Supporting information

Mangiferin improves hepatic damage-associated molecular patterns, lipid metabolic disorder and Mitochondrial Function in the alcohol hepatitis rats

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Table S1. Real-time quantitative PCR primer sequences.

Gene name	Sequence (5'-3')	Fragment size
Cas-1	For: CTGGAGCTTCAGTCAGGTCC Rev: CTTGAGGGAACCACTCGGTC	141bp
Cav-1	For: AACAGGGCAACATCTACAA Rev: TCCGCAATCACATCTTCA	163bp
EB1	For: TCACCTGTCCTCCTAACC Rev: GGCAGCGTCATAATCGT	83bp
IL-1β	For: TTGACTTGGGCTGTCCAGATGAGAG Rev: ATGAAGGAAAAGAAGGTGCT	175bp
NF-κB p65	For: ATGGACGATCTGTTTCCC Rev: GTCTTAGTGGTATCTGTGCT	136bp
NLRP3	For: GACCAGGTTCAGTGTGTTTT Rev: GGTTGGTGCTTAGACTTGAG	212bp
OPA-1	For: CTGCTGTTGGAGGTGGC Rev: CCAGGTCAGGAGCGAAA	189bp
PPAR-γ	For: TACCACGGTTGATTTCTC Rev: TCTACTTTGATCGCACTTT	132bp
β-actin	For: CTTGCATCCCTCAGCACCTT Rev: TCCTGTGGACAATGGATGGA	140bp

Table S2. The equation and the correlation coefficient of FFAs.

FFAs	Calibration equation	Regression coefficient
C16:1	Y=0.1948X+0.2052	0.9993
C16:0	Y=0.1545X+1.1075	1.0000
C17:3	Y=0.0214X+0.0348	0.9996
C18:2	Y=0.1651X+0.2302	1.0000
Trans C18:1	Y=0.0546X+0.0521	0.9994
C18:1	Y=0.0977X+0.0592	0.9998
C18:0	Y=0.0858X+0.3989	1.0000
C20:4	Y=0.0279X−0.0314	0.9997
C22:6	Y=0.0855X+0.0565	0.9994
C22:4	Y=0.01651X+ 0.0349	0.9991

*X: the quality of fatty acids; Y: relative peak area = peak area of fatty acid/peak area of internal standard.

Table S3. The equation and the correlation coefficient of alcohol.

Calibration equation	Regression coefficient
Y=1.4636X+0.0345	0.9996

*X: the quality of alcohol; Y: relative peak area = peak area of alcohol/peak area of internal standard.

Table S4. The equation and the correlation coefficient of metallic elements.

Metallic element	Calibration equation	Regression coefficient	Limit of detection
Na	Y=0.6896X+0.0256	0.9998	6.36
Mg	Y=0.3489X+0.0012	0.9998	1.94
K	Y=0.4106X+0.0185	0.9998	4.50
Ca	Y=0.006X+2.3516	0.9997	6.61
Fe	Y=0.00446X+0.003638	1.0000	0.82
Be	Y=0.001063X+0.000005	0.9999	0.04
B	Y=0.0005665X+0.001112	1.0000	1.25
Ti	Y=0.004803X+0.0001774	1.0000	0.46
V	Y=0.1471X+0.0008505	0.9999	0.08
Mn	Y=0.1295X+0.009352	1.0000	0.12
Co	Y=0.3130X+0.003812	1.0000	0.03
Ni	Y=0.08126X+0.1035	1.0000	0.06
Cu	Y=0.2311X+0.02113	1.0000	0.08
Zn	Y=0.04645X+0.03175	1.0000	0.67
Mo	Y=0.0026X+0.0000773	1.0000	0.06
Cd	Y=0.005893X+0.0000127	1.0000	0.05
Sb	Y=0.02207X+0.0002817	1.0000	0.15
Ba	Y=0.007617X+0.0005792	1.0000	0.20
Tl	Y=0.06783X+0.002180	0.9999	0.02
Pb	Y=0.048X+0.001218	1.0000	0.09

*X: the quality of metallic element; Y: relative peak area = peak area of metallic element /peak area of internal standard.

Table S5. Effects of MF on body weights in AH rats.

	NC	AL	SN	MFH	MFL
Initial body weight	251.18±18.02[a]	245.57±10.09[a]	242.39±14.58[a]	251.85±8.58[a]	251.99±10.79[a]
Final body weight (g)	509.24±33.02[a]	407.07±17.04[c]	459.63±24.89[b]	451.38±12.05[b]	440.40±34.46[b]
Body weight gain (g)	258.46±7.35[a]	161.50±23.14[d]	217.24±27.69[b]	199.53±14.65[bc]	188.41±20.49[c]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S6. The content of biochemical markers in the serum in the 6-week.

	NC	AL
ALT (U/L)	28.94±0.92[b]	46.78±1.88[a]
AST (U/L)	30.54±1.45[b]	94.14±4.50[a]
AST/ALT	1.06±0.080[b]	2.02±0.16[a]
GGT (U/L)	1.25±0.10[b]	1.75±0.060[a]
DBIL (µmol/L)	1.24±0.080[b]	2.01±0.090[a]
IBIL (µmol/L)	0.22±0.05[b]	1.54±0.10[a]
TBIL (µmol/L)	1.44±0.080[b]	3.54±0.19[a]
ALP (U/L)	86.90±8.04[b]	124.40±8.13[a]
CHE (KU/L)	0.10±0.020[b]	0.23±0.020[a]
TP (g/L)	50.18±1.34[a]	50.98±2.90[a]
ALB (g/L)	26.23±1.05[a]	26.90±0.55[a]
GLO (g/L)	23.94±4.72[a]	24.08±2.85[a]
ALB/GLO	1.10±0.050[a]	1.13±0.15[a]
TBA (µmol/L)	2.30±0.18[b]	4.11±0.17[a]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–b] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S7. Effects of MF on serum biochemical markers in AH rats.

	NC	AL	SN	MFH	MFL
ALT (U/L)	32.48±2.79[c]	54.44±8.28[a]	40.09±3.97[b]	39.90±5.58[b]	43.40±7.17[b]
AST (U/L)	30.32±1.97[d]	115.64±6.39[a]	63.47±8.99[c]	60.43±3.52[c]	83.02±6.79[b]
AST/ALT	0.94±0.11[c]	2.16±0.28[a]	1.59±0.21[b]	1.54±0.25[b]	1.95±0.34[a]
ALP (U/L)	103.35±5.27[b]	140.06±9.19[a]	110.40±12.28[b]	112.72±15.62[b]	132.98±11.19[a]
CHE (KU/L)	0.16±0.028[d]	0.35±0.048[a]	0.21±0.021[c]	0.22±0.036[c]	0.30±0.027[b]
TP (g/L)	52.93±3.49[a]	51.81±4.81[a]	51.67±2.72[a]	51.67±3.79[a]	50.75±1.13[a]
ALB (g/L)	25.17±2.22[a]	26.62±2.45[a]	26.47±1.48[a]	26.08±3.01[a]	26.23±1.30[a]
GLO (g/L)	27.77±3.42[a]	23.65±1.40[c]	25.20±1.73[b]	25.58±1.42[b]	25.07±1.14[b]
ALB/GLO	0.92±0.17[a]	1.10±0.070[a]	1.06±0.073[a]	1.02±0.12[a]	1.05±0.070[a]
TBA (μmol/L)	3.45±0.20[e]	8.10±0.18[a]	4.62±0.90[d]	4.98±0.13[c]	5.75±1.55[b]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–e] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S8. Effects of MF on serum SOD, GSH-PX, MDA, CAT and NO in AH rats.

	NC	AL	SN	MFH	MFL
SOD (U/mL)	245.11±9.61[a]	189.54±16.22[c]	210.52±9.82[b]	207.45±10.48[b]	203.39±4.40[b]
GSH-PX	36.00±1.94[a]	19.55±1.61[d]	31.37±2.59[b]	29.44±2.49[b]	22.68±3.25[c]
MDA (nmol/mL)	5.23±0.75[d]	8.38±0.72[a]	6.80±0.62[c]	6.99±0.21[bc]	7.57±0.40[b]
CAT (U/mL)	12.93±1.57[a]	6.16±0.55[c]	9.35±1.30[b]	11.55±0.96[a]	9.12±1.36[b]
NO (μmol/L)	40.90±5.67[c]	96.32±14.61[a]	60.28±6.09[b]	58.75±7.38[bc]	68.45±2.91[b]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S9. Effects of MF on liver SOD, GSH-PX, MDA and ROS in AH rats.

	NC	AL	SN	MFH	MFL
SOD (U/mgprot)	384.03±55.88[a]	193.93±29.57[c]	254.92±20.60[b]	250.53±24.75[b]	231.88±38.25[bc]
GSH-PX (μmol/gprot)	59.87±4.74[a]	17.06±3.12[c]	27.34±7.90[b]	26.82±5.39[b]	24.07±5.49[bc]
MDA (nmol/mgprot)	8.36±0.15[d]	16.41±0.21[a]	10.52±0.13[c]	12.42±0.20[b]	10.45±0.22[c]
ROS (IU/mL)	631.43±29.83[c]	918.53±108.38[a]	661.07±37.17[c]	684.07±41.02[c]	803.54±61.75[b]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S10. Effects of MF on brain SOD, MDA and GSH-PX in AH rats.

|	NC	AL	SN	MFH	MFL
SOD (U/mgprot)	4.43±0.41[a]	2.54±0.19[c]	4.18±0.25[a]	4.01±0.76[a]	3.46±0.16[b]
GSH-PX (μmol/gprot)	28.13±1.42[a]	17.01±0.91[d]	24.93±1.18[b]	24.43±1.31[b]	20.23±4.56[c]
MDA (nmol/mgprot)	0.069±0.017[c]	0.17±0.0086[a]	0.081±0.019[c]	0.089±0.015[c]	0.13±0.0048[b]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S11. Effects of MF on hepatic lipid measurements in AH rats.

	NC	AL	SN	MFH	MFL
TG (mmol/g)	3.71±0.92[c]	5.95±0.95[a]	4.01±0.86[bc]	4.08±0.86[bc]	4.93±0.21[b]
TC (mmol/g)	1.57±0.29[c]	2.70±0.43[a]	2.09±0.20[b]	2.11±0.28[b]	2.31±0.31[b]
HDL-C (mmol/g)	3.56±0.23[a]	2.66±0.17[d]	3.33±0.43[b]	3.24±0.14[c]	3.03±0.26[d]
LDL-C (mmol/g)	0.18±0.018[a]	0.32±0.029[b]	0.20±0.038[c]	0.21±0.013[c]	0.28±0.010[c]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

Table S12. Effects of MF on fecal lipid measurements in AH rats.

	NC	AL	SN	MFH	MFL
TG (mmol/g)	0.0048±0.00088[c]	0.0084±0.00059[a]	0.0054±0.0012[c]	0.0054±0.0012[c]	0.0067±0.00086[b]
TC (mmol/g)	0.0053±0.0011[c]	0.0083±0.0011[a]	0.0060±0.0013[bc]	0.0062±0.00092[bc]	0.0069±0.0010[b]

NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–c] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

A


B
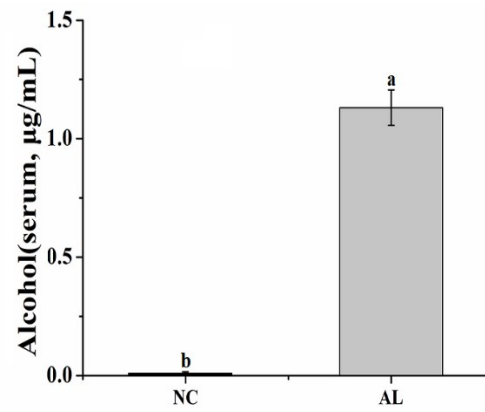
B	**Visual observation**	**H&E staining**	**Oil Red O staining**
NC			
AL			

Fig.S1. (A) The content of alcohol in the serum in the 6-week; (B) The liver visual observation, liver sections were stained with haematoxylin and eosin (H&E) and oil red O to demonstrate the histopathological morphology ($200\times$) in the middle of the experiment. NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day). Values are presented as means ± SD for all groups (n = 10). [a-b] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.
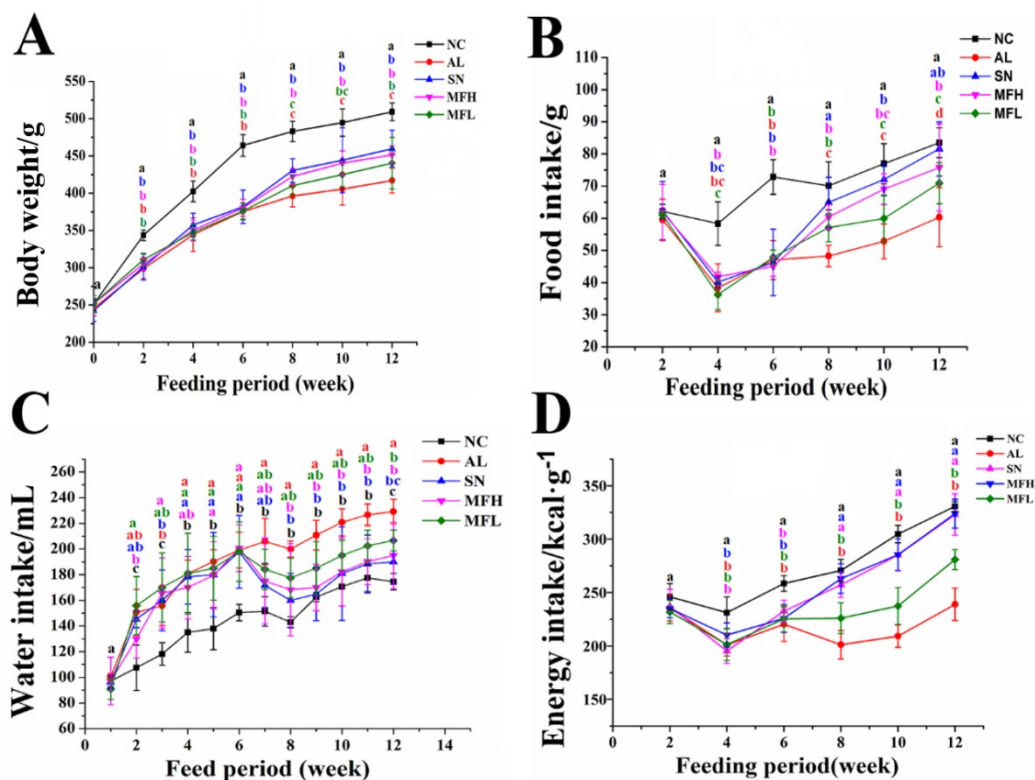
Fig.S2. Effects of MF on body mass, food intake, water and energy intake in AH rats. (A) Body weight; (B) Food intake; (C) Water intake; (D) Energy intake. NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.
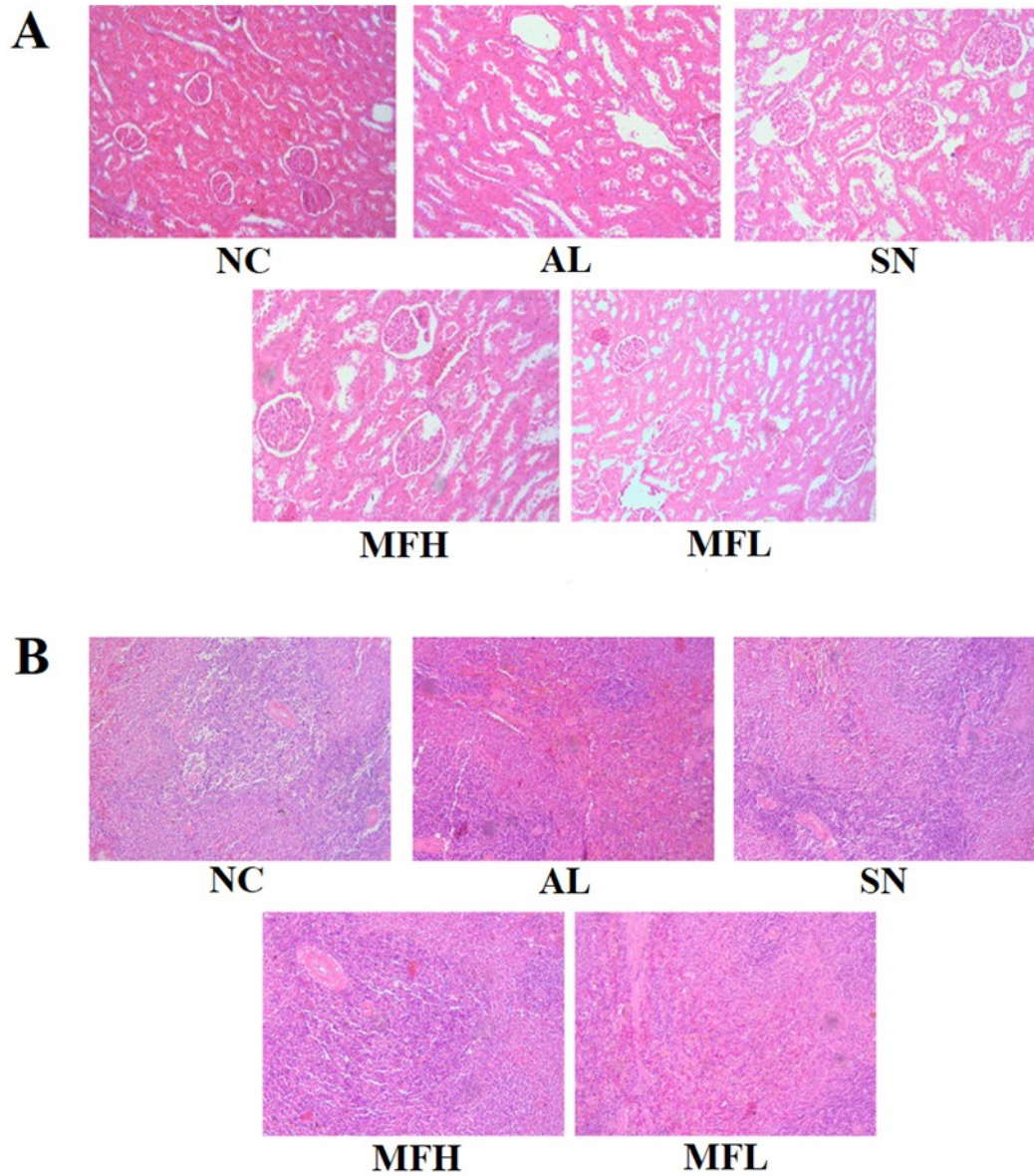
Fig.S3. The kidney and spleen sections were stained with haematoxylin and eosin (H&E) to demonstrate the histopathological morphology (200×) in the end of the experiment. NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day).
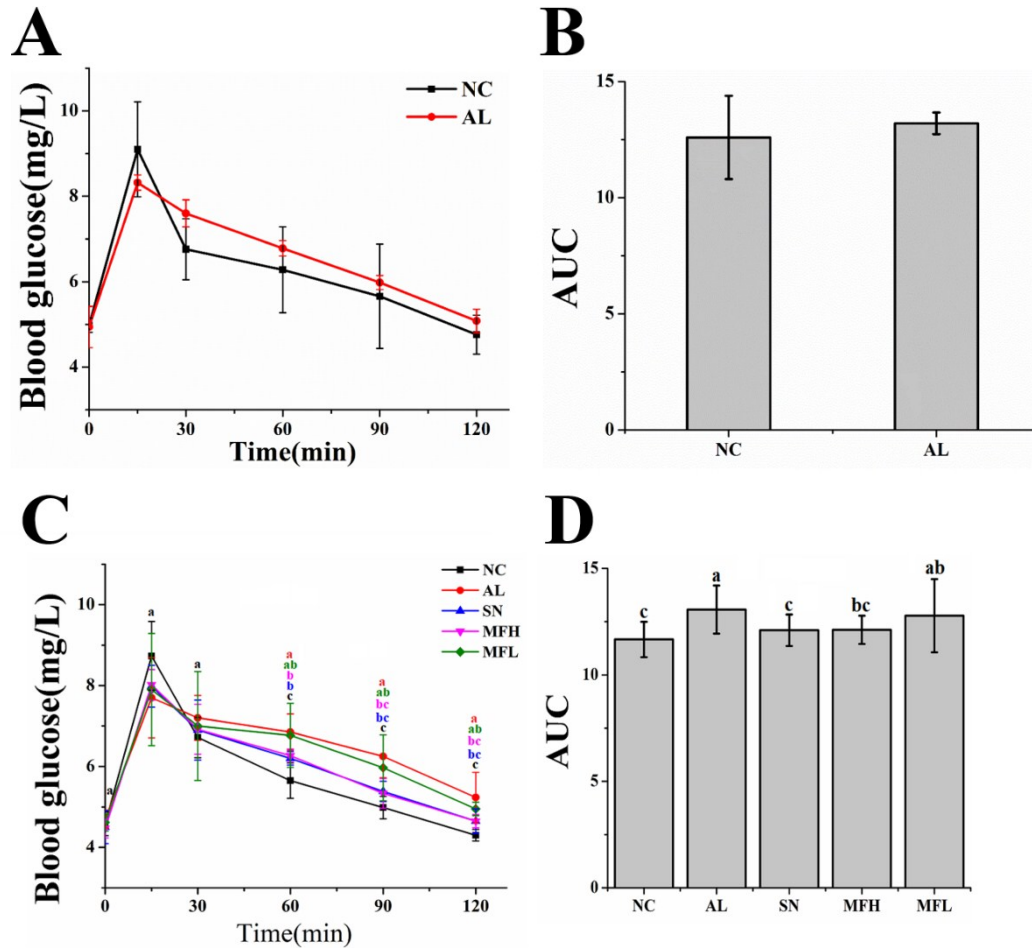
Fig.S4. Effects of MF on glucose intolerance in AH rats. (A) Blood glucose levels and (B) AUC in the middle of the experiment, (C) Blood glucose levels and (D) AUC in the end of the experiment. NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.
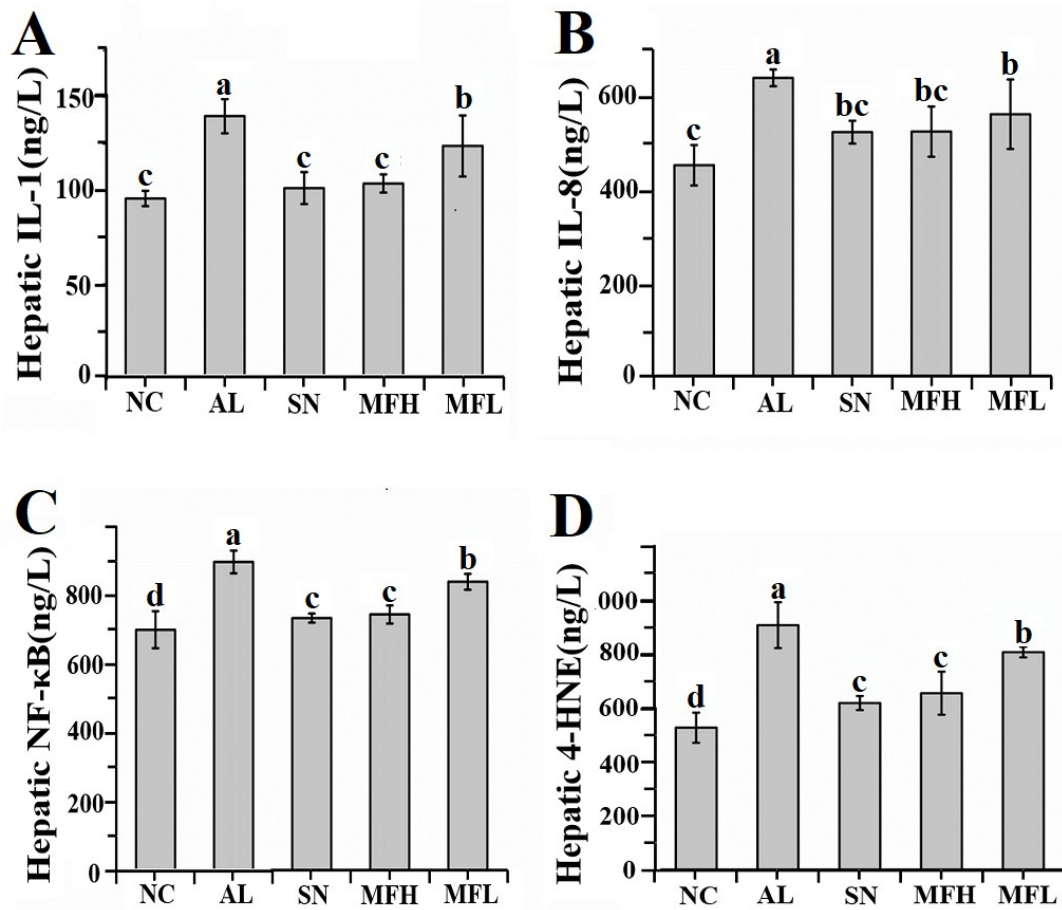
Fig.S5. Effects of MF on hepatic IL-1, IL-8, NF-κB and 4-HNE in AH rats. The levels of (A) IL-1, (B) IL-8, (C) NF-κB and (D) 4-HNE in liver at the end of the experiment. NC, normal group (0.9% normal saline 10 mL/kg/day); AL, alcohol group (40% alcohol 10 mL/kg/day); SN, silybin group (40% alcohol 10 mL/kg/day + SN 18.9 mg/kg/day); MFH, high-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 100 mg/kg/day); MFL, low-dose mangiferin group (40% alcohol 10 mL/kg/day + MF 50 mg/kg/day). Values are presented as means ± SD for all groups (n = 10). [a–d] Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.