Water extract of Goji berries improves neuroinflammation induced by high-fat high-fructose diet based on bile acid-mediated gut-brain axis pathway

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Supplementary Materials and methods

BAs analysis

The cortex, hippocampus and liver were collected and immediately frozen in chilled on dry ice. Samples were stored at 80℃ until analysis. BAs in serum, liver, and brain were measured according to previously reported methods with slight modifications.[1,2] Briefly, an aliquot of 100 μL of plasma was mixed with 200 μL aceonitrile (LC-MS grade), which contained internal standard (CA-d4, GCDCA-d4 and DCA-d4). Intestinal content, cortex, hippocampus, and liver samples were homogenized and extracted in acetonitrile containing deuterated internal standards (CA-d4, GCDCA-d4 and DCA-d4). Then the supernatant was collected by centrifugation at 12000 rpm and 4 ℃ for 20 min. The solvent of the supernatant is evaporated by termovap sample concentrator. The dried powder was reconstituted with 100 μL pure acetonitrile and filtered through a 0.45 μm membrane. Bile acids were then analyzed by UPLC-MS/MS (Sciex Triple Quad 5500 LC-MS/MS, USA).

References

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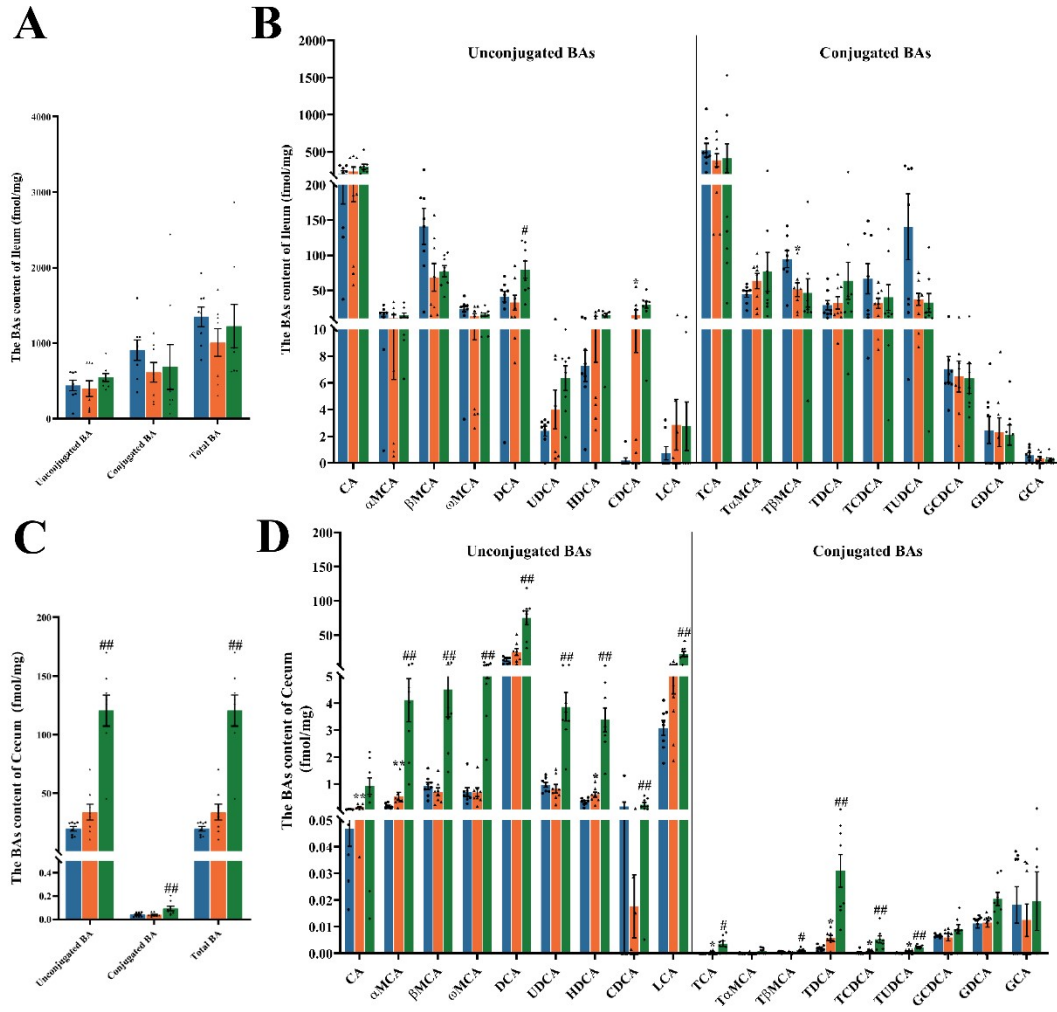
Fig. S1. Effects of LBE on ileum and cecum bile acid levels. (A) Effects of LBE on total feces BA levels (n = 8). (H) Feces BA classes and BA profile of mice after LBE intervention (n = 8). Data are presented as mean ± SEM, n = 8. *$p < 0.05$, **$p < 0.01$ compared to the NC group, #$p < 0.05$, ##$p < 0.01$ compared to the HFFD group.

Table S1 Primer sequences utilized in the RT-qPCR Experiments

Target gene	Primer Sequence (5´-3´)
TNF-α	FW: CTCATGCACCACCATCAAGG
	RV: ACCTGACCACTCTCCCTTTG
IL-1β	FW: AGCTTCAAATCTCGCAGCAG
	RV: TCTCCACAGCCACAATGAGT
IL-6	FW: CTCTGGCGGAGCTATTGAGA
	RV: AAGTCTCCTGCGTGGAGAAA
PSD-95	FW: TCTGTGCGAGAGGTAGCAGA
	RV: AAGCACTCCGTGAACTCCTG
CYP7A1	FW: AGCAACTAAACAACCTGCCAGTACTA
	RV: GTCCGGATATTCAAGGATGCA
CYP27A1	FW: GCCTCACCTATGGGATCTTCA
	RV: TCAAAGCCTGACGCAGATG
FXR	FW: AGGAGCCCCTGCTTGATGT
	RV: GCGGGTTCTCAGGCTGGTA
FGFR4	FW: GGCTCCATGACCGTCGTACA
	RV: ATGACCACTCGAGGAGCTGC
SHP	FW: AGGGTAGAGGCCATGAGGAG
	RV: ACGATCCTCTTCAACCCAGA
ZO-1	FW: TGAGTGCGTTTCTCTCCCTT
	RV: CCCTCTGTGTTCCTCATGGT
Occludin	FW: AGCACTTAACCTGCCTGGAT
	RV: AGCCTGTGGAAGCAAGAGAT
Claudin-1	FW: AGCTGCCTGTTCCATGTACT
	RV: CTCCCATTTGTCTGCTGCTC
GAPDH	FW: GGACTTACAGAGGTCCGCTT
	RV: CTATAGGGCCTGGGTCAGTG