Supplementary Material

Investigating the Effect and Potential Mechanism of Rhamnetin 3-*O*-α-Rhamnoside on Acute Liver Injury In Vivo and In Vitro

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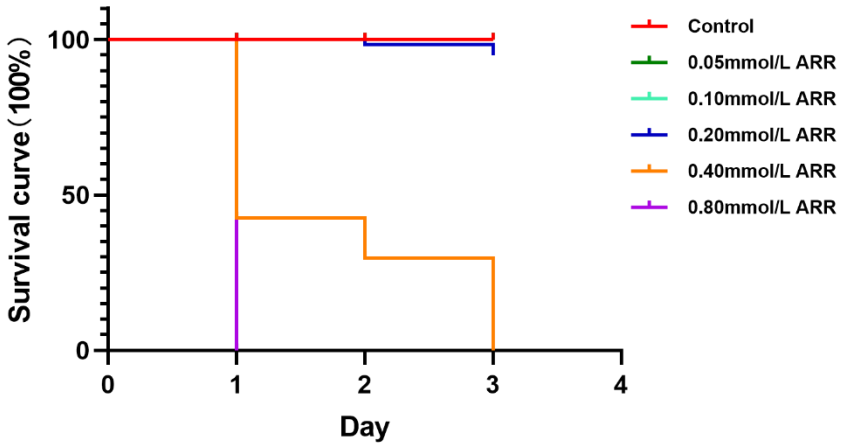
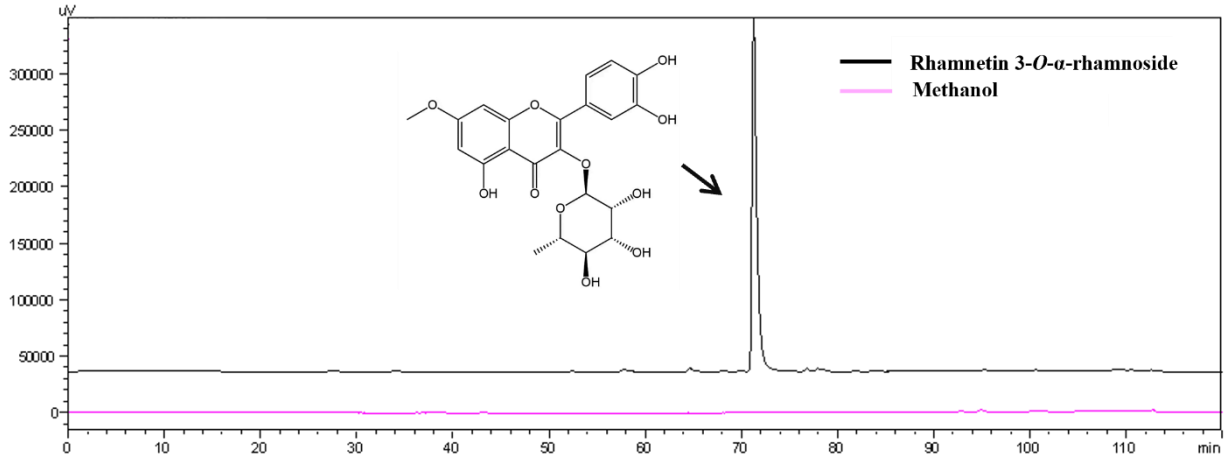
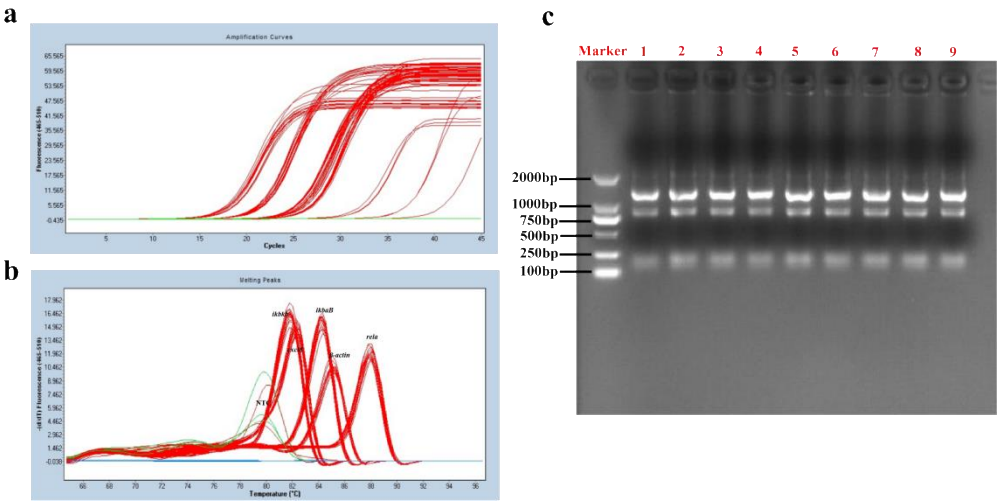


Supplementary Figure S1. Effect of ARR on the survival of zebrafish larvae.



Supplementary Figure S2. The HPLC chromatogram of rhamnetin 3-*O*-α-rhamnoside (ARR) (purity ≥ 95%). HPLC separation was performed on a Shimadzu LC-30AD pump with SPD-M20A detector (Shimadzu, Kyoto, Japan) in 280 nm. For the HPLC columns, Diamonsil 5μm C18 (Dikma, China; 250 mm × 4.6 mm). The column temperature was set to 35℃. The sample injection volume was 20 μL. The mobile phase was consisted of 0.1% phosphoric acid water (A) and acetonitrile (B) at a flow rate of 1mL/min. The gradient elution procedure was as follows: 0-15 min, 5%-10% B; 15-25 min, 10%-15% B; 25-35 min, 15%-19% B; 35-60 min, 19%-30% B; 60-75 min, 30%-45% B; 75-85 min, 45%-80% B; 85-100 min, 80%-100% B.



Supplementary Figure S3. Analysis of results related to qRT-PCR for each group. (**a**) Amplification curves. (**b**) Melt curves of *rela*, *ikbaB*, *ikbkb*, *cxcl8* and *β-actin*. (**c**) Results of RNA agarose gel electrophoresis. From left to right these are 2000 DNA Marker. 1: ARR1, 2: ARR2, 3: ARR3, 4: C1, 5: C2, 6: C3, 7: M1, 8: M2 and 9: M3.

Supplementary Tables

Supplementary Table S1. SYBR Green I PCR systems

Reagents	Additions
2×SYBR Green qPCR Mix	10 μL
Primer F (10 μM)	0.4 μL
Primer R (10 μM)	0.4 μL
cDNA	1 μL
RNase Free H_2O	up to 20 μL

Supplementary Table S2. SYBR Green I PCR procedures

Procedures	Temperature	Time
Pre-incubation	95 ℃	3 min
Amplification (45 cycles)	95 ℃	20 s
	61 ℃	15 s
Melting curves	95 ℃	5 s
	65 ℃	1 min
	97 ℃	Continuous

Supplementary Table S3. The concentration of RNA

Sample Name	OD260/280	OD260/230	Concentration (ng/μL)	Tape
ARR 1	1.96	1.77	1000.94	RNA
ARR 2	1.94	1.79	1346.89	RNA
ARR 3	1.92	1.68	1244.69	RNA
CON 1	1.88	1.66	1220.49	RNA
CON 2	1.92	1.56	1384.25	RNA
CON 3	1.95	1.74	839.509	RNA
MOD 1	1.9	1.6	909.244	RNA
MOD 2	1.83	1.35	851.17	RNA
MOD 3	1.9	1.5	826.627	RNA