Cyanidin-3-o-β-glucoside combined with its metabolite protocatechuic acid attenuated the activation of mice hepatic stellate cells

Electronic Supplementary Information

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Supplementary Materials and Methods

Measuring C3G and PCA by HPLC-PAD. The standard substance of C3G was kindly provided by Biosynth AS (Sandnes, Norway), standard PCA was purchased from Sigma-aldrich (St. Louis, MO, USA). Treated samples of serum and liver tissue were loaded to Agilent Zorbax SB-C^{18} column (2.1 mm × 50 mm, 1.8 μm) at a volume of 50 μL. The mobile phase was composed of eluent A (0.3% formic acid in water, v/v) and eluent B (methanol), the gradient elution program was performed as following supplemental table 1 (Table S1). Column temperature was 30℃, photodiode array detector determine wavelength was 520 nm for C3G and 260 for PCA. The standard curve were shown in Table S2 and S3.

Table S1. The gradient elution program of HPLC.

	Time (min)	Flow rate (mL/min)	%A	%B
1	0.01	1	15	85
2	1	1	20	80
3	3	1	40	60
4	6	1	60	40
5	10	1	65	35
6	12	1	75	25
7	18	1	100	0
8	22	1	15	85
9	23	0.2	15	85

Table S2. The standard curve of C3G.

Standard C3G (ng/μL)	Retention time (min)	Peak area
31.25	10.158	1086424
15.625	11.417	529078
7.813	10.004	303378
3.906	10.183	95285
1.953	10.145	56348
0.977	10.524	21814
0.488	-	-
0	-	-

Table S3. The standard curve of PCA.

Standard PCA (ng/μL)	Retention time (min)	Peak area
31.25	6.072	5500431
15.625	6.052	3040764
7.813	6.033	1509202
3.906	6.057	748310
1.953	6.067	368711
0.977	6.077	191576
0.488	6.064	95215
0	-	-