Supplementary Information

Integrative investigation of *Semen Strychni* nephrotoxicity and the protective effect of *Radix Glycyrrhizae* by a UPLC-MS/MS method based cell metabolomics strategy in HEK 293t cell lysates

Liqiang Gu [a], Shujuan Li [a], Ruowen Zhang [b], Yuanyuan Zhang [a], Xiaofan Wang [a], Kexia Zhang [c], Ziying Liu [a], Kaishun Bi [a], Xiaohui Chen [a*]

[a] *School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China*

[b] *Stem Cell Institute, Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294-0024, USA*

[c] *School of Traditional Chinese Materia MedicaShenyang Pharmaceutical University, Shenyang 110016, China*

[*] Corresponding author at: School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, 110016, China.

Email address: cxh_syphu@hotmail.com

Tel.: +8602423986259;

Fax: +8602423986259

Supplementary Information

1. Cell morphology in the dose selection of *Semen Strychni*

The cell morphology changes were observed by treating with a series of concentrations (5, 10, 20, 40, 80 mg/L) of *Semen Strychni*.

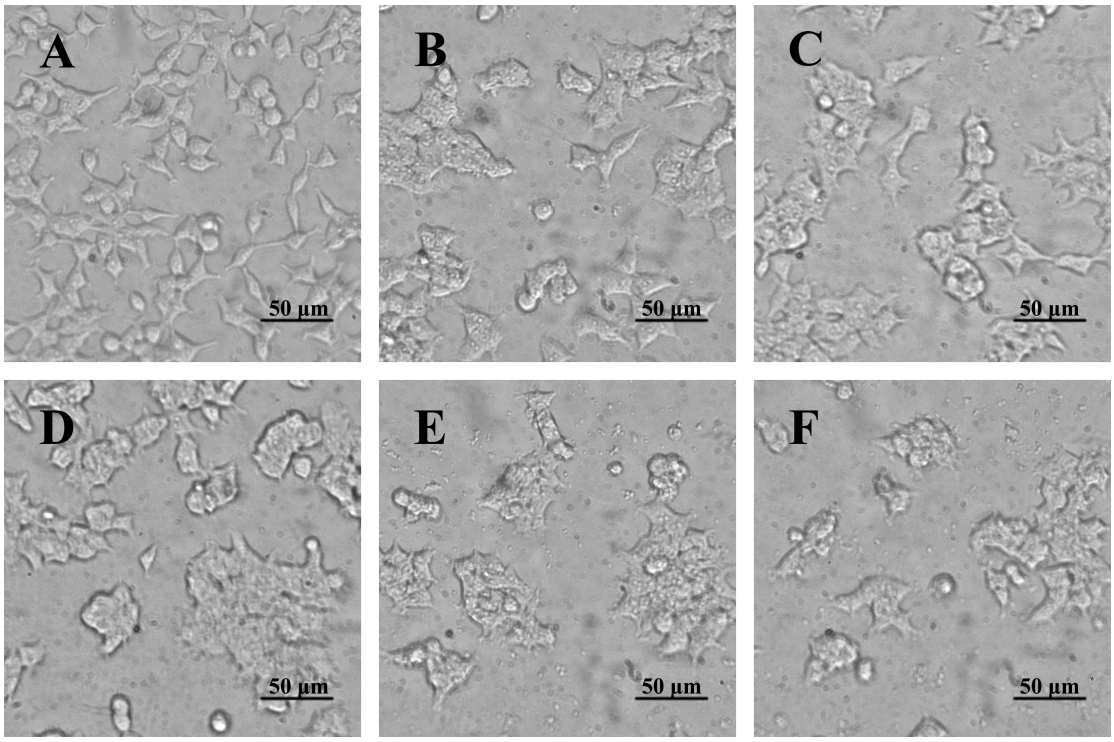

Fig. S1 Representative morphology photographs of HEK 293t cells (200 ×). Cells treated with blank medium (A), showed normal appearances of the scalene triangle or fusiform shapes with the smooth edges. Cells treated with medium of 5, 10 and 20 mg/L *Semen Strychni* (B-D), showed some toxic appearances of the round shapes and the blurred cell edges, while some cells were still in normal appearance. Cells treated with medium of 40 and 80 mg/L *Semen Strychni* (E-F), showed severe toxic appearances of the clustered cell forms, the round shapes and the blurred cell edges. All cells in E-F became round, diopter degraded and easily sheded.

Supplementary Information

2. Identification of the putative biomarkers

The structures of the putative biomarkers were identified by referring to several online databases (for example, Metlin, HMDB and KEGG [Ref.1-3]) and comparing to our acquired commercial standards. The example (*m/z* = 205.0, finally be identified as tryptophan) are shown as follows:

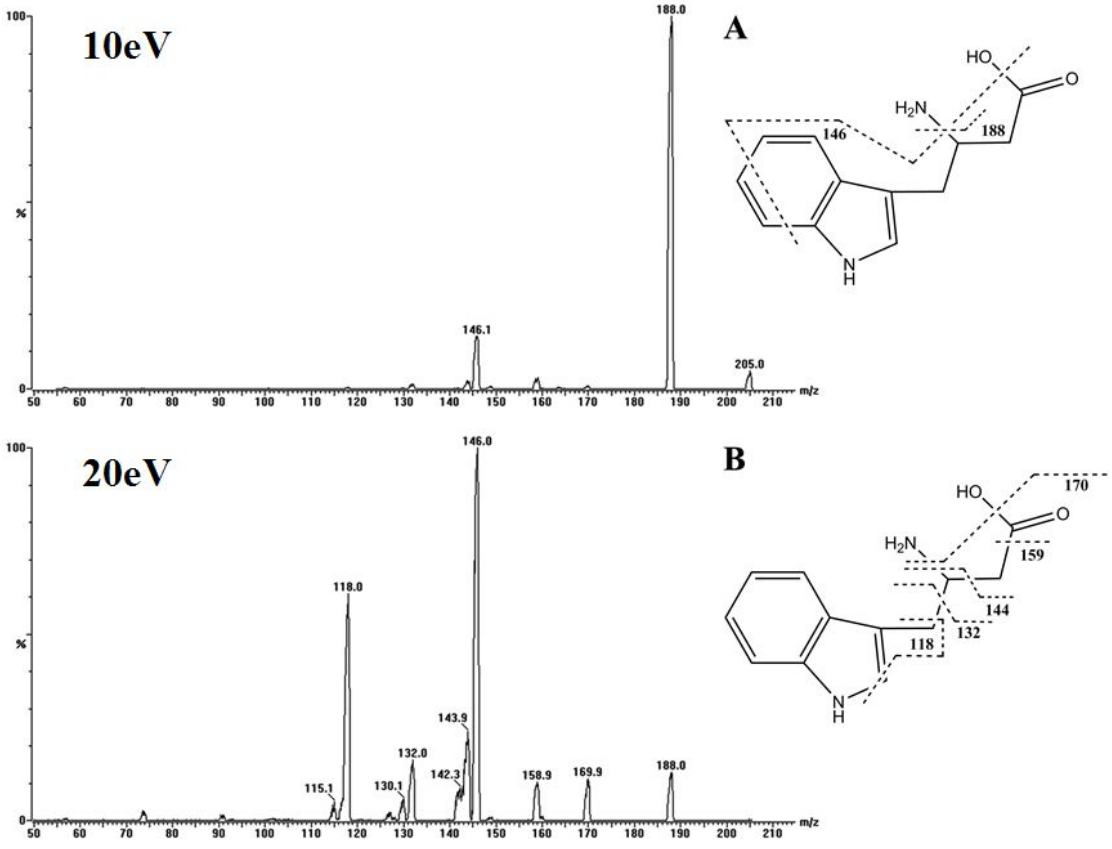

Fig. S2 Product ion spectrum of *m/z* 205.0 with collision energy of 10 eV (A) and 20 eV (B) in positive ion mode and possible MS fragmentation mechanism.

Supplementary Information

3. Cell culturing after 24 hours

We had cultured the cells after 24 hours with fresh drug free medium in the PCG and SSG groups (till 72 hours). The results showed no obvious reverse in cell morphology and viability assays (the values of cell viability after culturing for 48 and 72 h were even lower than that for 24 h) between cell culturing for 24, 48 and 72 hours. It seemed that the toxicity is not reversible by culturing with merely the fresh drug free medium in the PCG and SSG groups.

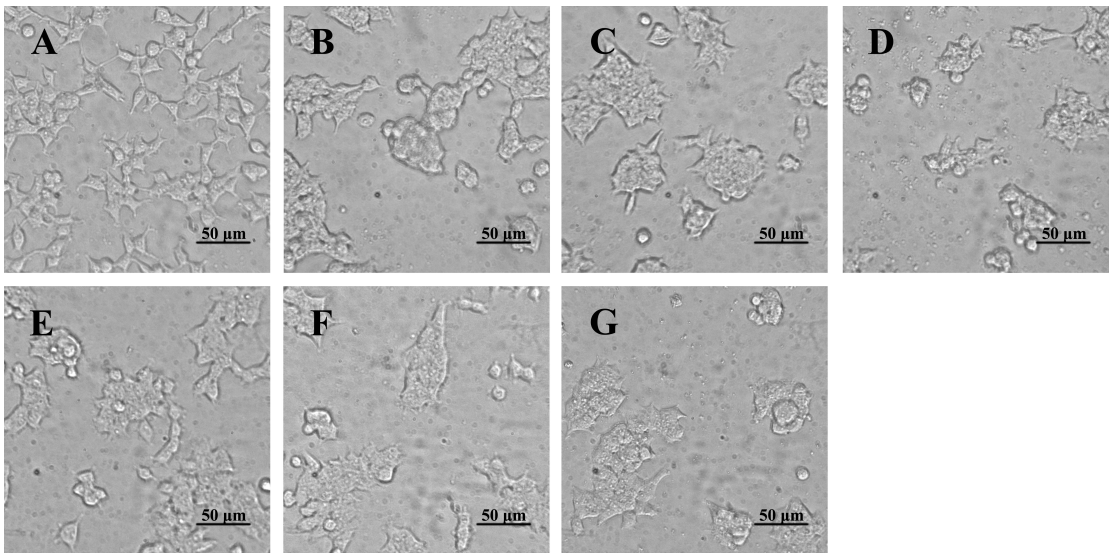

Fig. S3 Representative morphology photographs of HEK 293t cells (200 ×). Cells treated with blank medium (A), showed normal appearances of the scalene triangle or fusiform shapes with the smooth edges. Cells in PCG and SSG after culturing for 24, 48 and 72 h (B-D for PCG and E-G for SSG), showed some toxic appearances of the round shapes and the blurred cell edges, while some cells were still in normal appearance.

Supplementary Information

4. The metabolites in rat serum after treating with *Semen Strychni*

The herb metabolites in serum were identified and semiquantified (for the similar structures of these constituents) by a UPLC-MS/MS method. Results showed that compared with the precursor constituents, the intensities of the metabolites are much lower.

Table S1 Identification of the metabolites in rat serum after treating with *Semen Strychni*

Precursor constituents	**$[M+H]^+$ (m/z)**	**Intensity**
Strychnine	335.17505	6.02E7
Brucine	395.19611	3.92E7
Metabolites	**$[M+H]^+$ (m/z)**	**Intensity**
Isostrychnine	335.17508	4.07E6
11,12-dehydrostrychnine	333.15933	6.73E6
Dehydrostrychnine	333.15543	4.60E6
Strychnine 21,22-epoxide	351.16989	6.82E6
22-OH-strychnine	351.16989	6.07E6
16-OH-strychnine	351.16977	5.56E6
Hydroxyl-strychnine	351.15674	6.75E6
Hydroxyl-strychnine-N-oxide	367.16425	6.68E6
Dihydroxystrychnine	367.16483	4.12E6
21,22-Dihydroxy-22-hydrostrychnine	369.18036	5.58E6
2-OH-3-methoxystrychnine	381.18066	3.15E6
Glucuronide conjugate of strychnine	511.05209	4.89E6
Glucuronide conjugate of hydroxyl-strychnine	527.20166	6.59E5
Demethylated brucine	381.18066	6.56E6
22-OH--brucine	411.19113	4.06E6
16-OH-brucine	411.19083	4.47E6
21,22-Dihydroxy-22-hydrobrucine	429.20175	3.19E6
Glucuronide conjugate of brucine	571.25751	4.46E6

Supplementary Information

References

1 Scripps Center For Metabolomics - Metlin: Meabolite and Tandem MS Database (https://metlin.scripps.edu/index.php).

2 HMDB: The Human Metabolome Database (http://www.hmdb.ca/).

3 KEGG: Kyoto Encyclopedia of Genes and Genomes (http://www.kegg.jp/).