Electronic Supplementary Material (ESI) for Food & Function.
This journal is © The Royal Society of Chemistry 2023

Supporting information

Materials and Methods

Animal experiment

C57BL/6 male mice (8 weeks of age) were purchased from STJ Laboratory Animal Co., LTD (Hunan, China). Then, the mice were housed in 1/mice per cage under the same condition (temperature, 23±2℃; lighting cycle, 12h:12h light/dark; 8:00-20:00 for light) and allowed free access to water and feed.These 18 male mice were randomly divided into 3 groups (6 mice per group). Then the mice were orally given distilled water (CON group) or 100 mg/kg, 200 mg/kg (according to the body weight) of TCDCA for 10 days.

Targeted metabolomics of bile acids

A 70 uL volume of serum was transferred to an EP tube. After the addition of 280 uL extract solvent (acetonitrile-methanol, 1:1), the samples were vortexed for 30 s, sonicated for 15 min in ice-water bath followed by incubation at -40 oC for 1 h and centrifugation at 12000 rpm (RCF=13800(×g), R= 8.6 cm) and 4 oC for 15 min. The clear supernatants were transferred to an auto-sampler vial for UHPLC-MS/MS analysis. Stock solutions were individually prepared by dissolving or diluting each standard substance to give a final concentration of 1 mg/mL. An aliquot of each of the stock solutions was transferred to a flask to form a mixed working standard solution. A series of calibration standard solutions were then prepared by stepwise dilution of this mixed standard solution (containing isotopically-labelled internal standard mixture in identical concentrations with the samples). The UHPLC separation was carried out using an UHPLC System (Vanquish, Thermo Fisher Scientific), equipped with a Waters ACQUITY UPLC BEH C18 column (150 * 2.1 mm, 1.7 μm, Waters). The mobile phase A was 5 mmol/L ammonium acetate in water, and the mobile phase

B was acetonitrile. The column temperature was set at 45 ℃. The auto-sampler temperature was set at 4 ℃ and the injection volume was 1 μL.

A Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific) was applied for assay development. Typical ion source parameters were: spray voltage = +3500/-3200 V, sheath gas (N_2) flow rate = 40, aux gas (N_2) flow rate = 15, sweep gas (N_2) flow rate = 0, aux gas (N_2) temperature = 350 ℃, capillary temperature = 320 ℃.

The parallel reaction monitoring (PRM) parameters for each of the targeted analytes were optimized, by injecting the standard solutions of the individual analytes, into the API source of the mass spectrometer. Since most of the analytes did not show product ion acceptable for quantification, the precursor ion in high resolution was selected for quantification.

Construction of FXR overexpression IPEC-J2 cell line

The construction of cells was conducted by Cyagen Biology (Suzhou, China) Biotechnology Co., Ltd. Add lentivirus vector containing LV-EFS>pNR1H4/FLAG tag-CMV>mCherry/T2A/Puro (overexpression group) or LV-CMV>mCherry/T2A/Puro (control group) into IPEC-J2 cells at the ratio of MOI=1:10. At 48-72 hours post-infection, cells with successful overexpression were selected using puromycin (3 mg/mL). Following the completion of drug screening, the cells were maintained with a concentration of 1.5μg/mL to ensure stable expression. The expression of the FXR gene was determined through fluorescence quantitative PCR analysis.

Table S1 The primer sequence of pig

Genes	Sense（5‘-3’）	Anti-sense(5'-3')	Product length
β-actin	GAGATTGGCATGGCTTTATTTG	ACTGCTGTCACCTTCACCGTT	127
TGR5	TGCTGTCCCTCATCTCATTGG	TGTGTAGCGATGATCACCCAG	80
FXR	ACCACTGCTTGAGGTGCTACA	ATGTCCGCAACTCAGTCAGG	101
CDX2	AGGACGAAAGACAAATACCGAGT	CCTTTCTCCGAATGGTGATGT	100
Ki67	AGAACAAAGCCGTCAAGCG	TGCTGAACTGGTTCTGAAGGAT	105
PCNA	TAATGCAGACACCTTGGCACT	GCAAATTCACCAGAAGGCATC	152
Caspase 3	GGAATGGCATGTCGATCTGGT	ACTGTCCGTCTCAATCCCAC	351
Caspase 9	TGGAACTCAAGCCAGAGGAG	CTGCATTCAGGACGTAAGCC	195
Bcl-2	TATTGGTGAGTCGGATCGCA	CTCTCAGCTGCTGCATTGTT	127
Bax	AAGCGCATTGGAGATGAACT	CGATCTCGAAGGAAGTCCAG	251

Table S2 The primer sequence of mice

Genes	Sense（5‘-3’）	Anti-sense(5'-3')	Product length
β-actin	GTGACGTTGACATCCGTAAAGA	GTAACAGTCCGCCTAGAAGCAC	287
TGR5	CAGTCTTGGCCTATGAGCGT	CTGCCCAATGAGATGAGCGA	70
FXR	CAAAATGACTCAGGAGGAGTACG	GCCTCTCTGTCCTTGATGTATTG	84
CDX2	CCGAGAGGCAGGTTAAAATTTGG	CTGAGGCTGGGAAGGTTGTG	100
Ki67	ACCGTGGAGTAGTTTATCTGGG	TGTTTCCAGTCCGCTTACTTCT	126
PCNA	GAAGTTTTCTGCAAGTGGAGAG	CAGGCTCATTCATCTCTATGGT	107
Caspase 3	TGGAATGTCATCTCGCTCTGGT	GAAGAGTTTCGGCTTTCCAGTC	298
Caspase 9	GACCAATGGGACTCACAGCAA	GTTCACATTGTTGATGATGAGGCA	281
Bcl-2	GCTACCGTCGTGACTTCGCA	CATCCCAGCCTCCGTTATCC	270
Bax	GCCTTTTTGCTACAGGGTTTCAT	TATTGCTGTCCAGTTCATCTCCA	151

Table

Table S3. The bile acid composition and concentration in the serum of mice (nmol/L)

id	Compound name	CON	200 mg/kg TCDCA	*P*-Value
1	Taurocholic acid	756.2 ± 442.01	12917.61 ± 7442.75	0.343
2	Cholic acid	436.74 ± 242.50	5937.50 ± 3682.55	0.486
3	Chenodeoxycholic acid	139.00 ± 20.24	676.5375 ± 281.98	0.029
4	Taurochenodeoxycholic acid	186.59 ± 33.40	1382.35 ± 663.13	0.029
5	Taurolithocholic acid	ND	1.43 ± 0.32	0.057
6	Lithocholic acid	12.68 ± 7.17	19.3 ± 10.22	0.343
7	Tauro ω-muricholic acid	146.15 ± 76.17	1315.16 ± 880.66	0.686
8	Tauro α-Muricholic acid	351.71 ± 66.30	6516.94 ± 3718.28	0.486
9	Tauro β-Muricholic acid	1181.8 ± 657.78	23645.3 ± 13818.50	0.343
10	ω-Muricholic Acid	254.375 ± 104.24	684.99 ± 440.81	0.686
11	α-Muricholic acid	46.66 ± 6.02	540.10 ± 302.99	0.486
12	β-Muricholic acid	584.15 ± 235.50	5191.05 ± 3362.10	1.000
13	Tauroursodeoxycholic acid	223.56 ± 41.65	1756.39 ± 909.22	0.200
14	6-Ketolithocholic acid	1.35 ± 1.00	4.81 ± 2.91	0.686
15	7-Ketolithocholic acid	18.54 ± 1.67	131.65 ± 74.52	0.057
16	12-Ketolithocholic acid	0.34 ± 0.08	0.84 ± 0.29	0.486
17	Ursodeoxycholic acid	216.60 ± 20.51	550.69 ± 186.85	0.200
18	Hyodeoxycholic acid	4.93 ± 2.67	9.69 ± 1.85	0.343
19	6,7-Diketolithocholic acid	3.82 ± 2.00	10.57 ± 7.19	1.000
20	7-Ketodeoxycholic acid	72.8± 41.86	1156.98 ±743.20	1.000
21	12-Dehydrocholic acid	27.98±16.17	344.68±241.82	1.000
22	3-Dehydrocholic acid	0.34 ± 0.083	87.35 ± 59.18	0.486
23	Ursocholic acid	3.84 ± 2.61	48.33 ± 33.25	1.000
24	3β-Cholic Acid	9.66 ± 1.71	25.95 ± 5.64	0.029
25	Hyocholic acid	0.34 ± 0.083	17.85 ± 11.60	0.486
26	Allocholic acid	24.25 ± 15.16	340.04 ± 252.26	0.486
27	Glycocholic acid	2.32 ± 1.98	14.65 ± 8.41	0.686
28	Taurohyodeoxycholic acid	6.30 ± 2.98	15.68 ± 3.72	0.114
29	Taurodeoxycholic acid	5.07 ± 2.75	11.78 ± 5.10	0.686
30	Glycolithocholic Acid-3-Sulfate	4.67 ± 2.62	64.18 ± 37.53	1.000
31	Taurohyocholic acid	ND	48.60 ± 28.70	0.000
32	Glycochenodeoxycholic Acid-3-O-β-glucuronide	1.33±0.63	1.33±0.64	1.000

Figure S1

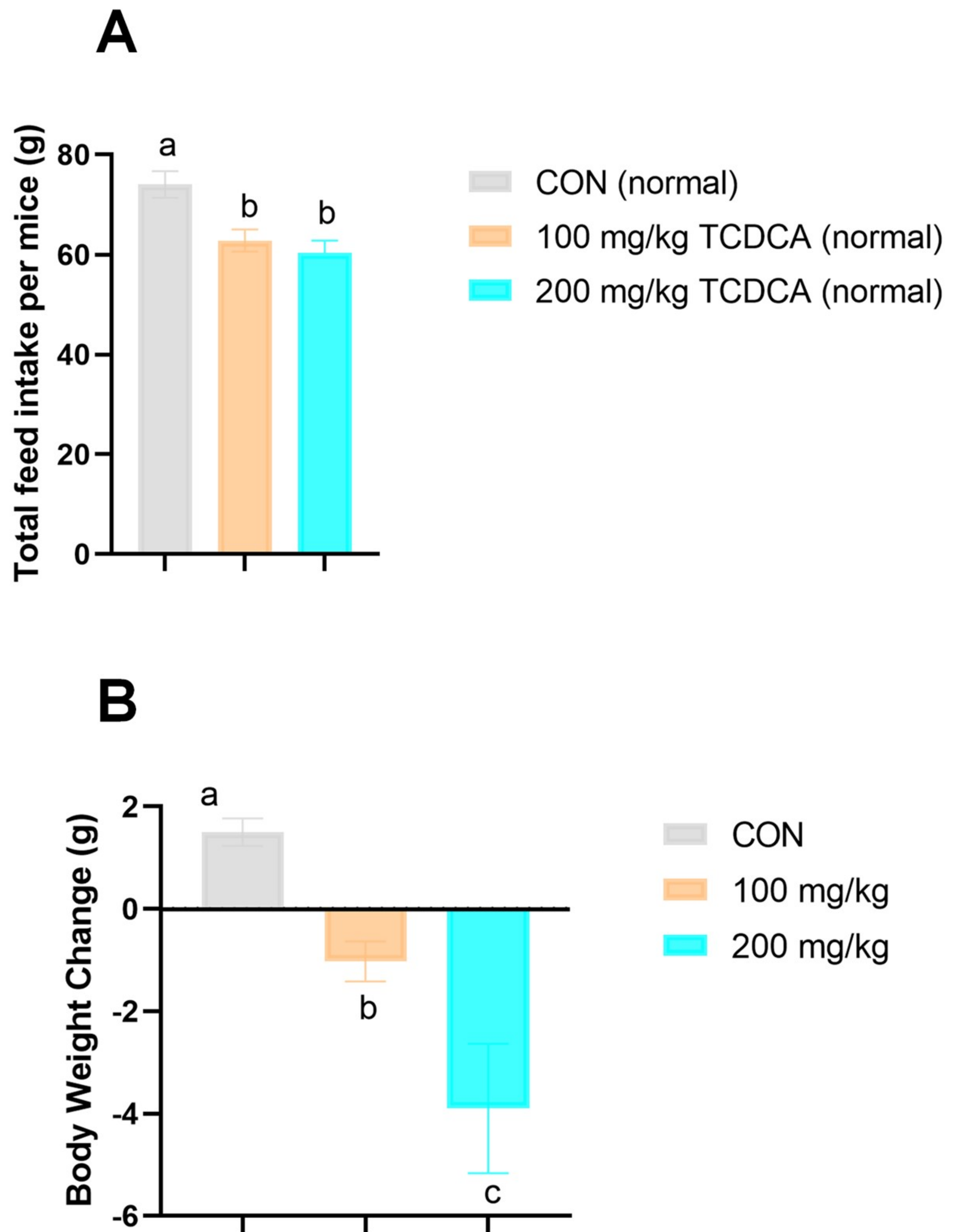

Figure S1.Effects of different doses of TCDCA on body weight and food intake of normal mice.

Figure S2

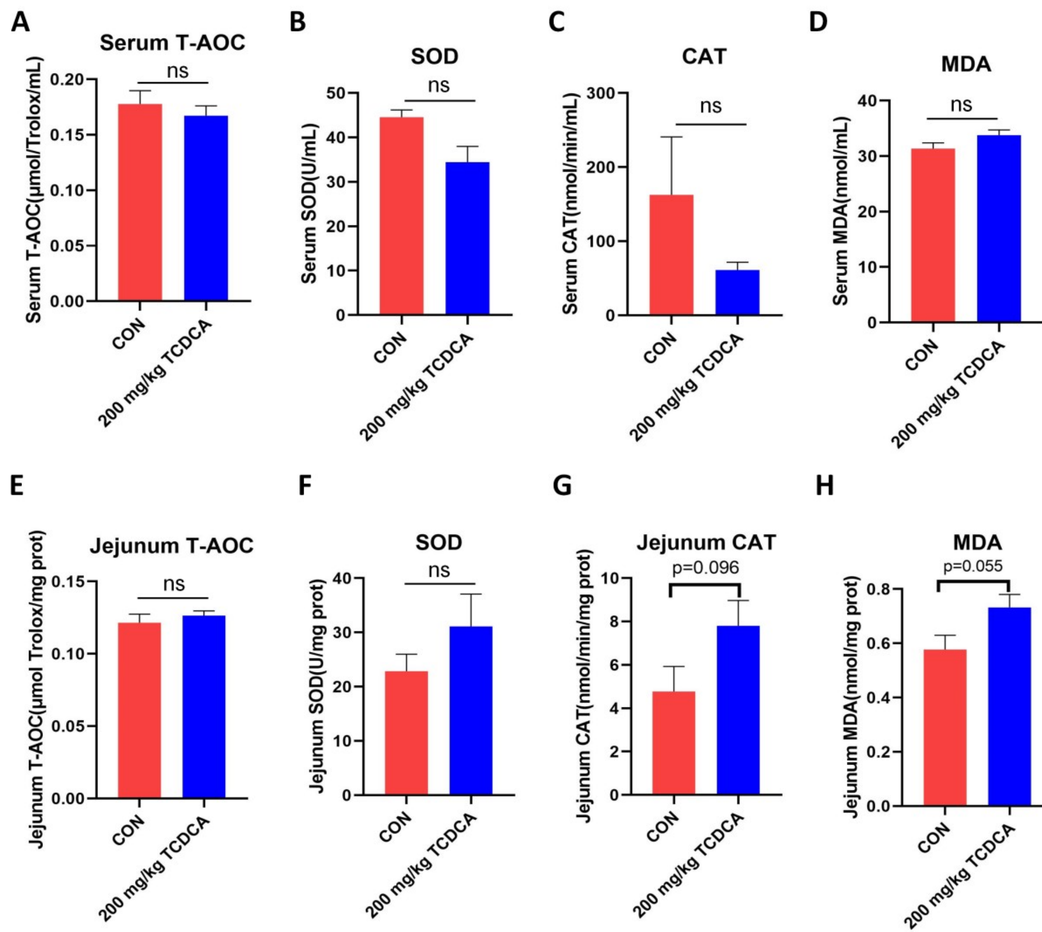

Fig. S2 The changes of oxidative stress related index in the serum and Jejunum of mice after TCDCA oral administration.(A-D) The indexes related to oxidative stress in serum. (E-H) The indexes related to oxidative stress in Jejunum (ns: no significant difference).

Figure S3

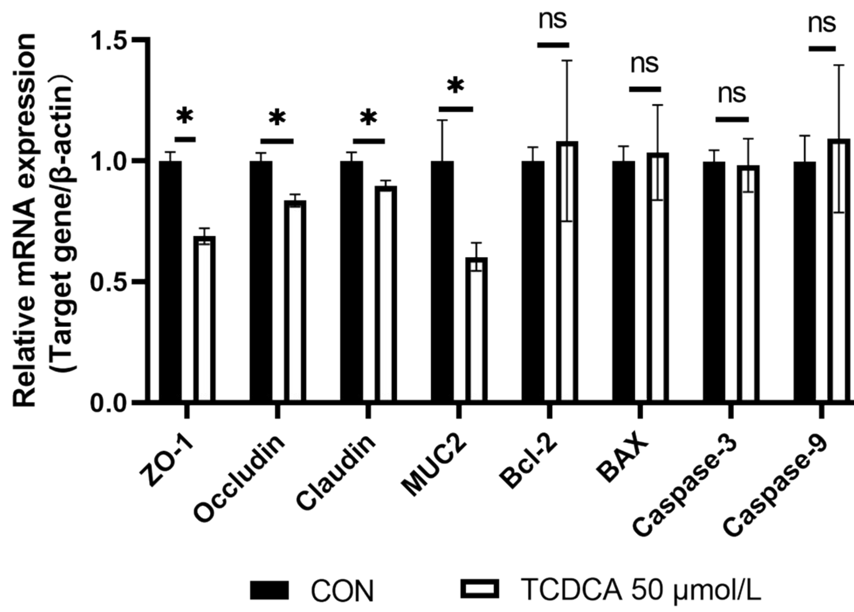

Fig.S3 The effect of 50 μmol/L TCDCA treatment on the gene expression related to tight junction and apoptosis. * P<0.05, ns: no significant difference.

.

Figure S4

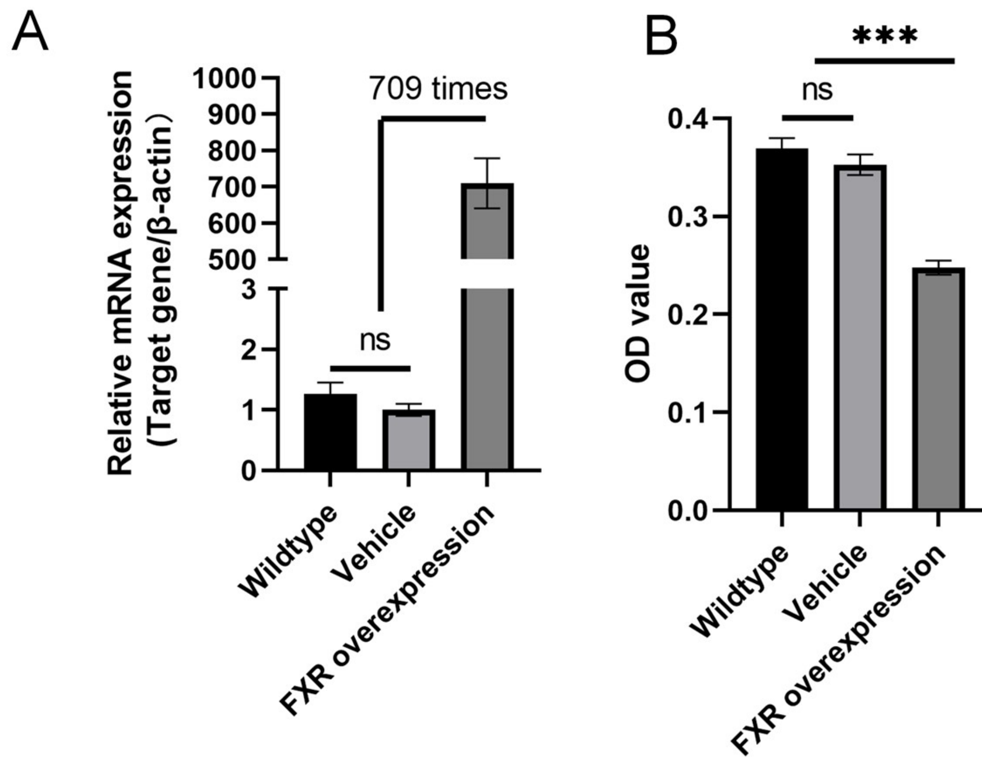

Fig. S4 Effect of FXR overexpression on cell viability. (A) The FXR gene expression level among wild type, empty vehicle transfection group and FXR overexpression group. (B) Overexpression of FXR significantly inhibited cell viability. *** $P<0.001$, ns: no significant difference