b.w./day, Cat# 3915, Sigma-Aldrich) for a week based on our previous studies (Shi et al., 2021, 2022, 2020). COL was prepared with 0.9% NaCl solution, and the gavage volume of each mouse was determined based on its body weight. All experiments in this study were conducted in strict accordance with the ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals. The study was approved by the Ethics Committee of School of Life Sciences, Lanzhou University (Approval no: EAF2023052).

2.2 | Sample harvesting

On the eighth day, three pellets of fresh feces were collected from each mouse, weighed, and dried in an oven at 37°C for 24 h, the dry weight of feces was weighed, and the water content of feces was calculated. Each mouse was then placed in a clean cage, and the number of pellets or dots that the mice defecated within 2 h was counted to calculate the frequency of their defecation. Next, blood was taken from the posterior orbital sinuses of mice using capillary glass tubes. The blood was kept at 4°C overnight and centrifuged at 3500 rpm for 20 min. The supernatant was carefully collected to obtain mouse serum for assessment of relevant serum enzymes for the evaluation of hepatotoxicity. Finally, the mice were euthanized with CO_2

inhalation, and the liver tissue, ileum tissue, and colon contents were flash-frozen in liquid nitrogen and stored in −80°C for subsequent gene expression and bile acid content analysis. Furthermore, a fraction of liver (5 mm^2) needed to be fixed with 4% paraformaldehyde for 24 h to assess histological changes.

2.3 | Liver histological evaluation

After dehydration, the liver tissues were embedded in paraffin and cut into 6 μm serially sections by microtome and stained with hematoxylin and eosin (H&E). Images were acquired on a microscopy imaging system (AxioScope.A1, Oberkochen, Germany). The sections were submitted to professional pathologists for evaluation of pathological changes.

2.4 | Serum biochemical analysis

The serum levels of alanine aminotransferase (ALT, Cat# S03030), aspartate transaminase (AST, Cat# S03040), alkaline phosphatase (ALP, Cat# S03038), and total bile acid (TBA, Cat# S03074) were determined using commercially available kits (Rayto Life and Analytical Sciences Co., Ltd, Shenzhen, China).

2.5 | Real-time quantitative PCR

Total RNA was extracted from liver and ileum tissues by Trizol Reagent (Cat# 9109, TaKaRa, Japan). RNA concentration and absorbance ratio were determined by NanoDrop 2000, and the ratio of A260 to A280 should be between 1.8 and 2.0. cDNA was reverse transcribed with an iScript cDNA Synthesis Kit according to the manufacturer's protocol (Cat# KR118, TIANGEN, China). Real-time quantitative PCR reactions were performed on an Applied Biosystem instrument (ThermoFisher, Q5, USA) using SYBR Green SuperReal PreMix Plus (Cat# FP205, TIANGEN, China). The PCR conditions were set as follows: pre-denaturing at 94°C for 15 min, 40 cycles of denaturation at 95°C for 15 s, and annealing at 60°C for 32 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene, and the relative expression levels of the target genes were analyzed by the delta–delta Ct method. Primer sequences are shown in Table 1.

2.6 | Targeting bile acid metabolomics

The fecal samples were ground with liquid nitrogen and added with 200-μL pre-cooled ultra-pure water. The feces homogenate was added with 800-μL methanol/acetonitrile (1:1, v/v), vortex oscillated, sonicated in ice for 60 min, incubated at −20°C for 1 h to precipitate proteins, and then centrifuged at 14000×*g* at 4°C for 20 min. After vacuum drying, the supernatant was redissolved by adding 100-μL acetonitrile-aqueous solution (1:1, v/v), centrifuging at 14000×*g* at 4°C for 15 min. The final supernatant was collected for LC–MS/MS analysis.

The samples were separated by Agilent 1290 Infinity LC ultra-high performance liquid chromatography system. In brief, 2 μL of sample was injected onto a Waters ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm, 45°C) using an automatic injector

TABLE 1 Primer list for real-time quantitative PCR amplification.

Primer	Forward primer (5′–3′)	Reverse primer (5′–3′)
GAPDH	ACGGCAAATTCAACGGCACAG	AGACTCCACGACATACTCAGCAC
Cyp7a1	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
Cyp8b1	GGCTGGCTTCCTGAGCTTATT	ACTTCCTGAACAGCTCATCGG
Cyp7b1	TAGCCCTCTTTCCTCCACTCATA	GAACCGATCGAACCTAAATTCCT
Cyp27a1	GCCTCACCTATGGGATCTTCA	TCAAAGCCTGACGCAGATG
Akr1d1	TGCACACCACCAAATATCCCT	CTTCACTGCCACATAGGTCTTC
Fxr	TCCAGGGTTTCAGACACTGG	GCCGAACGAAGAAACATGG
Shp	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Fgf15	ACGTCCTTGATGGCAATCG	GAGGACCAAAACGAACGAAATT
Lrh1	TTGAGTGGGCCAGGAGTAGT	ACGCGACTTCTGTGTGTGAG
Ntcp	ATGACCACCTGCTCCAGCTT	GCCTTTGTAGGGCACCTTGT
Oatp1	CAGTCTTACGAGTGTGCTCCAGAT	ATGAGGAATACTGCCTCTGAAGTG
Mrp2	GGATGGTGACTGTGGGCTGAT	GGCTGTTCTCCCTTCTCATGG
Mrp3	TCCCACTTTTCGGAGACAGTAAC	ACTGAGGACCTTGAAGTCTTGGA
Bsep	CTGCCAAGGATGCTAATGCA	CGATGGCTACCCTTTGCTTCT
Ibat	ACCACTTGCTCCACACTGCTT	CGTTCCTGAGTCAACCCACAT
Ibabp	CAGGAGACGTGATTGAAAGGG	GCCCCCAGAGTAAGACTGGG
Osta	TGTTCCAGGTGCTTGTCATCC	CCACTGTTAGCCAAGATGGAGAA
Ostb	GATGCGGCTCCTTGGAATTA	GGAGGAACATGCTTGTCATGAC

(4°C). The mobile phase, consisting of 15-mM ammonium acetate aqueous solution (solvent A) and acetonitrile (solvent B), was delivered at a flow rate of 300 μL/min. The following solvent gradient was used: 90%–40% B, 0–18 min; 40%–90% B, 18–18.1 min; 90% B, 18.1–23 min. A QC sample is set up at certain intervals in the sample cohort to test and evaluate the stability and repeatability of the system. In addition, a mixture of bile acid standards was set up in the sample cohort for correction of chromatographic retention times.

The 5500 QTRAP mass spectrometer (AB SCIEX) was used for mass spectrometry in negative multiple reaction mode (MRM). Parameters were set as follows: source temperature, 450°C; ion Source

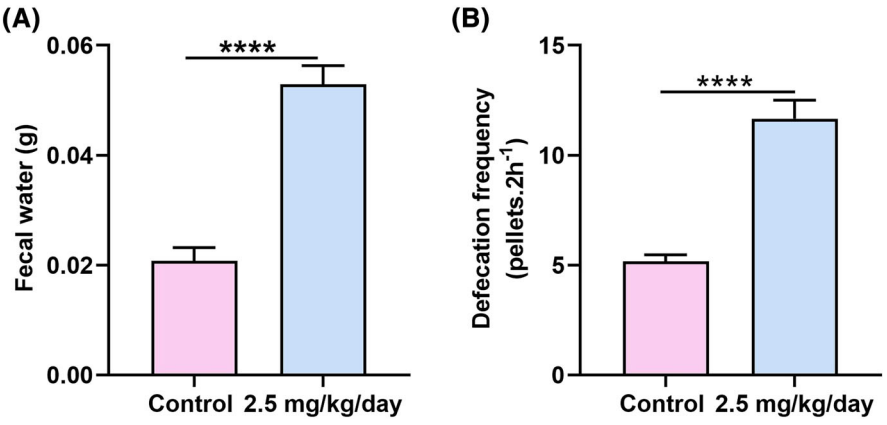

FIGURE 1 Evaluations of COL-induced diarrhea in mice. (A) Fecal water content. (B) Frequency of bowel movements. Data are represented by mean ± SEM, $n = 6$. **** denote $p < 0.0001$ compared with the control mice.

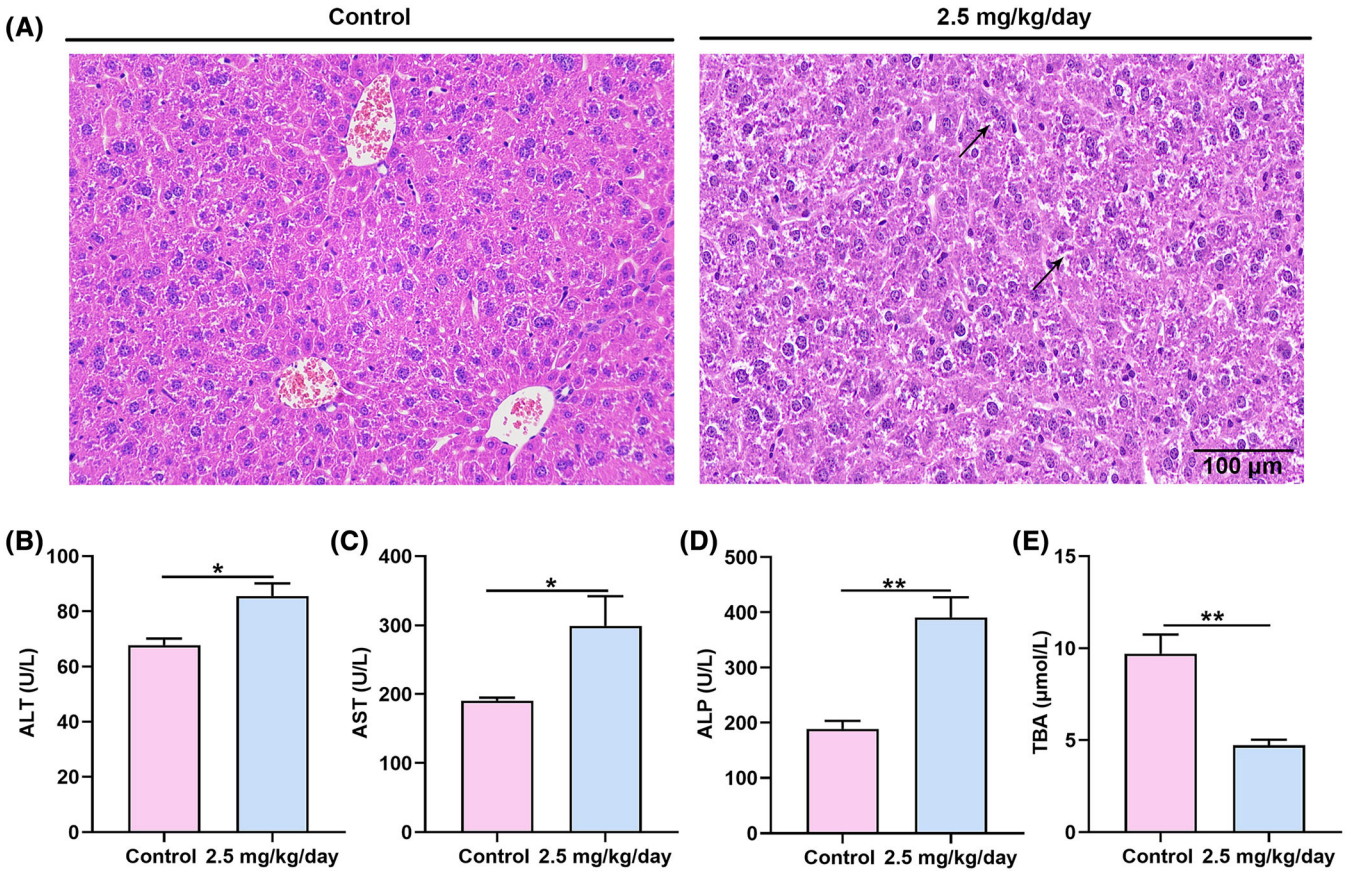

FIGURE 2 Effects of COL treatment on mouse liver. (A) Toxicity assessments of COL treatment on mouse liver morphology. The black arrows indicate hepatocyte granular degeneration, and the cytoplasm was loose and lightly stained. (B–E) Effects of COL treatment on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bile acid (TBA) in mice. Data were expressed as mean ± SEM, $n = 3$. * and ** denote $p < 0.05$ and $p < 0.01$ compared with the control mice, respectively.

Gas1, 45 psi; Ion Source Gas2, 45 psi; Curtain Gas, 30 psi; ionSapary Voltage Floating, −4500 V.

Bile acids were identified by extracting peak areas and retention times using MultiQuant software and correcting retention times using bile acid standards. All samples were quantitatively analyzed according to the established sample pre-treatment and instrumental analysis methods.

2.7 | Statistical analysis

Statistical analysis and data visualization were conducted using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The results were expressed as mean ± SEM. Each trial was repeated for at least three times. Significance analysis was performed using the Student's *t*-test, and *p*-values of <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Evaluations of diarrhea model

To evaluate whether a COL-induced diarrhea model was successfully constructed, we quantified the degree of diarrhea in mice using fecal water content and frequency of defecation. Compared with the control group, fecal water content and defecation frequency of mice were significantly increased after COL treatment (Figure 1A,B, $p < 0.0001$), indicating that the experimental model was successfully constructed.

3.2 | Effects of COL treatment on the liver of mice

To determine the effects of COL on bile acid metabolism in mice, we first evaluated the effects of COL on the biosynthetic site of bile acid (the liver). Compared with the control group, COL treatment caused a large number of mild granular degeneration of hepatocytes, and the cytoplasm was loose and lightly stained (Figure 2A). By further measuring the conventional liver function evaluation indicators, we found that COL treatment significantly increased the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (Figure 2B–D, $p < 0.05$), while significantly decreasing the level of total bile acid (TBA) in the serum (Figure 2E, $p < 0.01$). The increase of ALT and AST activity further indicated that COL treatment caused hepatocyte injury, and the increase of ALP activity in the serum could specifically reflect cholestatic liver injury, and this injury resulted in the observed decrease of total bile acid level in the serum.

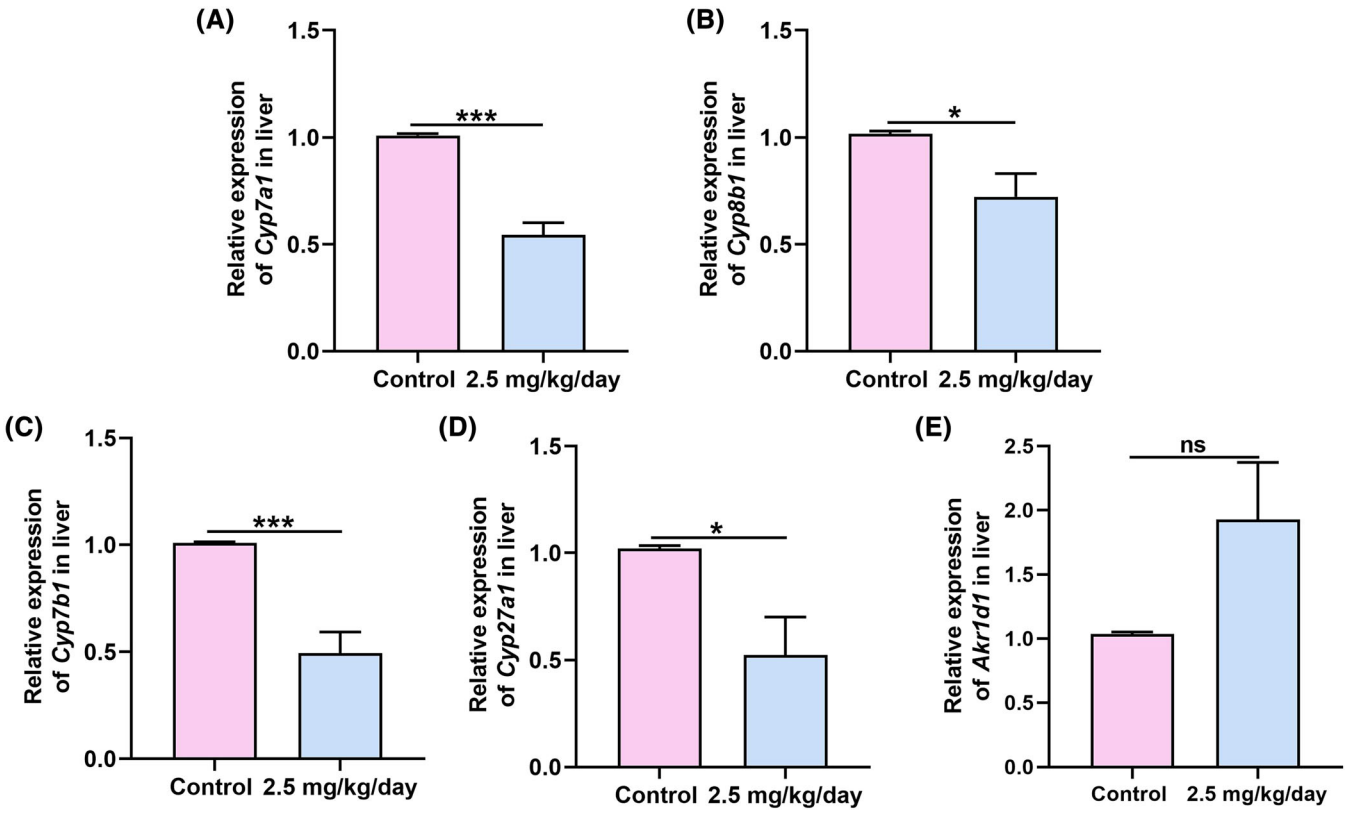

FIGURE 3 Effects of COL treatment on regulatory enzymes related to bile acid biosynthesis in mouse liver. (A) Cholesterol 7α-hydroxylase (*Cyp7a1*). (B) Sterol 12α-hydroxylase (*Cyp8b1*). (C) Oxysterol 7α-hydroxylase (*Cyp7b1*). (D) Sterol 27-hydroxylase (*Cyp27a1*). (E) Aldo-keto reductase family 1 member D1 (*Akr1d1*). Data were expressed as mean ± SEM, $n = 3–6$. * and *** denote $p < 0.05$ and $p < 0.001$ compared with the control mice, respectively. ns indicates that there is no significance between the two groups.

3.3 | Effects of COL treatment on liver bile acid biosynthesis in mice

To assess the effects of COL on bile acid synthesis pathways in mice, the mRNA expression levels of regulatory enzymes associated with bile acid biosynthesis were quantified in mouse liver. Compared with the control group, COL treatment significantly reduced mRNA expression levels of most enzymes in the bile acid biosynthesis pathway, including *Cyp7a1*, *Cyp8b1*, *Cyp7b1*, and *Cyp27a1* (Figure 3A–D, $p < 0.05$). Conversely, the mRNA expression level of *Akr1d1* showed an increasing trend after COL treatment but did not show statistical significance (Figure 3E, $p > 0.05$). These results suggest that COL treatment affects the biosynthetic pathway of hepatic bile acids in mice.

3.4 | Effects of COL treatment on Farnesoid X receptor (FXR) in the liver and ileum of mice

FXR is one of the 48 members of the human nuclear receptor transcription factor superfamily, which plays an important role in regulating bile acid biosynthesis and homeostasis (Sinal et al., 2000), and we therefore investigated whether the effects of the COL on bile acid biosynthesis are mediated through FXR. The results showed that COL treatment significantly decreased the expression levels of *Fxr* and its downstream target genes *Shp* and *Lrh1* in the liver (Figure 4A–C, $p < 0.01$), and *Fxr* and its downstream target genes *Shp* and *Fgf15* in the ileum were also significantly decreased after COL treatment (Figure 4D–F, $p < 0.01$). These results indicated that COL treatment affected FXR-mediated bile acid feedback regulation in liver and ileum tissues, which then decreased the bile acid biosynthesis in the liver.

3.5 | Effects of COL treatment on bile acid transporters in the ileum and liver of mice

Bile acids are released into the duodenum after synthesis in the liver, travel along the small intestine, and are reabsorbed mainly in the distal ileum and transported back to the liver (Dawson et al., 2009). To investigate the effects of COL treatment on bile acid reabsorption and reuptake in mice, we examined the mRNA expression of apical and internal transporters (Mrp2, Ibabp, and Ibat) and basolateral transporters (Mrp3, Osta, and Ostb) in the ileum. Compared with the control group, the mRNA expression levels of the apical and internal

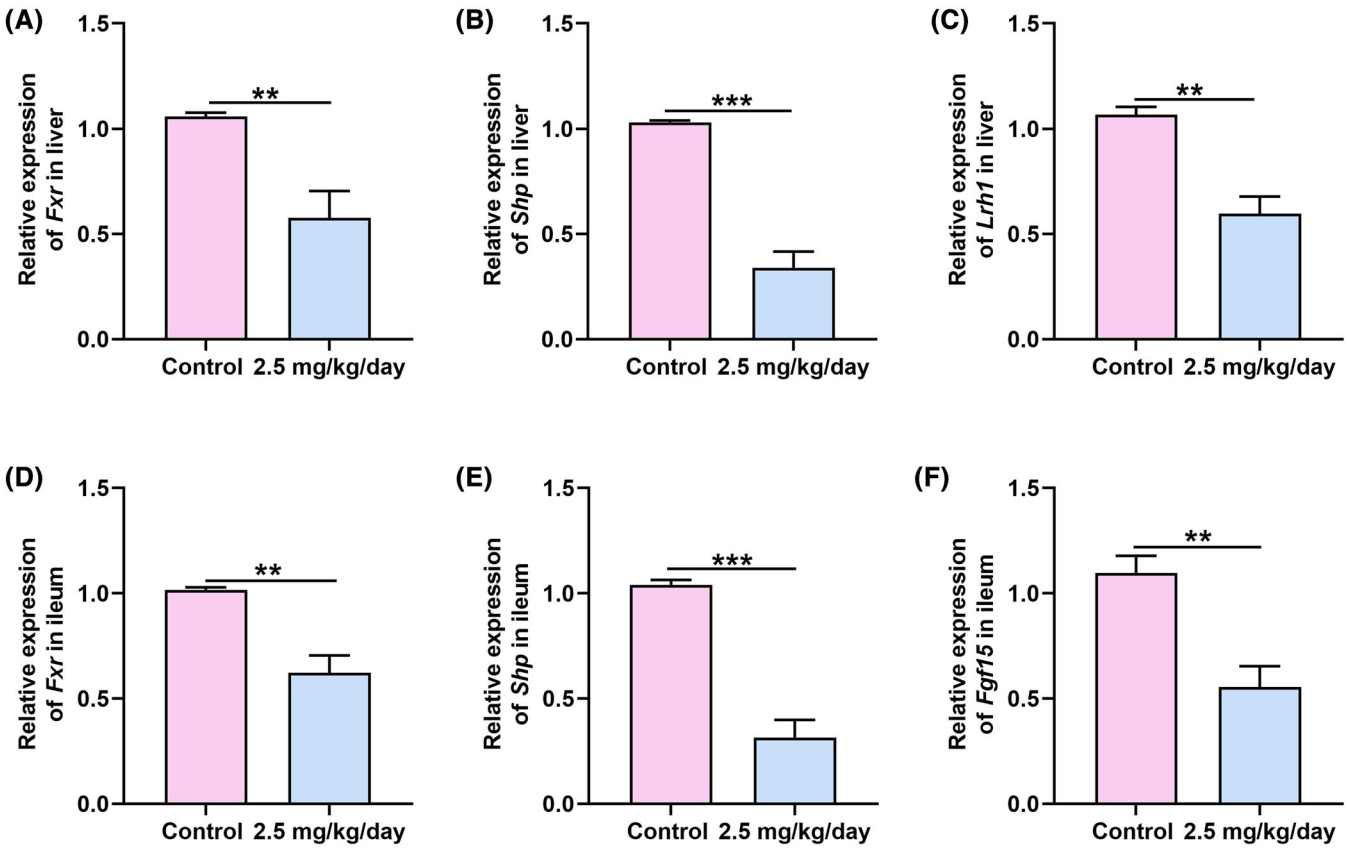

FIGURE 4 Effects of COL treatment on Farnesoid X receptor (FXR) and its molecular targets (*Shp*, *Lrh1*, and *Fgf15*) in mouse liver (A–C) and ileal tissue (D–F). *Shp*, short heterodimer partner; *Lrh1*, liver receptor homolog-1; *Fgf15*, fibroblast growth factor 15. Data were expressed as mean ± SEM, $n = 3–6$. **and *** denote $p < 0.01$ and $p < 0.001$ compared with the control mice, respectively. ns indicates that there is no significance between the two groups.

transporters *Mrp2* and *Ibabp* in the ileum were significantly down-regulated after COL treatment (Figure 5A,B, $p < 0.01$), whereas the mRNA expression levels of *Ibat* showed an upward trend but were not significant (Figure 5C, $p > 0.05$). Similarly, the mRNA expression levels of basolateral transporters *Mrp3*, *Osta*, and *Ostb* were significantly down-regulated after COL treatment (Figure 5D–F, $p < 0.01$). These genes are closely related to bile acid reabsorption in the ileum. The effect of COL in suppressing these genes supports the conclusion that the reabsorption of bile acids would be inhibited in the ileum, inducing the consequent accumulation of bile acids within the intestine.

Next, we examined the mRNA expression levels of hepatic canalicular bile acid transporters (Bsep and Mrp2) and portal bile acid transporters (Ntcp, Oatp1, and Mrp3) in the liver. The results showed that although COL treatment caused fluctuations in the mRNA expression levels of *Bsep* and *Mrp2* in the liver, there was no significant change (Figure 6A,B, $p > 0.05$). Interestingly, the mRNA expression levels of *Ntcp* and *Oatp1* were significantly down-regulated in the liver after COL treatment (Figure 6C,D, $p < 0.05$), whereas the expression level of *Mrp3* was significantly up-regulated (Figure 6E, $p < 0.01$). These genes are closely related to the reuptake of bile acids in the liver, and the fluctuations caused by COL interfere with the hepatic reuptake process of bile acids.

3.6 | Effects of COL treatment on intestinal bile acid content in mice

By examining the expression of bile acid reabsorption genes in the ileum and bile acid reuptake genes in the liver, we found that COL treatment significantly inhibited the expression levels of these genes, and the double inhibition may lead to the accumulations of bile acid in the intestine. To confirm this speculation, we performed targeted bile acid metabolomics to examine the content of bile acids in the colon. First, the results showed that no matter PCA analysis or PLS-DA analysis, the bile acid metabolic profiles of the control group and the COL treatment group were significantly different. The samples within the group were clustered together, and the samples between the groups were significantly separated (Figure 7A,B). Second, a total of 25 bile acids were identified, and the contents of five bile acids were significantly increased after COL treatment compared with the control group, including LCA, isoLCA, 12-ketoLCA, HDCA, and GCA (Figure 7C–G, $p < 0.05$). The content of 14 bile acids was increasing, but not significant, namely, alloLCA, 6,7-diketoLCA, TCA, NorDCA, DCA, CDCA, NorCA, alpha-MCA, beta-MCA, THDCA+TUDCA, TDCA, TDCA, T-α-MCA, and T-β-MCA (Figure S1). In contrast, six bile acids, including 7-ketolca, 7,12-diketolca, UCA, CA, ACA, and GDCA, showed a decrease in content after COL treatment, but no significant

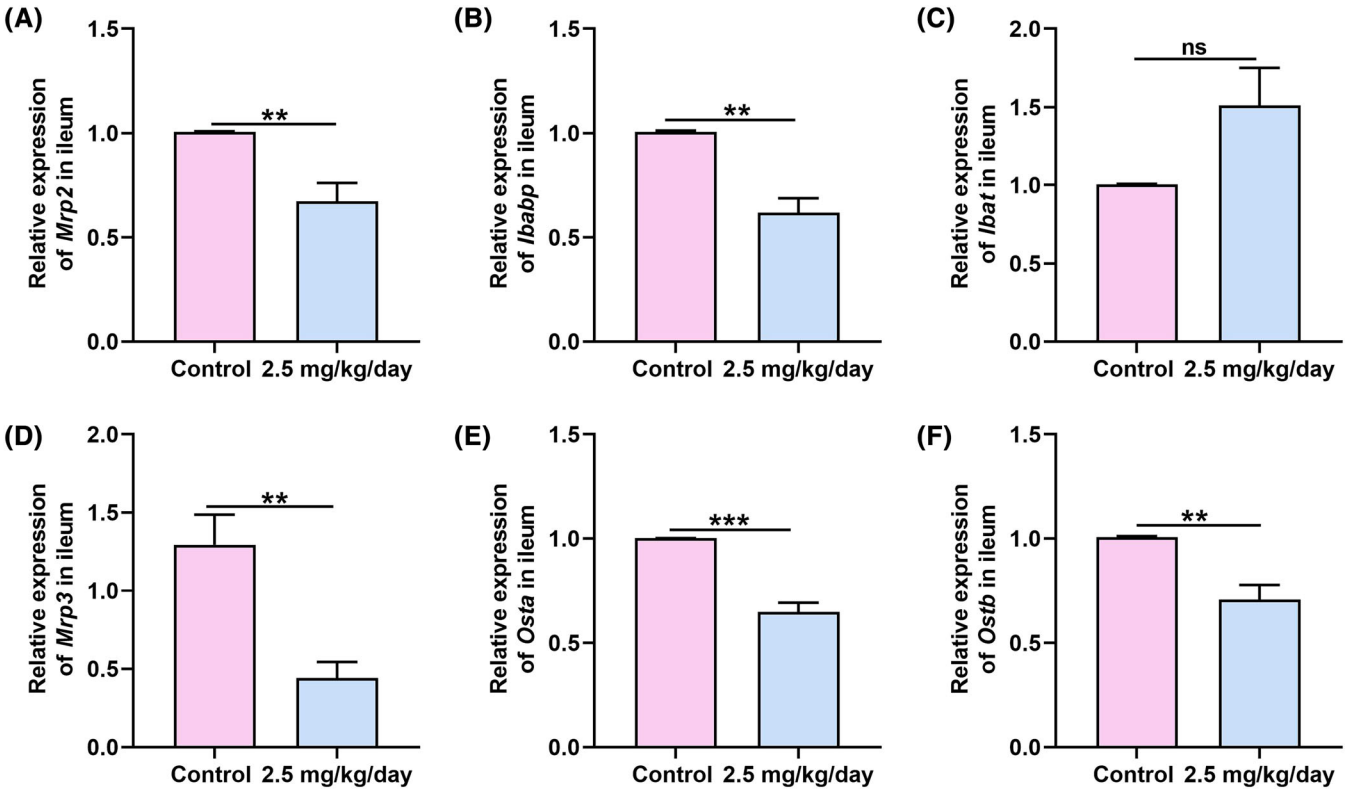

FIGURE 5 Effects of COL treatment on ileal transporters in mice. (A) Multidrug resistance-associated protein 2 (*Mrp2*). (B) Ileal bile acid-binding protein (*Ibabp*). (C) Ileal bile acid transporter (*Ibat*). Mrp2, Ibabp, and Ibat are apical and internal transporters. (D) Multidrug resistance-associated protein 3 (*Mrp3*). (E) Organic solute transporter α (*Osta*). (F) Organic solute transporter β (*Ostb*). Mrp3, Osta, and Ostb are basolateral transporters. Data were expressed as mean ± SEM, $n = 3–6$. **and *** denote $p < 0.01$ and $p < 0.001$ compared with the control mice, respectively. ns indicates that there is no significance between the two groups.

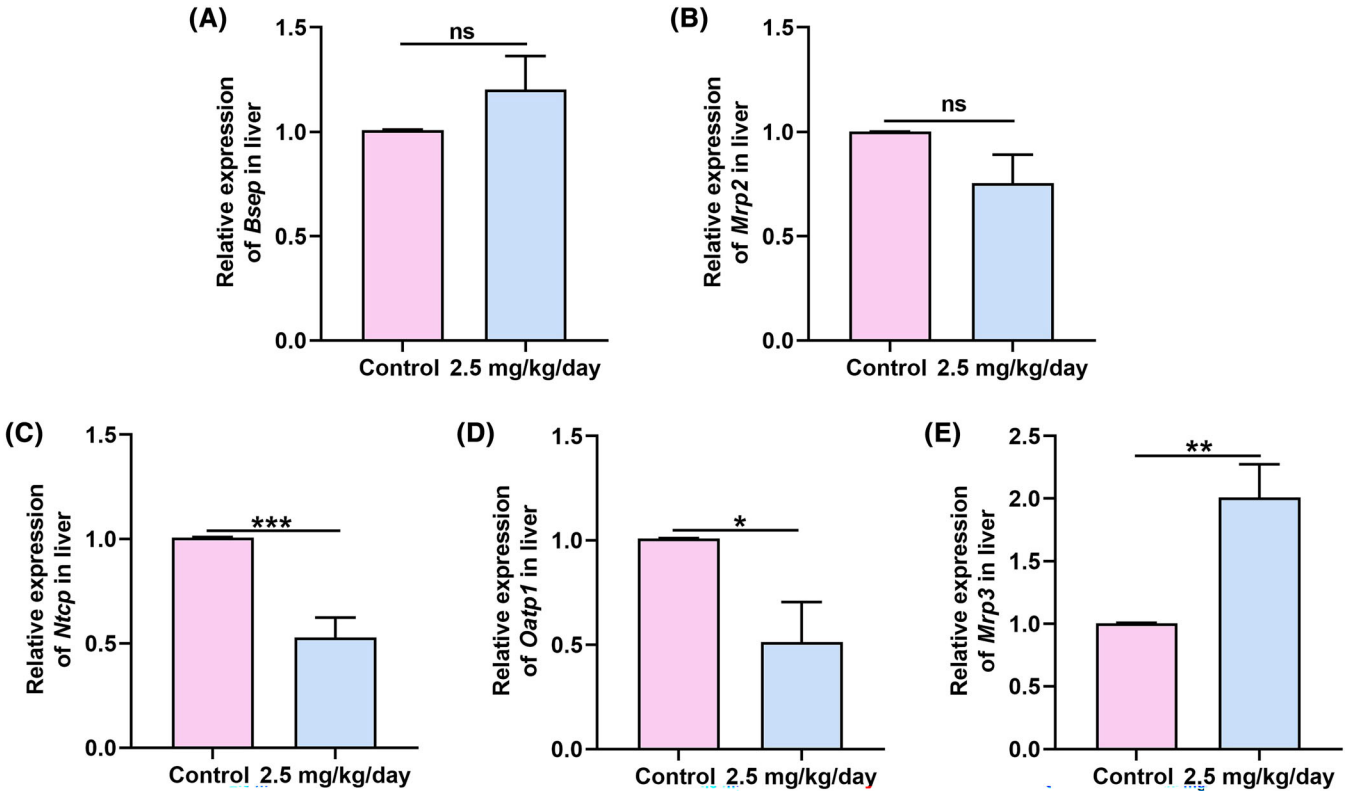

FIGURE 6 Effects of COL treatment on liver transporters in mice. (A) Bile salt export pump (*Bsep*). (B) Multidrug resistance-associated protein 2 (*Mrp2*). Bsep and Mrp2 are canalicular bile acid transporters. (C) Na^+/taurocholate cotransporter (*Ntcp*). (D) Organic anion transporting protein 1 (*Oatp1*). (E) Multidrug resistance-associated protein 3 (*Mrp3*). Ntcp, Oatp1, and Mrp3 are portal bile acid transporters. Data were expressed as mean ± SEM, $n = 3–6$. *, **, and *** denote $p < 0.05$, $p < 0.01$, and $p < 0.001$ compared with the control mice, respectively. ns indicates that there is no significance between the two groups.

difference (Figure S2). Overall, COL treatment resulted in an increase in the levels of 19 bile acids in the colon of mice, accounting for 76% of the total identified bile acids. On the other hand, targeted bile acid metabolomics data validated the accuracy of real-time fluorescence quantitative PCR results, suggesting that COL treatment did affect the reabsorption of bile acids in the ileum and the reuptake in the liver, leading to an accumulation of bile acids in the intestine.

4 | DISCUSSION

Given the predominance of bile in the drug excretion of COL, the present study was designed to investigate the toxicological profiles of COL from the perspective of bile acid metabolism. Here, we found that COL treatment damaged the structure of the bile acid synthesis site, which was reflected in significant increases in serum levels of ALT, AST, and ALP. This is consistent with the results of other researchers, and this toxicity is time-dependent and dose-dependent (Guo et al., 2019). In addition, the level of TBA in the serum of mice also decreased significantly after COL treatment, indicating that COL treatment may affect the biosynthesis of bile acids.

The results also showed that COL treatment significantly inhibited the expression levels of several key enzymes in mouse liver, including *Cyp7a1*, *Cyp27a1*, and *Cyp7b1*. Cyp7a1 is the rate-limiting enzyme in the classical pathway of bile acid synthesis, and Cyp27a1 can initiate an alternative pathway of bile acids (Di Ciaula et al., 2017; Zhang et al., 2022). The changes of these key enzymes would be expected to affect the biosynthesis pathway of bile acids and the size of bile acid pool.

FXR is a highly expressed member of the nuclear receptor superfamily in the gastrointestinal tract, which strictly regulates the enterohepatic circulation of bile acids by controlling the transcription of key regulatory genes involved in bile acid biosynthesis, biliary bile acid secretion, and intestinal transport of bile acids to the liver via the portal blood circulation (Chiang, 2017; Martinot et al., 2017). In the present study, we found that COL treatment not only significantly inhibited the mRNA expression levels of *Fxr* and its molecular targets *Shp* and *Lrh1* in the liver but also significantly inhibited the mRNA expression levels of *Fxr* and its molecular targets *Shp* and *Fgf15* in the ileum. These results suggest that COL treatment disrupts FXR-mediated feedback regulation mechanism of bile acids, resulting in changes in bile acid biosynthesis.

The bile acids synthesized by the liver then pass into the intestine (Joyce & Gahan, 2016). Approximately 95% of bile acids are actively absorbed at the end of the ileum via the apical sodium-dependent bile acid transporter (Asbt) and ileal bile acid-binding protein (Ibabp) or

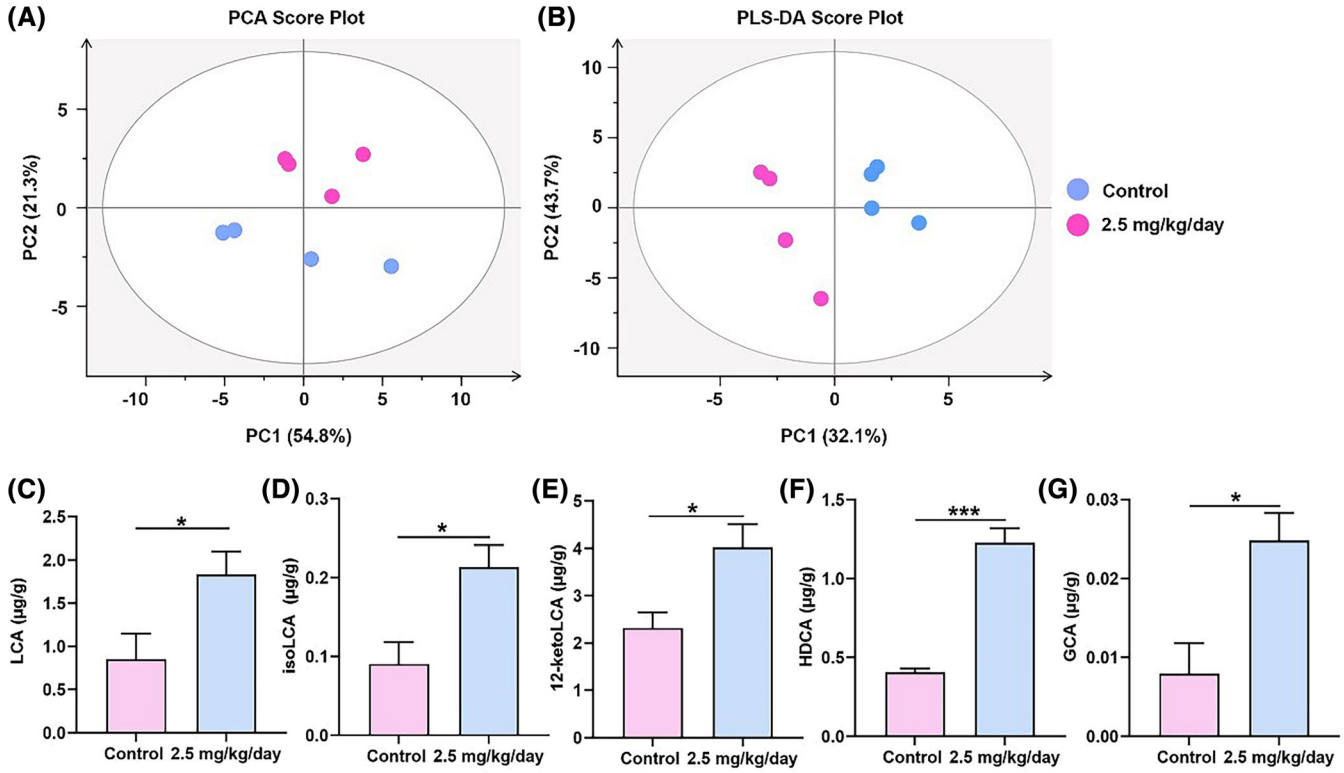

FIGURE 7 Effects of COL treatment on bile acid content in the colon of mice. (A) PCA analysis of bile acid targeted metabolomics. (B) PLS-DA analysis of bile acid targeted metabolomics. (C–G) Significant rise in bile acids. LCA, lithocholic acid; isoLCA, isolithocholic acid; 12-ketoLCA, 12-ketolithocholic acid; HDCA, hyodeoxycholic acid; GCA, sodium glycocholate hydrate. Data were expressed as mean ± SEM, n = 4. *and *** denote $p < 0.05$ and $p < 0.001$ compared with the control mice, respectively.

passively absorbed in the colon. The reabsorbed bile acids are released into the bloodstream via the organic solute transport proteins Osta or Ostb in the basolateral membrane and transported back to the liver for recirculation via the portal circulation (Di Ciaula et al., 2017). Reuptake in the liver is mediated by the sodium taurocholate cotransporting polypeptide (Ntcp) for bile salts and organic anion transporting proteins (Oatps) for bile acids, which are recombined to taurine or glycine and recycled once recovered (Alrefai & Gill, 2007; Dawson et al., 2009). Real-time fluorescence quantitative PCR detection showed that COL treatment significantly reduced the mRNA expression levels of *Mrp2*, *Ibabp*, *Osta*, and *Ostb* in the ileum. Inhibition of the expression of these key transporters would result in ineffective reabsorption of primary bile acids into the enterocytes. Further examination of transporters associated with bile salt and bile acid reuptake in the liver revealed that COL treatment significantly inhibited the expression levels of *Ntcp* and *Oatp1*, suggesting that COL treatment affected bile acid reuptake in the liver. On the other hand, the inhibitory effect of COL on the enterohepatic circulation of bile acids may be an important cause for the decrease of serum TBA. Overall, inhibition of the expression of reabsorption- and reuptake-related transporters may lead to the accumulation of bile acids in the intestine, as defects in any uptake system in the gastrointestinal tract can lead to the accumulation of bile acids in the gastrointestinal tract (Joyce & Gahan, 2014).

Gut microbiota plays an important role in the deconjugation, dehydrogenation, and dehydroxylation of bile acids (Begley et al., 2005; Jones et al., 2008; Ridlon et al., 2006). In our previous study, we found that COL treatment significantly altered the relative abundance of Firmicutes, Bacteroidetes, and Actinobacteria in the gut (Shi et al., 2020). The changes in the abundance of these phyla will inevitably affect the deconjugation of bile acids, which will cause the disorders of bile acid metabolism. Other studies have shown that disruption of the gut microbial community can rapidly alter the size, composition, and concentration of bile acid pools. Typical examples are that changes in the community structure and function of gut microbiota after antibiotic administration led to rapid changes in bile acids (Theriot et al., 2014; Zhang et al., 2014). Targeted bile acid metabolomics results showed that COL treatment caused an increase in 76% (19/25) of bile acids and a decrease in 24% (6/25) of bile acids in the colon of mice, suggesting that COL treatment did cause intestinal microbiome-mediated bile acid metabolism disorders. Furthermore, the 76% rise in bile acids in the colon also validates the accuracy of the previous real-time fluorescence quantitative PCR results that the inhibition of bile acid reabsorption- and reuptake-related transporters expression by COL leads to bioaccumulation of bile acids in the gut.

Our findings suggest that COL treatment disrupts the enterohepatic circulation of bile acids in mice and represents an important step toward understanding how COL treatment affects bile acid

metabolism. The metabolic disorder of bile acids is bound to lead to the poor excretion of COL in the body, which will further aggravate the toxicity of COL in the body, thereby poisoning tissues and organs. Furthermore, the abnormal accumulations of bile acids in the gut can also activate some receptor proteins related to diarrhea, such as TGR5, which may be an important cause of gastrointestinal adverse reactions induced by COL administration. Therefore, future studies could delve into the mechanism of COL-induced diarrhea from this perspective.

5 | CONCLUSION

In summary, we have demonstrated that COL treatment has a profound effect on bile acid metabolism. Not only does COL exert its toxic effects within bile acid biosynthesis in the liver but also in the biological transport of bile acids. COL treatment can cause defects in the reabsorption and reuptake system of bile acids in mice, resulting in a large accumulation of bile acids in the gut, thereby disrupting bile acid metabolism in mice and possibly contributing to the adverse intestinal reactions observed in humans treated with the drug.

AUTHOR CONTRIBUTIONS

Yongpeng Shi: Experimentation, Sample collection, Data analysis, Writing—original draft. Li Wei: Experimentation, Writing—Review and Editing. Fang Jin: Writing—Review and Editing. Ji Wang: Experimentation. Hanwen Cao: Experimentation. Ying Yang: Experimentation. Lan Gao: Project administration, Funding acquisition, Conceptualization, Supervision, Methodology, Writing—Review and Editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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