**Components** **synergy between stilbenes and emodin derivatives contributes to hepatotoxicity induced by *Polygonum multiflorum***

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**SUPPLEMENTARY MATERIALS**

**Supplementary methods**

***Animals and study design***



Fig S1 Dosage screening experiment for 2,3,5,4´-tetrahydroxyl diphenylethylene-2-*O*-glucoside (TSG) and emodin-8-*O*-*β*-D-glucoside (EG). \*P<0.05 and \*\*P<0.01 *vs* Normal group, respectively. #P<0.05 and ##P<0.01 *vs* lipopolysaccharide (LPS) group, respectively. n=6.

**Supplementary results**

***LC-MS analysis of serum sample from patient with liver injury of PM***

*Case report*

The patient is a 55-year-old male, who was hospitalized in 302 hospital on March 21, 2012 for "1 month of yellowing of urine and 1 week of yellowing of his eyes". In order to turn his hair black, the patient took *Polygonum multiflorum* Thunb. (PM) for nearly 3 months. In March 22, 2012, his serum liver function tests revealed as alanine aminotransferase (ALT) 938 U/L, aspartate transaminase (AST) 396 U/L, total bilirubin (TBil) 107.8μmol/L, direct bilirubin (DBil) 85.4μmol/L, alkaline phosphatase (ALP) 167 U/L, total bile acid (TBA) 194μmol/L, albumin (ALB) 36g/L, total protein (TP) 55g/L, alpha-fetoprotein (AFP) 9 ng/mL.

The patient stated that he had no history of liver disease and denial of alcohol abuse. In the first three months of his illness, he did not take other known drugs except for PM, nor did he have a history of unclean diet and food allergies. The patient's family history was negative for inherited metabolic diseases. The serologic markers of hepatitis viruses (HBVAg, HCVAb, HAVAb, HEVAb, anti-CMV, anti-EB), immunological antibodies (IgA, IgG, IgM, ANA, anti-AMA, anti-SMA) were all negative.

The withdrawal of PM had been absolutely done under the requisition of clinicians, when the patient was admitted to 302 hospital. Symptomatic and supportive treatments such as liver-protecting were applied. His symptoms of yellowing of the urine and yellowing of the eyes were significantly alleviated, and his serum aminotransferase and bilirubin was decreasing gradually (Supplementary Table 1). According to Roussel Uclaf Causality Assessment Method (RUCAM) score system and American College of Gastroenterology (ACG) clinical guideline about the diagnosis and management of idiosyncratic drug-induced liver injury, the RUCAM scale greater than 8, which means that the liver damage is highly correlated with PM.

Table S1 The recovery of liver function after withdrawal of PM

|  |  |  |  |
| --- | --- | --- | --- |
|  | March 22, 2012 | March 28, 2012 | April 04, 2012 |
| ALT (U/L) | 938 | 287 | 132 |
| AST (U/L) | 396 | 80 | 55 |
| ALP (U/L) | 167 | 158 | - |
| TBil (μmol/L) | 107.8 | 55.8 | 40.4 |
| DBil (μmol/L) | 85.4 | 42.7 | 29.3 |
| ALB (g/L) | 36 | 37 | 37 |
| TP (g/L) | 55 | 57 | - |

***Multivariate statistical analysis of metabolomics***



Fig S2 (A) PCA score plots of different groups in ESI- and ESI+ mode for PCA1 v PCA3. (B) PCA score plots of different groups in ESI+ and ESI- for PCA2 v PCA3. (C) OPLS-DA score plots (left) and S-plots (right) of LPS group and LPS combination drug groups in ESI+.

Table S2 Differential metabolites for each group

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | Differential metabolites | Groups | Differential metabolites |
| Normal *vs* TSG | 422 | Normal *vs* LPS/EG | 742 |
| Normal *vs* EG | 436 | Normal *vs* LPS/TSG/EG | 1465 |
| Normal *vs* TSG/EG | 1087 | LPS *vs* LPS/TSG | 1158 |
| Normal *vs* LPS | 610 | LPS *vs* LPS/EG | 298 |
| Normal *vs* LPS/TSG | 1287 | LPS *vs* LPS/TSG/EG | 1407 |

***MS of identified metabolites***



Fig S3 MS of identified metabolites. Reference substance (left), metabolites in rat plasma (right).