Supplementary Materials

Ferulic acid identification in brown rice using untargeted metabolomics and network analysis and its effects in mitigating AFB1-induced hepatic damage

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Supplementary Materials and Methods

Polyphenol metabolome between white rice and brown rice

UPLC-ESI-MS/MS conditions

The sample extracts were analyzed using an UPLC-ESI-MS/MS system (UPLC, ExionLC™ AD; MS, Applied Biosystems 4500 Q TRAP, https://sciex.com.cn/). The analytical conditions were as follows, UPLC: column, Agilent SB-C18 (1.8 μm, 2.1 mm * 100 mm); The mobile phase was consisted of solvent A, pure water with 0.1% formic acid, and solvent B, acetonitrile with 0.1% formic acid. Sample measurements were performed with a gradient program that employed the starting conditions of 95% A, 5% B. Within 9 min, a linear gradient to 5% A, 95% B was programmed, and a composition of 5% A, 95% B was kept for 1 min. Subsequently, a composition of 95% A, 5.0% B was adjusted within 1.1 min and kept for 2.9 min. The flow velocity was set as 0.35 mL per minute; The column oven was set to 40°C; The injection volume was 2 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS. The ESI source operation parameters were as follows: source temperature 550°C; ion spray voltage (IS) 5500 V (positive ion mode)/-4500 V (negative ion mode); ion source gas I (GSI), gas II(GSII) and curtain gas (CUR) were set at 50, 60, and 25 psi, respectively; the collision-activated dissociation (CAD) was high. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to medium.

Cell viability assay

The cell viability was determined with a CCK-8 assay. Briefly, cells (1×10^4 cells per well) were seeded in 96-well plates for 24 h, then cells were treated with different concentrations of reagents for 24 h. The CCK-8 assay was performed according to

previous study [1].

ROS determination

The production of ROS in AML12 cells was measured with a reactive oxygen species assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. Briefly, the treated cells were incubated with DCF-DA (10 mM) for 30 min at 37 °C. Then cells were observed with the fluorescence microscope.

Mitochondrial membrane potential determination

AML12 cells were incubated with JC-1 solution (Beyotime, Shanghai, China) for 20 min at 37 °C in the murk environment and were aspirated with the staining solution. Then the fluorescence intensity of cells was tested by an inverted fluorescence microscope.

Cell apoptosis detection

Terminal-deoxynucleoitidyl Transferase/(TdT-) Mediated Nick End Labeling (TUNEL) assay was used to detect apoptotic cells in the liver of mice. Liver Section (5 μm) were dewaxed with xylene, hydrated with graded ethanol solutions, and then treated with proteinase K. After treatment with equilibration buffer, liver sections were marked by FITC-12-dUTP, recombined with TdT enzyme, and stained by DAPI. The stained liver sections were observed under a fluorescence microscope. Cells with green fluorescence are apoptotic cells, while cells with blue fluorescence are living cells.

RNA extraction and quantitative real-time PCR analysis

Total RNA in AML12 cells and liver of mice were isolated by FastPure® Cell/Tissue

Total RNA Isolation Kit V2 (RC112; Vazyme Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions. The concentration of the RNA was measured in a NanoDrop-2000 spectrophotometer (ThermoFisher Scientific Co., Waltham MA, USA). Reverse transcription was done using the HiScript® II Q RT SuperMix for qPCR (R223-01; Vazyme Biotech Co., Ltd., Nanjing, China) following the manufacturer's instructions. Two-step quantitative real-time PCR was performed with SYBR qPCR Master Mix (Q712-02; Vazyme Biotech Co., Ltd., Nanjing, China) on a Real-Time PCR Detection Systems (Bio-Rad, Hercules, California, USA, CFX Connect™) according to the manufacturer's instructions. GenBank from the National Center for Biotechnology Information was used to design pairs of primers. PCR conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 10s, 58°C for 30s, with a final extension at 72°C for 5 min. The mRNA expression levels of genes were determined based on the expression of the GAPDH using the $2^{-\Delta\Delta Ct}$ method [2].

Table S1. The quantitative real-time PCR primers used in this study.

Target genes	**Primer sequence (5' to 3')**
GAPDH	F: AGGTCGGTGTGAACGGATTTG
	R: TGTAGACCATGTAGTTGAGGTCA
Caspase-3	F: AGAGACATTCATGGGCCTGAAATAC
	R: CACCATGGCTTAGAATCACACACAC
Bax	F: GATCAGCTCGGGCACTTTAG
	R: TTGCTGATGGCAACTTCAAC
Bcl-2	F: GCCACCTGTGGTCCATCT
	R: CATCCCAGCCTCCGTTAT

F: forward; R, reverse. *GAPDH*, *Glyceraldehyde-3-phosphate dehydrogenase*; *Caspase-3*, *cysteine aspartate-specific protease 3*; *Bax, B-cell lymphoma-2-associated X protein*; *Bcl-2, B-cell lymphoma-2*.

Table S2. The differential metabolites identified between brown rice and white rice.

No.	Compounds	Q1 (Da)	Q3 (Da)	Molecular weight	Formula	Ionization model	Class I
1	Cyanidin 3-O-(6-O-p-coumaroyl) glucoside	595.14	287.06	595.14	C30H27O13+	[M]+	Flavonoids
2	1-O-Caffeoyl Galactonic Acid	357.08	195.05	358.09	C15H18O10	[M-H]-	Phenolic acids
3	3,4'-Dihydroxy-3',5'-dimethoxypropiophenone	227.0917	181.0493	226.08358	C11H14O5	[M+H]+	Phenolic acids
4	3'-Hydroxy-4'-O-methylglabridin	353.14	295.06	354.1467	C21H22O5	[M-H]-	Flavonoids
5	Genistein-7-O-galactoside-rhamnose*	579.17	271.06	578.1636	C27H30O14	[M+H]+	Flavonoids
6	Vitexin-7-O-(6''-feruloyl)glucoside	771.21	177.1	770.2058	C37H38O18	[M+H]+	Flavonoids
7	Tamarixetin-3-O-glucoside-7-O-rhamnoside*	625.1763	317.0698	624.169	C28H32O16	[M+H]+	Flavonoids
8	Nepetin-4'-O-diglucoside	641.17	317.06	640.1639	C28H32O17	[M+H]+	Flavonoids
9	Aureusidin-4-O-glucoside	449.11	287.06	448.1006	C21H20O11	[M+H]+	Flavonoids
10	O-Feruloyl 7-hydroxycoumarin*	339.09	177.05	338.079	C19H14O6	[M+H]+	Lignans and Coumarins
11	Chrysoeriol feruloyl glucosyl glucoside	801.23	301.07	800.2164	C38H40O19	[M+H]+	Flavonoids
12	sophorabioside*	579.17	271.06	578.1636	C27H30O14	[M+H]+	Flavonoids
13	Kaempferol-4'-O-glucoside*	449.11	287.06	448.1006	C21H20O11	[M+H]+	Flavonoids
14	3-Hydroxy-1-(4-Hydroxy-3-Methoxyphenyl)Propa	197.08	151.04	196.0736	C10H12O4	[M+H]+	Phenolic acids

15	n-1-One Kaempferol-3-O-glucoside (Astragalin)*	449.1078	287.0553	448.1006	C21H20O11	[M+H]+	Flavonoids
16	Luteolin-7-O-neohesperidoside (Lonicerin)*	595.1658	287.0584	594.1585	C27H30O15	[M+H]+	Flavonoids
17	Hesperetin-7-O-glucoside	465.14	303.09	464.1319	C22H24O11	[M+H]+	Flavonoids
18	Hispidulin-8-C-glucoside*	463.13	343.09	462.1162	C22H22O11	[M+H]+	Flavonoids
19	Methylhesperidin	625.21	317.1	624.2054	C29H36O15	[M+H]+	Flavonoids
20	Petunidin-3-O-(6"-O-p-Coumaroyl) glucoside	625.16	317.07	625.1552	C31H29O14+	[M]+	Flavonoids
21	Arillanin A	723.21	547.17	724.2215	C33H40O18	[M-H]-	Phenolic acids
22	6-O-Feruloyl- β -D-glucose	355.1	193.05	356.1107	C16H20O9	[M-H]-	Phenolic acids
23	3-Hydroxy-4-methoxybenzoic acid; Isovanillic Acid	169.05	65.04	168.0423	C8H8O4	[M+H]+	Phenolic acids
24	Kaempferol-3-O-Rutinoside (Nicotiflorin)*	595.1658	287.0532	594.1585	C27H30O15	[M+H]+	Flavonoids
25	Rhamnetin-3-O-Rutinoside*	625.1661	317.066	624.169	C28H32O16	[M+H]+	Flavonoids
26	5,7,4'-Trihydroxy-6,8-dimethoxyisoflavone-7-O-galactoside-rhamnose*	655.19	331.08	654.1796	C29H34O17	[M+H]+	Flavonoids
27	Syringic acid	197.05	123.01	198.0528	C9H10O5	[M-H]-	Phenolic acids
28	Swertiajaponin	463.12	313.07	462.1162	C22H22O11	[M+H]+	Flavonoids
29	Isorhamnetin-3-O-neohesperidoside*	625.17	317.07	624.169	C28H32O16	[M+H]+	Flavonoids

30	Isosaponarin (Isovitexin-4'-O-glucoside)	595.17	313.07	594.1585	C27H30O15	[M+H]+	Flavonoids
31	Luteolin-7-O-glucoside (Cynaroside)*	449.1078	287.0622	448.1006	C21H20O11	[M+H]+	Flavonoids
32	Sibiricose A5*	517.16	175.04	518.1636	C22H30O14	[M-H]-	Phenolic acids
33	6-C-Methylquercetin-3-O-rutinoside	625.18	317.06	624.169	C28H32O16	[M+H]+	Flavonoids
34	Chrysoeriol xylosyl glucoside	595.17	301.07	594.1585	C27H30O15	[M+H]+	Flavonoids
35	Chrysoeriol-7-O-rutinoside-5-O-glucoside	771.23	463.2	770.2269	C34H42O20	[M+H]+	Flavonoids

Supplementary References

1. Q. Wang, T. Liu, M. Koci, Y. Wang, Y. Fu, M. Ma, Q. Ma and L. Zhao, Chlorogenic Acid Alleviated AFB1-Induced Hepatotoxicity by Regulating Mitochondrial Function, Activating Nrf2/HO-1, and Inhibiting Noncanonical NF-kappaB Signaling Pathway, *Antioxidants (Basel)*, 2023, **12**.
2. K. J. Livak and T. D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods*, 2001, **25**, 402-408.