Liver metabonomics study on the protective effect of glycyrrhetinic acid against realgar-induced liver injury

Huo Taoguang, Fang Ying, Zhang Yinghua, Feng Cong, Jiang Hong

Department of Health Laboratory Technology, School of Public Health, China Medical University, Shenyang, 110122, PR China

[ABSTRACT] Glycyrrhetinic acid (GA) is the bioactive ingredient in Glycyrrhizae Rads et Rhizoma. Our previous study has reported that GA has protective effect on realgar-induced hepatotoxicity. However, the details of the hepatoprotective mechanisms of GA on realgar-induced liver injury remain to be elucidated. In the study, mice were divided into control, GA-control, realgar, and co-treated groups. Their liver tissues were used for metabonomics study by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) method. The results illustrate that GA significantly ameliorate the liver injury and metabolic perturbations caused by realgar. Some metabolites, such as phenylalanine, pyroglutamic acid (PGA), proline, carnitine, nicotinamide, choline, lysophosphatidylcholine (LPC) 16:0 and LPC 18:2 were found responsible for the hepatoprotective effect of GA. These metabolites are associated with the methylation metabolism of arsenic, cell membrane structure, energy metabolism and oxidative stress. From the results of this study, we infer that the potential hepatoprotective mechanism of GA on realgar-induced liver injury may be associated with reducing arsenic accumulation and its methylation metabolism in the liver, promoting the conjugation of arsenic and GSH to play detoxification effect, and ameliorating the liver metabolic perturbations caused by realgar.

(KEY WORDS) Metabonomics; Glycyrrhetinic acid; Realgar; Liver; UPLC-MS


Introduction

Realgar (Species: Sulfide; English name: red orpiment; Chinese name: Xionghuang) is a mineral drug that contains more than 90% arsenic disulfide (As₂S₂) and a trace level of soluble arsenic [1]. It is often used in the treatment of variety of skin problems and abdominal pains due to parastic infestation, convulsive epilepsy and malaria according to Chinese Pharmacopeia (2010 edition) [2]. Although the water solubility and bioavailability of realgar is poor, considering the usage and dosage of realgar-containing medicines and the extremely long elimination half-life of arsenic, the potential of arsenic poisoning cannot be ignored [3]. Liver is the major site for arsenic metabolism and it has been regarded as the key target of realgar toxicity [4, 5]. Long-term use or overdose of realgar-containing medicines could cause hepatotoxicity due to the accumulation of arsenic in the liver [5, 6].

Realgar is seldom used alone, and it is frequently used in combination with other herbs in traditional medicines to achieve desired therapeutic effects and to reduce its toxicity.

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[CORRESPONDING AUTHOR] These authors have no conflict of interest to declare.
The accumulation rate = \( \frac{\text{GA content measured in the liver}}{\text{Liver weight}} \times \frac{\text{GA dosage}}{\text{body weight}} \)


Material and reagents

Realgar was purchased from Sanmenxia Yuhuangshan Pharmaceutical Company (batch number: w1-180321, A₃S₂ content > 90.4%, Henan, PR China). GA was purchased from Shanghai Yuanye Biotechnology (CAS#471-53-4; batch number: T09A7X1277; Purity > 97%, Shanghai, PR China). The standard solution of arsenic was provided by Agilent Corporation (Part:5183-4688, USA). Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutathione-S-transferase (GST) were purchased from Ji- an Cheng Biological Engineering Institute (Nanjing, China). Assay kit for S-adenosylmethionine (SAM) was purchased from Enzyme-linked Biotechnology Co., Ltd (Shanghai, China). Acetonitrile and formic acid (HPLC grade) were purchased from Enzyme-linked Biotechnology Co., Ltd (Shanghai, China). Acetonitrile and formic acid (HPLC grade) were purchased from ChemFaces (Wuhan, China). Arsenic solutions were prepared by dissolving realgar in aqueous cold acetonitrile by Tissuelyser II homogenizer, the supernatant was centrifuged at 12 000 rpm for 15 min at 4°C and diluted with deionized water to 5 mL to give a final nitric acid concentration of 10% v/v. The arsenic solution used was 100 μM. Membrane filtration was performed using 0.22 μm membrane filter before use. All other chemicals were of analytical grade and commercially available.

Animal treatment

This is a follow-up study on the hepatoprotective effect of GA on realgar-induced hepatotoxicity with the same set of animals as we previously described [11]. Briefly, healthy, adult, male Institute of Cancer Research (ICR) mice (23-25 g) obtained from the Experimental Animal Center, China Medical University (Permission code: SCXK2015-0001; Liaoning, China) were used and housed in standard conditions. After acclimatization, mice were randomly divided into control (treated with 0.5 % sodium carboxymethylcellulose, CMC-Na), GA-control (treated with 48 mg/kg GA), realgar (treated with 1.35 g/kg realgar) and GA-L (co-treated with 1.35 g/kg realgar and 16 mg/kg GA), GA-H (co-treated with 1.35 g/kg realgar and 48 mg/kg GA) groups, respectively. All the mice were treated for 8 consecutive weeks and sacrificed 12 h after the last administration. The liver tissues were collected and rapidly washed by phosphate buffered saline (PBS) to remove as much blood as possible and were then rapidly frozen in liquid nitrogen to quench metabolism and stored at -70°C until analysis. All animal treatments were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the China Medical University (Publication No. 85-23, revised 1985). All efforts were made to minimize the number and suffering of the animals.

Determination of GA content and accumulation rate in the liver tissues

The contents of GA in liver tissues of GA-control and co-treated groups were measured by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method reported by us before [12]. Briefly, the liver tissues (about 0.2 g) were precipitated with acetonitrile and caffeic acid was used as the internal standard. The analysis was conducted on an Agilent 7700x Inductively Coupled Plasma Mass (ICP-MS) Spectrometer (Agilent Technologies, Waldbronn, Germany). The operating conditions were 1500 W generator power, 15 L/min outer gas flow, 1 L/min intermediate gas flow and 1.2 L/min nebulizer gas flow.

Determination of ASL, AST and GST activities in the liver tissues

The activities of ALT, AST and GST in the liver tissues were measured according to the instructions of assay kits.

Liver metabonomics study

Liver samples of 0.1 g were homogenized in 1.0 mL of 50% aqueous cold acetonitrile by Tissuelyser II homogenizer (QIAGEN, Germany) for 3 min. Quality control (QC) samples were prepared by pooling equal volumes of homogenate from every sample and divided into aliquots. The homogenate was centrifuged at 12 000 rpm for 15 min at 4°C to precipitate proteins. The supernatant (500 μL) was transferred into a 1.5 mL tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The dried residues from the liver extracts were reconstituted in 200 μL water/acetonitrile
Control
GA-control
Realgar GA-L GA-H
0
1
2
3
4
A
**
##
##
Arsenic content (μg·g−1)
0
1 000
2 000
3 000
5 000

realgar group, (GA-L P vs P realgar group, = 0.37; GA-H P = 0.055) and AST activities
realgar group, 0.77; GA-H vs P = GA-H group, elevation of ALT (GA-L realgar group,
pared with the control group (Fig. 2). No obvious changes of ALT and AST activities in the
ALT, AST and GST activities in the liver tissues
The content of GA and its accumulation rate in the liver tissues were given in Fig1B and C. Compared with the GA-control group, significantly decrease of the GA accumulation rate in GA-L and GA-H groups were observed.
ALT, AST and GST activities in the liver tissues
The activities of ALT, AST and GST in the liver tissues were shown in Figure 2. No obvious changes of ALT and AST activities were found between control and GA-control group. Significantly decrease of ALT and AST activities in the liver tissues were found in realgar exposed mice as compared with the control group (P < 0.01). For the GA-L and GA-H group, elevation of ALT (GA-L vs realgar group, P = 0.77; GA-H vs realgar group, P = 0.055) and AST activities (GA-L vs realgar group, P = 0.37; GA-H vs realgar group, P = 0.0035) in the liver tissues were found in comparison to the realgar group.
The activities of GST in the liver tissues of realgar exposed mice were significantly increased as compared with controls (P < 0.05), while the activities were significantly decreased in liver tissues of GA-H group as compared to the realgar group (P < 0.05).
Effect of GA on metabolite profiles in the liver of realgar-exposed mice
A partial least square-discriminant analysis (PLS-DA) analysis was performed on the liver metabolic profiles data from control, GA-control, realgar, GA-L and GA-H groups to reveal their metabolic distinction. The GA-control group was clustered together with the control group (Fig.3A), which in-

(50:50, v/v). The resulting solution was then centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was transferred to autosampler vials and analyzed on an ACQUITY UPLC™ BEH C18 column (50 mm × 2.1 mm, i.d., 1.7 μm) maintained at 40 °C. The metabolites in the liver tissues were profiling with the mobile phase consisted of water (A, containing 0.1% formic acid) and acetonitrile (B, containing 0.1% formic acid) under gradient conditions (15 to 50% B over 0-10 min, 50% to 95% B over 10-15 min, and held at 95% B for 5 min) at a flow rate of 0.25 mL/min. The mass detection was carried out on a Micromass Quattro micro™ API mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) interface set in positive ion mode. The following parameters were set up: capillary voltage of 3.0 kV, cone voltage of 35 V, source temperature of 120 °C and desolvation temperature of 400 °C. Nitrogen was used as the desolvation and cone gas with the flow rates of 450 and 30 L/h respectively. Full scan mode was employed in the mass range of 100-1000 amu. The MS/MS analysis was conducted with collision energy ranging from 10 to 35 eV. Samples from five groups were analyzed randomly and successively. QC samples were analyzed every 6 samples within run.

The data were processed using the Markerlynx Applications Manager version 4.1 (Waters, Manchester, U.K.) to give a table of retention time and mass pairs (tR_m/z) with corresponding intensities for all the detected peaks. The text file (.csv) generated were further exported to MetaboAnalyst (version 4.0, http://metaboanalyst.ca). After the process of data check and missing value estimation, each detected peak signal was normalized to the sum of the total signal response in the metabolic profile. The normalized dataset was pareto-scaled for multivariate data analysis.

Statistical analysis
The data were analyzed with the SPSS software program (version 17.0, Chicago, IL, USA). ANOVA followed by Student’s t-test was used for the evaluation of significant differences of the results. The differences were considered to be statistically significant when P < 0.05.

Result
Arsenic and GA accumulated in the liver tissues
Figure 1A illustrates the arsenic contents in the liver tissues of five groups. The arsenic contents were significantly higher in the liver tissues of the realgar group than those in the control group (P < 0.01). Significantly decrease of arsenic contents were found in GA-L and GA-H groups as compared to the realgar group (P < 0.01). No significant changes in arsenic contents were observed between the control and GA-control groups.

![Fig. 1](image-url)

Fig. 1  (A) Contents of total arsenic in the liver tissues of five groups (n = 6, **P < 0.01, compared with controls; ***P < 0.01, compared with the realgar group); (B) Contents of GA in the liver tissues from GA-control, GA-L and GA-H groups (n = 6); (C) The accumulation rate of GA in the liver tissues from GA-control, GA-L and GA-H groups (n = 6, compared with GA-control group; **P < 0.01)
indicates that the liver metabolic profiles were not significantly changed in normal mice after GA administration. The realgar group was well distinguished from the control group in the PLS-DA score plot (Fig.3A), which suggested that treatment with realgar induced prominent changes in liver metabolites. The data points of GA-L and GA-H groups were clustered between the realgar and control groups in the PLS-DA score plot (Fig.3A), and the data points of GA-L group is more nearer to the realgar group (Fig.3B), which might suggest a dose-dependent protective effect of GA on realgar-induced liver injury.

To delineate putative metabolites that contribute to the toxicity reducing effect of GA, a PLS-DA model (5 components, $R^2=1.0$, $Q^2=0.92$) was established using the data from the liver metabolic profiles of realgar and GA-H groups (Fig.3C and D). Variables were highlighted as important for
discriminant according to variable importance (VIP) values (>1.0) and P-value less than 0.05. Finally, seventeen variables were selected (shown in Table 1). To ensure the reliability of these variables, the stability of these variables in QC samples were studied. The relative standard deviations (RSDs) of peak intensities, retention times and m/z of these variables in QC samples were all less than 9.6% (shown in Table 2). The identification of these variables was conducted by matching retention time and fragmentation pattern with standards or the compounds against a spectral library. Take the ion at 1.58_123.0 in positive ion mode as an example. The base peak ion at m/z 122.7 ([M+H]+) and the ion at m/z 144.6 ([M+Na]+) were observed (Fig.4A). The main fragment ions analyzed via the MS/MS screening were observed at m/z 105.8, 96.0, 79.7, 77.8 and 52.9, which could be the [M+H]+ lost of -NH3, -CHN, -C\_2H\_N, -CH\_2NO, -C\_H\_3N\_2, respectively. Finally, the metabolite was identified as nicotinamide by comparing fragmentation patterns with online HMDB database (HMDB0001406). Its MS/MS spectrum and proposed fragmentation pathway are displayed in Fig.4. By the way, ten of the variables were identified as choline, nicotinamide, phenylalanine, fragmentation of phenylalanine, isotope of phenylalanine, proline, LPC 16:0, pyroglutamic acid (PGA), LPC 18:2 and carnitine. Table 1 shows the identification results.

Table 1  The identification result of potential biomarkers

<table>
<thead>
<tr>
<th>t_r, m/z (min)</th>
<th>Metabolites</th>
<th>VIP</th>
<th>P-Value</th>
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<td>Choline</td>
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<td>Not identified</td>
<td>4.35</td>
<td>4.10E-05</td>
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<tr>
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<td>Nicotinamide</td>
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<td>2.13E-07</td>
</tr>
<tr>
<td>4.11_166.1</td>
<td>Phenylalanine</td>
<td>3.10</td>
<td>2.03E-04</td>
</tr>
<tr>
<td>1.59_103.9</td>
<td>Not identified</td>
<td>2.06</td>
<td>8.07E-03</td>
</tr>
<tr>
<td>1.09_116.0</td>
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<td>5.32E-03</td>
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<td>4.11_120.0</td>
<td>Fragmentation of phenylalanine</td>
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<td>4.12E-02</td>
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<td>5.81E-04</td>
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<td>LPC 16:0</td>
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<td>1.04E-02</td>
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<tr>
<td>1.08_130.1</td>
<td>Pyroglutamic acid</td>
<td>1.49</td>
<td>1.86E-02</td>
</tr>
<tr>
<td>21.8_103.9</td>
<td>Not identified</td>
<td>1.38</td>
<td>4.32E-02</td>
</tr>
<tr>
<td>15.6_520.4</td>
<td>LPC 18:2</td>
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<td>1.53E-02</td>
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<td>22.6_143.0</td>
<td>Not identified</td>
<td>1.21</td>
<td>1.09E-03</td>
</tr>
<tr>
<td>4.11_167.1</td>
<td>Isotope of phenylalanine</td>
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<td>9.38E-04</td>
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<tr>
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<td>1.15</td>
<td>4.47E-03</td>
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<tr>
<td>1.28_162.1</td>
<td>Carnitine</td>
<td>1.07</td>
<td>5.23E-03</td>
</tr>
<tr>
<td>1.59_119.0</td>
<td>Not identified</td>
<td>1.05</td>
<td>1.13E-03</td>
</tr>
</tbody>
</table>

A heatmap colored according to the relative abundances of these significantly changed metabolites was constructed to visualize the differences between realgar and GA-H groups (Fig. 5A). Among all these metabolites, the abundances of phenylalanine, choline, LPC 16:0, carnitine were significantly decreased and the abundances of pyroglutamic acid (PGA), LPC 18:2, proline, nicotinamide were significantly increased in the liver tissues of realgar exposed mice as compared to controls, while GA can partly reverse the alterations of these metabolites in the liver caused by realgar (Fig.6). A Pearson’s correlation analysis was conducted using the abundances of altered metabolites (Fig.5B). The yellow colors represent positive correlations and the blue colors represent negative correlations. The green colors represents no strong correlations between metabolites. The correlation coefficients and significances were given in Table 3.

**Discussion**

The soluble forms of arsenic in realgar colud be absorbed and accumulated in the liver when the animals were exposed continuously and chronically to realgar, therefore, significantly increase of arsenic contents were observed in the liver tissues of realgar exposed mice. Decrease of ALT and AST activities were found in the liver tissues of realgar exposed mice, which indicates the liver injury occurred in mice after 8 weeks realgar exposure. GST is a key enzyme catalyzes the conjugation of reactive electrophile species with glutathione (GSH) to play detoxification function [14]. It has been proposed as a more sensitive indicator of liver damage...
Fig. 4  The mass spectrum of nicotinamide in ESI+ mode proposed (A) and MS fragmentation mechanism (B)

Fig. 5  The hierarchically clustered heatmap of metabolites abundances in realgar and GA-H group. The columns represent samples in different experimental conditions, and the rows represent altered metabolites. (B) Pearson’s correlation plot showing the correlations between the abundance levels of altered metabolites
than ALT [15]. The activities of GST in liver tissues were significantly increased as compared with controls ($P < 0.05$). Elevated activities of ALT and AST and down-regulation of GST activities were found in the liver tissues of GA co-treated groups, indicating a protective effect of GA on realgar-induced liver injury. We found that GA can reduce the accumulation of arsenic in the liver as the arsenic contents were significantly decreased in GA-L and GA-H groups. Interestingly, we found realgar can also reduce GA accumulation in the liver tissues for the GA accumulation rate in GA-L and GA-H groups were significantly decreased as compared with GA-control group. It has been reported that Glycyrrhizae Radix et Rhizoma can reduce the dissolution of arsenic from realgar in artificial gastric or intestine solutions [7] and the function groups of carboxyl groups and hydroxyl groups contained in the structure of GA can interact with arsenic to

Fig. 6 The relative intensities of potential biomarkers in liver tissues of mice from control, realgar and GA-H groups. ($P<0.05$, $P<0.01$, compared with controls; $P<0.05$, $P<0.01$, compared with realgar group)
<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>1.59_103.9</th>
<th>22.6_143.0</th>
<th>LPC 16:0</th>
<th>21.8_103.9</th>
<th>Fragment of Phenylalanine</th>
<th>Choline</th>
<th>Isotope of phenylalanine</th>
<th>1.59_150.0</th>
<th>Phenylalanine</th>
<th>Camitine</th>
<th>LPC 18:2</th>
<th>Pyroglutamic acid</th>
<th>Proline</th>
<th>1.59_119.0</th>
<th>1.35_116.0</th>
<th>Nicotinamide</th>
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<td>1.59_103.9</td>
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<td>0.825**</td>
<td>0.541</td>
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<td>0.537</td>
<td>0.560</td>
<td>0.561</td>
<td>0.584*</td>
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<td>0.292</td>
<td>-0.413</td>
<td>-0.561</td>
<td>-0.741**</td>
<td>-0.921**</td>
<td>-0.645*</td>
<td>-0.742**</td>
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<tr>
<td>22.6_143.0</td>
<td>1</td>
<td>0.667*</td>
<td>0.793**</td>
<td>0.518</td>
<td>0.627*</td>
<td>0.587*</td>
<td>0.635*</td>
<td>0.576*</td>
<td>0.364</td>
<td>-0.182</td>
<td>-0.647*</td>
<td>-0.634*</td>
<td>-0.718**</td>
<td>-0.826**</td>
<td>-0.780**</td>
<td>-0.807**</td>
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<td>1</td>
<td>0.685*</td>
<td>0.516</td>
<td>0.523</td>
<td>0.622*</td>
<td>0.434</td>
<td>0.358</td>
<td>0.402</td>
<td>0.154</td>
<td>-0.338</td>
<td>-0.395</td>
<td>-0.386</td>
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<td>-0.658*</td>
<td>-0.656**</td>
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<td>0.395</td>
<td>0.171</td>
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<td>-0.614*</td>
<td>-0.583*</td>
<td>-0.671*</td>
<td>-0.699*</td>
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<td>Fragment of Phenylalanine</td>
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<td>0.871**</td>
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<td>0.719**</td>
<td>0.465</td>
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<td>-0.397</td>
<td>-0.575</td>
<td>-0.768**</td>
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<tr>
<td>Choline</td>
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<td>0.925**</td>
<td>0.646*</td>
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<td>0.777**</td>
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<td>0.786**</td>
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<td>Camitine</td>
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<td>-0.477</td>
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<tr>
<td>LPC 18:2</td>
<td>1</td>
<td>0.620*</td>
<td>0.436</td>
<td>0.603*</td>
<td>0.120</td>
<td>0.388</td>
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*P<0.05, **P<0.01
form complexes [16]. Therefore, we speculated that the reason of decreased accumulation of arsenic and GA in the liver tissues of GA-L and GA-H groups may be due to the counteract of GA and realgar, which resulted in the reduction of the dissolution of arsenic from realgar and the protective effect of GA on realgar-induced liver injury may be partly associated with decreased arsenic accumulation in the liver.

Liver metabolic profiling study suggests GA restores the liver metabolic disturbances caused by realgar exposure. Some metabolites, including LPCs, choline, phenylalanine, nicotinamide, proline, carnitine and pyroglutamic acid were found to be responsible for the protective effect of GA.

Choline is the most significantly changed metabolites found responsible for the discrimination of realgar and GA-H group. The liver is the major site of choline metabolism. In the liver, choline can be oxidized to betaine then supply a methyl group in the form of S-adenosylmethionine (SAM), which is important for the methylation metabolism of arsenic [17]. The concentrations of SAM in plasma were determined by ELISA method. Significantly decrease of SAM concentrations were found in plasma of realgar-exposed mice as compared with controls (Control: 9.50 ± 0.27 μg/mL; Realgar group: 8.87 ± 0.43 μg/mL; Control vs Realgar group, P = 0.0054). The significantly decreased choline levels in the liver tissues and SAM concentrations in plasma of realgar exposed mice may indicate a reduction of arsenic methylation metabolism, which is considered to be a detoxification pathway [18]. Choline is also a major membrane constituent and is important to the integrity of cell membranes [19]. The down-regulation of choline levels in the liver tissues may also denote the disruption of membrane integrity caused by realgar [11]. Up-regulation of choline levels in the liver tissues and SAM concentrations (Realgar group: 8.87 ± 0.43 μg/mL; GA-L group: 8.92 ± 0.61 μg/mL; GA-H group: 9.31 ± 0.33 μg/mL; GA-L group vs Realgar group, P = 0.88; GA-H group vs Realgar group, P = 0.041) in plasma were found in GA-H group as compared with realgar group, which may suggest an enhancement of arsenic methylation metabolism and a protective effect of GA on realgar-induced membrane toxicity.

LPCs are formed during low-density-lipoprotein oxidation or by degradation of phosphatidylcholine via the enzyme of phospholipase A2 [20]. It has been reported to activate multiple signaling pathways that are involved in oxidative stress and inflammatory responses [21]. In this study, significantly increase of LPC 18:2 levels were observed in the live tissues of realgar exposed mice. GA has anti-oxidant and anti-inflammation effects, thus, decrease of LPC 18:2 levels were found in liver tissues of GA-H group. It has been reported that, in the liver, LPCs can down-regulate hepatic fatty acid oxidation [22]. Consistent with the up-regulation of LPC 18:2 levels in the liver tissues of realgar exposed mice, lower levels of carnitine, which aids the translocation of fatty acids into the mitochondria for β-oxidation were found. The hepatic fatty acids oxidation supplies the tricarboxylic acid (TCA) cycle with acetyl groups [23]. Significantly positive correlation between carnitine and phenylalanine, a TCA cycle-associated amino acids were found. Therefore, the decreased liver carnitine and phenylalanine levels may indicate a suppression of TCA cycle caused by realgar [24, 25]. The suppression of TCA cycle caused ATP depletion. It has been reported that ATP depletion could affect the nicotinamide adenine dinucleotide (NAD+) regeneration resulting in the accumulation of its precursor nicotinamide [26, 27]. Elevated levels of nicotinamide were observed in the liver tissues of realgar exposed mice and negative correlations of nicotinamide between phenylalanine and carnitine were also found. Our previous paper has reported GA can restore the disturbances of the energy metabolism caused by realgar by regulating the levels of plasma D-glucose, lactate and 3-hydroxybutyric acid. In this study, we further suggested that GA can reverse the changes of energy metabolism caused by realgar by regulating liver carnitine, phenylalanine and nicotinamide levels.

Realgar has been reported to induce oxidative stress [28], PGA, which has been proposed as a potential biomarker for enhanced oxidative stress were found significantly increased in the liver tissues of realgar exposed mice [29, 30]. Accumulation of PGA may also suggest deficiencies of GSH synthesis or degradation [31, 32]. Decrease of GSH contents were found in the liver tissues of realgar exposed mice reported in our previous paper [11]. Furthermore, increased contents of GST were found in liver tissues of realgar exposed mice, which may due to the decreased conjugation of GSH and arsenic. Our previous paper has reported that GA can increase liver GSH content and the activities of super-oxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The down-regulation of PGA level found in liver tissues of GA-H group may due to the antioxidant effect of GA. GA decreased the contents of GST in liver tissues of realgar exposed mice, which may promote the conjugation of GSH and arsenic to play detoxification function.

The summary of the effect of GA on liver metabolic profiles of realgar exposed mice is shown in Figure 7.

**Conclusion**

In this study, we found that GA can alleviate the liver injury and liver metabolic profiles perturbations caused by realgar. The most regulated metabolites were those associated with energy metabolism, oxidative stress and membrane structure. Through this study, we infer that the potential hepatoprotective mechanism of GA on realgar-induced liver injury may be associated with reducing arsenic accumulation and its methylation metabolism in the liver, promoting the conjugation of arsenic and GSH to play detoxification effect and ameliorating the liver metabolic perturbations.

**Abbreviations**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CMC-Na: sodium carboxymethylcellulose; ESI: electrospray ionization; GA: glycyrrhetinic acid; GSH: glutathione; GSH-Px: glutathione peroxidase; GST: glutathione-S-
Fig. 7 Summary of the effect of GA on liver metabolic profiles in realgar exposed mice. Red arrows represent the change in realgar exposed mice compared with controls and the blue arrows represent the change in GA treatment group compared with realgar group.


