Chemico-Biological Interactions 273 (2017) 11–17

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

# Metabolic profiling of the hepatotoxicity and nephrotoxicity of Ginkgolic acids in rats using ultra-performance liquid chromatography-high-definition mass spectrometry

Yiyun Qian [a], Yunru Peng [b], Erxin Shang [a], Ming Zhao [a], Liang Yan [c], Zhenhua Zhu [a], Jinhua Tao [d], Shulan Su [a], Sheng Guo [a], Jin-ao Duan [a, *]

[a] Jiangsu Collaborative Innovation Center of Chinese Medicinal Resource Industrialization, National and Local Collaborative Engineering Center of Chinese Medicinal Resources Industrialization, Formulae Innovative Medicine, Nanjing University of Chinese Medicine, China
[b] Jiangsu Provincial Institute of Traditional Chinese Medicine, Nanjing 210028, China
[c] Nanjing Sanhome Pharmaceutical Co.,Ltd, NO. 99 West Yunlianghe Road, Xuanwu District, Nanjing, Jiangsu Province, China
[d] School of Pharmacy, Nantong University, Nantong 226001, China

ARTICLE INFO

*Article history:*
Received 11 January 2017
Received in revised form
11 May 2017
Accepted 26 May 2017
Available online 28 May 2017

*Keywords:*
Ginkgolic acids
Metabolomics
Hepatotoxicity
Nephrotoxicity
UPLC-QTOF/MS

ABSTRACT

Ginkgolic acids (GAs) are thought to be the potentially hazardous constituents corresponding to the toxic side effects of Ginkgo products. In this study, toxicological and metabolomics studies of GAs were carried out by ultra-performance liquid chromatography-high-definition mass spectrometry (UPLC-HDMS). Significant changes in serum clinical chemistry were observed in the both low (100 mg/kg) and high (900 mg/kg) doses. Especially the serum enzyme of ALT, AST, LDH, and CK decreased in treated groups. The histopathological observation demonstrated hepatic steatosis in liver and tubular vacuolar degeneration in kidney. These results demonstrated the hepatotoxicity and nephrotoxicity of GAs. Functional disorders are more likely to be toxic induced by GAs. Metabolic profiling within seven days revealed the change of the body status after oral administration. The results indicated the body function was significantly influenced at the 3rd day and could recover in seven days. Metabolomic analysis showed alterations in 14 metabolites from plasma such as LysoPC(18:0), LysoPC(18:2) and other lipids. The results suggested that exposure to GAs could cause disturbances in liver and kidney function associated with the metabolisms of lipids, glucose and the enzyme activity.



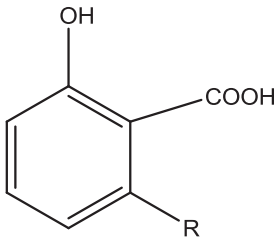
## 1. Introduction

Ginkgolic acids (GAs), such as GA (13:0), GA (15:1), GA (17:2), GA (15:0), and GA (17:1) (Fig. 1), exist in leaves, nuts, and external seed coats of *Ginkgo biloba* L. [1], possessing manypharmacological activities such as anti-tumor activity [2,3], HIV inhibition [4] and protein sumoylation inhibition [5]. Meanwhile, GAs were thought to be the potentially hazardous constituents corresponding to the toxic side effects of Ginkgo extracts [6], which should be less than 5 ppm according to the UE and US pharmacopoeias [7,8]. Many *in vivo* studies focusing on the metabolites identification [9,10], pharmacokinetic study, and tissue distribution of Gas [11,12] have been reported, while its toxicity is still not completely clear.

Traditional medicines play an important role in disease prevention and intervention all over the world, but their various toxicities severely affects their widely use [13,14]. In order to uncover toxic effect and ensure traditional medicine safely, more systematic studies of various toxicities are urgently needed. The techniques of system biology including transcriptomics, proteomics and metabolomics were widely used for the disease diagnosis, biomarker discovery, new drug discovery, toxicity evaluation, pharmacology, human nutrition and environmental science [15–18]. Nuclear magnetic resonance spectroscopy and mass spectrometry (MS) are two mainly analytical tools used in metabolomic-based on the studies of hepatotoxicity and nephrotoxicity of herbal medicines [19–21]. In the MS-based metabolomics, ultra performance liquid chromatography-high-definition mass spectrometry (UPLC-HDMS) is established to be suitable for the analysis of metabolic profiling due to its enhanced reproducibility of retention time [22–26]. Wrona et al. introduced $MS^E$ technique for the first time [27]. $MS^E$

* Corresponding author.
*E-mail address:* dja@njutcm.edu.cn (J.-a. Duan).

http://dx.doi.org/10.1016/j.cbi.2017.05.020
0009-2797/




C13:0 R $C_{13}H_{27}$
C15:0 R $C_{15}H_{31}$
C15:1 R $C_7H_{14}CH{=}CHC_6H_{13}$
C17:1 R $C_9H_{18}CH{=}CHC_6H_{13}$
C17:2 R $C_8H_{16}CH{=}CHCH_2CH{=}CHC_4H_9$

**Fig. 1.** The chemical structures of ginkgolic acids.

provide parallel alternating scans for acquisition in both low collision energy obtained precursor ion in formation and high collision energy obtained full-scan accurate precursor ion, mass fragment and neutral loss information [28–31]. $MS^E$ based on metabolomics technique were widely applied to for biomarker discovery, disease diagnosis and drug evaluation [32–36]. In agreement with the holistic thinking of traditional Chinese medicine, metabolomics has shown potential in evaluation of therapeutic effects and toxicity of traditional Chinese medicine such as *Rheum officinale* [37,38], *Kansui radix* [39], aristolochic acids [40–42] and *Poriacocos* [43–45]. In the current study, UPLC-QTOF/HDMS-based metabolomics and multivariate data analysis were utilized to investigate the hepatotoxicity and nephrotoxicity of Ginkgolic acids in rats after oral administration of GAs at high (900 mg/kg) and low (100 mg/kg) doses, respectively. The serum of rats at 0-day, 1-day, 3-day and 7-day were collected for metabolomic analysis. The potential exposure biomarkers of GAs toxicity were also examined.

## 2. Methods

### 2.1. Chemicals and reagents

Ginkgolic acids (GAs) extract (95.66% purity) were prepared by our laboratory. Acetonitrile, methanol and formic acid were HPLC-grade, and from Merck (Darmstadt, Germany). Deionized water was purified by the Milli-Q system (Millipore, Billerica, MA, USA).

### 2.2. Animal treatment and samples collection

A total of 42 male SD rats weighing approximately 180–200 g were purchased from the vital river experimental animal Co., Ltd., China. They were kept at a controlled humidity (50–60%) and temperature (22 ± 2 °C) with a 12 h light-dark cycle. The rats were randomly divided into seven groups: a control group (6 rats, B), three high-dose groups (900 mg/kg, 6 rats each, H), and three low-dose groups (100 mg/kg, 6 rats each, L). Each group were intragastric administration once and then fed normally. Plasma samples were collected from abdominal aorta at 1d (1H, 1L), 3d (3H, 3L) and 7d (7H, 7L), respectively.

### 2.3. Sample preparation

After the rats were anesthetized with chloral hydrat via intraperitoneal injection, blood samples were collected from the aorta abdominalis. Serum was collected after centrifugation at 3000 rpm for 15 min, which was divided into two parts for immediate analysis of clinical chemistry and later metabolomics analysis, respectively. After blood collection, the rats were sacrificed for histopathological assessment of heart, liver, spleen, lung, kidney and brain. Samples for histology were fixed in 10% formalin, embedded in paraffin wax, cutinto4-mm sections, and examined by light microscopy.

### 2.4. Chromatography

Chromatographic analysis was performed on a Waters Acquity UPLC system (Waters, Corp., Milford, MA, USA), equipped with a binary pump solvent management system, an online degasser, an auto-sampler, and an ACQUITY UPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm) column. The column temperature and flow rate were set at 35 °C and 0.4 mL/min, respectively. The mobile phase was composed of (A) formic acid aqueous solution (0.1%) and (B) acetonitrile using a gradient elution of 5–45% B at 0–3 min, 45–95% B at 3–13 min, 95% B at 13–14 min, and 95-5% B at 14–14.1 min.

### 2.5. Mass spectrometry

The MS analysis was performed on a Waters ACQUITY™ Synapt Q-TOF mass spectrometer connected to the Waters Acquity UPLC system via an electrospray ionization interface (ESI). ESI mass spectra were acquired in both positive and negative ionization modes by scanning over the *m/z* range 100–1000. The optimized conditions were set as follows: (1) $ESI^+$ mode, capillary voltage 3 kV; sample cone voltage 30 V; extraction cone 4.0 V; source temperature 120 °C; desolvation temperature 400 °C; cone gas flow 50 L/h; desolvation gas flow 900 L/h. (2) $ESI^-$ mode, capillary voltage 2.5 kV; sample cone voltage 40 V; extraction cone 4.0 V; source temperature 120 °C; desolvation temperature 400 °C; cone gas flow 50 L/h; desolvation gas flow 800 L/h.

### 2.6. Data analysis

The UPLC-QTOF/HDMS data of samples were analyzed to identify potential discriminating variables. The peak finding, peak alignment, and peak filtering of raw data were carried out with MarkerLynx applications manager version 4.1 (Waters). The parameters used were set as Rt range 0–15min, mass range 100–1000 Da, and mass tolerance0.05 Da. Internal standard detection parameters were deselected for peak retention time alignment, isotopic peaks were excluded for analysis, and noise elimination level was set at 6.00.

The data were mean-centered and pareto-scaled by EZinfo software [46,47], followed by principal component analysis (PCA) for multivariate statistical analysis. Potential biomarkers were explored on the basis of variable importance in the project value (VIP > 1). For the identification of potential markers, the following databases have been used: HMDB (http://www.hmdb.ca/), PubChem (http://ncbi.nlm.nih.gov/), METLIN (http://metlin.scripps.edu/),Massbank (http://www.massbank.jp), and KEGG (http://www.genome.jp/kegg/).