Chinese Herbal Medicines 14 (2022) 422–431

Contents lists available at ScienceDirect

Chinese Herbal Medicines

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

Original Article

# Metabonomics study of liver and kidney subacute toxicity induced by garidi-5 in rats

Wurihan [a,b], Aodungerle [b], Bilige [b], Lili [b], Sirguleng [c], Aduqinfu [d], Meirong Bai [a,b,*]

[a] Key Laboratory of Mongolian Medicine Research and Development Engineering, Ministry Education, Inner Mongolia Minzu University, Tongliao 028000, China
[b] Mongolian Medical College, Inner Mongolia Minzu University, Tongliao 028000, China
[c] Tongliao City Hospital of Inner Mongolia, Tongliao 028000, China
[d] School of Nursing, Inner Mongolia Minzu University, Tongliao 028000, China

A R T I C L E I N F O

*Article history:*
Received 20 October 2021
Revised 8 April 2022
Accepted 10 May 2022
Available online 22 July 2022

*Keywords:*
garidi-5
kidney
liver
metabonomics
subacute toxicity

A B S T R A C T

*Objective:* Metabonomics was used to analyze and explore the biomarkers and possible mechanisms of liver and kidney subacute toxicity induced by garidi-5 in rats.
*Methods:* Taking garidi-5 as the target drug and SD rats as the research objects, each administration group except the normal group was intragastric administration of the corresponding drug solution for 28 d. The serum, liver and kidney samples of rats were detected by metabolomics and characterized by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) to identify the sensitive markers and metabolic pathways of liver and kidney subacute toxicity.
*Results:* Metabolomics analysis showed that compared with the normal group (Z), the 52, 64 and 54 different metabolites were identified in the serum, liver and kidney samples of garidi-5 high dose group (GG), which were mainly enriched in ABC transporters, arginine and proline metabolism, nicotinate and nicotinamide metabolism, central carbon metabolism in cancer pathways.
*Conclusion:* The preliminarily suggested that garidi-5 can damage the liver and kidney by affecting the ABC transporters, arginine and proline metabolism, nicotinate and nicotinamide metabolism pathways, etc. Trimethylamine *N*-oxide, L-pyroglutamic acid, glycine-betaine, xanthine, glutathione, L-leucine, cytidine, L-arginine, spermidine, urea, 5-aminovaleric acid, creatine, L-glutamic acid, 1-methylnicotinamide and *S*-adenosyl-L-methionine can be used as potential biomarkers of liver and kidney toxicity sensitivity.
© 2022 Tianjin Press of Chinese Herbal Medicines. Published by ELSEVIER B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## 1. Introduction

The mongolian medicine garidi-5, synonymed chong'a and zhachong'ava, originated from the "*Terminalia chebula* Beaded prescription", and is still used as the regional first prescription for eliminating sticky and dry yellow water diseases in Mongolian medicine of all dynasties. It has the effect of eliminate sticky, relieve pain, drying xieriwusu, detumescence etc. The major functions of it are sticky stinging pain, sticky epidemic, diphtheria, erysipelas, anthrax, Heruhu, Wuyaman, Taolai, Xieriwusu disease, swelling, etc. (Inner Mongolia Health Department, 1984). However, because of its toxicity, it is listed as the category of drugs with caution, and so far there is no new understanding and research report on the toxicity of garidi-5. However, according to reviewing relevant research findings, it was found that all the five medicinal of garidi-5 compounds have certain toxicity, and *Aconiti Kusnezoffii Radix* also has gastrointestinal, reproductive and genetic toxicity in addition to cardiotoxicity and neurotoxicity (Wurihan et al., 2021). *Musk* has embryotoxicity and cytotoxicity (Chen et al., 2014). *Terminalia chebula Retz.* has obvious liver toxicity (Wang et al., 2016). *Acorus tatarinowii* Schott has cardiotoxicity and neurotoxicity (Yang, 2018). *Aucklandia* has embryotoxicity, hepatotoxicity and nephrotoxicity (He et al., 2016). It can be seen that the garidi-5 toxicity evaluation is an urgent research work.

Metabolomics can provide such a method for toxicity assessment and mechanism study. It can sensitively monitor the abnormal metabolic changes caused by drugs through dynamic analysis of endogenous metabolites, and clarify the toxic effect and mechanism of drugs (Liu et al., 2010). The rapid and efficient detection technology of metabonomics provides a strong basis for promoting toxicological research, clarifying the potential markers of drug toxicity and safe drug use. Therefore, in this study, LC–MS technology was used to analyze the changes of metabolites in the liver and

* Corresponding author.
*E-mail address:* baimeirong@126.com (M. Bai).

https://doi.org/10.1016/j.chmed.2022.05.003
1674-6384/© 2022 Tianjin Press of Chinese Herbal Medicines. Published by ELSEVIER B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

kidney of Mongolian medicine garidi-5, and to explore the subacute toxicity and mechanism of garidi-5 on the liver and kidney of rats from the perspective of endogenous metabolites, so as to lay a foundation for its in-depth research and safe drug use.

## 2. Methods and materials

### 2.1. Animals

SD male rat, SPF grade, weight (200 ± 20) g, provided by Liaoning Changsheng Biotechnology Co., Ltd. (SCXK (Liao) 2015-0001). Feeding conditions: room temperature (23 ± 1) °C, light for 12 h, free drinking and feeding. Animal welfare and experimental process are in accordance with the provisions of the Medical Ethics Committee of the Affiliated Hospital of Inner Mongolia University for Nationalities.

### 2.2. Drugs and reagents

Garidi-5 is provided by Mongolian medicine preparation room of Affiliated Hospital of Inner Mongolia Minzu University. Alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), UREA nitrogen (UREA), creatinine (CREP), creatine kinase (CK), creatine kinase isoenzyme (CKMB) kits were purchased from Shanghai Roche Diagnostic Products Diagnostic Products Co., Ltd.; purified water (Watsons Group Co., Ltd.), formic acid (Sigma-Aldrich Co.), mass spectrometry acetonitrile (Thermo Fisher Scientific Co., USA).

### 2.3. Instruments

Pipette (EppendorfN13462C, Eppendorf, Germany); Mass ectrometer (TripleTOF5600+, AB SCIEX™); Table-top High-speed Refrigerated Centrifuge (Mikro 220R, Hettich); Roche automatic biochemical analyzer (cobasc 311, Shanghai Roche diagnostic products Diagnostic Products Co., Ltd.); Comminution grinder (8R, Shanghai Anbai Biotechnology Co., Ltd.) Chromatographic UHPLC (Nexera UHPLC LC-30A, SHIMADZU).

### 2.4. Animal grouping and sampling

Thirty-two SD rats were randomly divided into normal group (Z), garidi-5 high-dose group (0.3429 g/kg, clinical equivalent 4 times, GG), garidi-5 medium-dose group (0.0857 g/kg, clinical equivalent, GZ) and garidi-5 low-dose group (0.0214 g/kg, clinical equivalent 1/4 times, GD) according to body weight, with eight rats in each group. From the first day of the experiment, each administration group was intragastric administration of the corresponding drug solution for 28 d, while the normal group was intragastric administration of the equal volume normal saline. After the last administration, anesthetize the animals, the serum was centrifuged at 3500 r/min for 10 min to separate, and part of the supernatant was taken to measure the contents of AST, ALT, CK, CKMB, UREA, CREP and ALP. Collect 1/2 liver and kidney of rats in each group and store them in refrigerator at −80 °C for test.

### 2.5. Metabolite extraction

The serum sample was thawed at 4 °C, 100 μL was taken, 300 μL of methanol was added, the shock extraction was extracted at 12,000 r/min, centrifuged at 4 °C for 10 min, the supernatant was taken over a 0.22 μm organic membrane, and 10 μL was injected, and detected with UPLC-MS.

The Kidney and the liver samples were weighed 0.3 g, 1000 μL of methanol was added to the PE tube, ground to a homogenate with a glass grinder, centrifuged for 15 min (4 °C, 12,000 r/min), and the supernatant was taken 10 μL.

### 2.6. UPLC-MS detection

Chromatographic conditions: SHIMADZU InerSustain $C_{18}$ chromatographic column (100 mm × 2.1 mm, 2 μm). Mobile phase A: acetonitrile, B: 0.1% formic acid aqueous solution, column temperature 35 °C, flow rate 0.3 mL/min, injection volume 10 μL. Elution gradient: 95 %–30% B, 0–7 min; 30%–0% B, 7–13 min; 0%–95% B, 13–16 min.

Mass spectrum parameter conditions: Electrospray ionization (ESI) positive and negative ion modes were used for detection. ESI source conditions are as follows: ion source Gas1:50, ion source Gas2:50, curtain gas (CUR): 25, interface heating temperature: 500 °C (positive ion) and 450 °C (negative ion), ion spray voltage floating (ISVF) 5500 V (positive ion) and 4400 V (negative ion), TOF mass scanning range: 100–1200 Da, product ion scanning range: 50–1000 Da, TOF MS scanning cumulative time 0.2 s, product ion scanning cumulative time 0.01 s, the secondary mass spectrum was obtained by Information Dependent Acquisition (IDA) and high sensitivity mode. Declustering Potential (DP): ± 60 V, collision energy: (35 ± 15) eV.

### 2.7. Data processing

SPSS 22.0 was used for statistical analysis, and the biochemical indexes of each group were expressed as Mean ± SEM. *T-test*, rank sum test and one-way ANOVA were used for comparison between groups, with $P < 0.05$ as the difference, which was statistically significant. The difference was statistically significant with $P < 0.05$.

Use the Analysis Base File Converter software to convert the obtained original data into ABF format, and then import MS-Dial 4.60 software to extract the peak information. Compare with MassBank, Respect and GNPs databases. Multivariate analysis (PCA and PLS-DA) was used to analyze the samples of each group, and $P < 0.05$, FC > 1.5 (or < 0.5) and VIP > 1 were screened as differential metabolites. Metabolic pathways were analyzed using MBRole 2.0.

## 3. Results

### 3.1. Evaluation of serum biochemical indexes in each group

Compared with Z, the contents of ALT, UREA, CK and CKMB in GG group were significantly increased ($P < 0.05$), the contents of UREA, CREP, CKMB and ALP in GZ and GD groups were significantly decreased ($P < 0.05$, $< 0.01$), and the content of AST in the GD group was significantly decreased ($P < 0.05$). It is suggested that garidi-5 has certain toxicity to heart, liver and kidney, as shown in Fig. 1.

### 3.2. Multivariate statistical analysis

#### 3.2.1. PCA analysis results

PCA classifies the data of each group according to the main new variables, removes the heavy abnormal and outlier samples, and can observe the aggregation and dispersion of the samples. As shown in Fig. 2, serum, liver and kidney metabolites of rats in Z and GG group showed a separation trend, indicating that there were certain differences in metabolites. Metabo Analyst was used for cluster analysis, and the difference between groups was analyzed by clustering results of heat map. The color depth indicates the level of metabolites, red is increased and blue is decreased. As shown in Fig. 3, there was significant difference in the relative abundance of metabolites between Z and GG group. The results

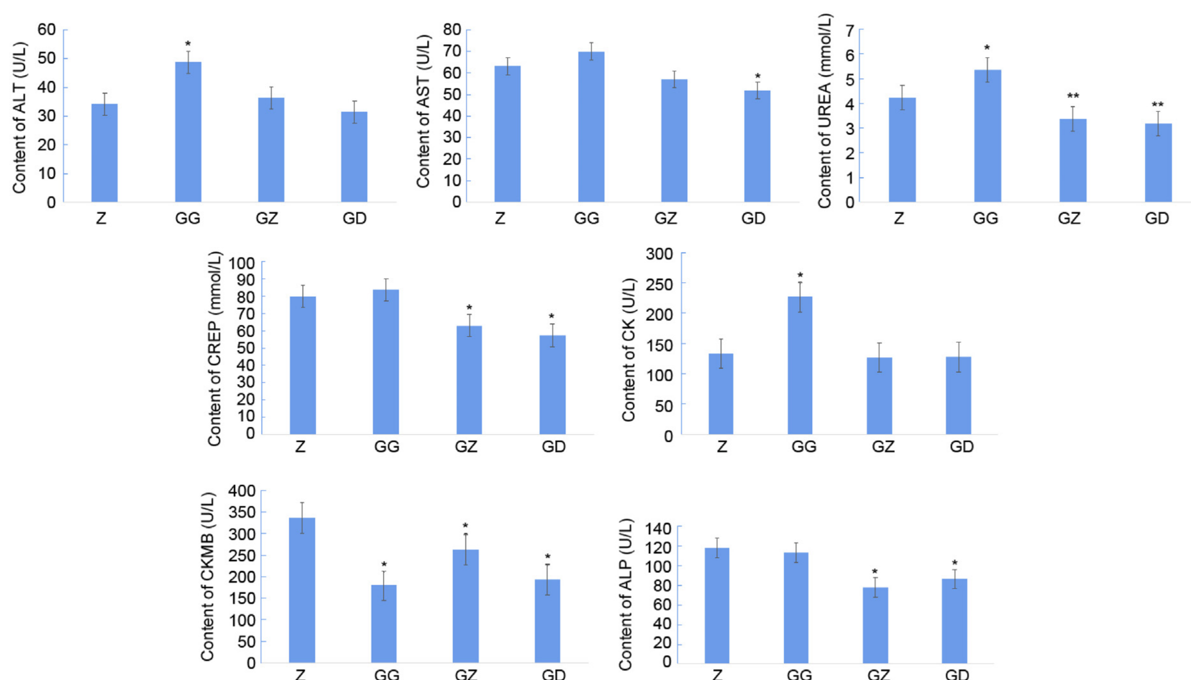


**Fig. 1.** Effects of garidi-5 on biochemical indexes (blood extraction from orbit) of rats. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* normal group (Z).

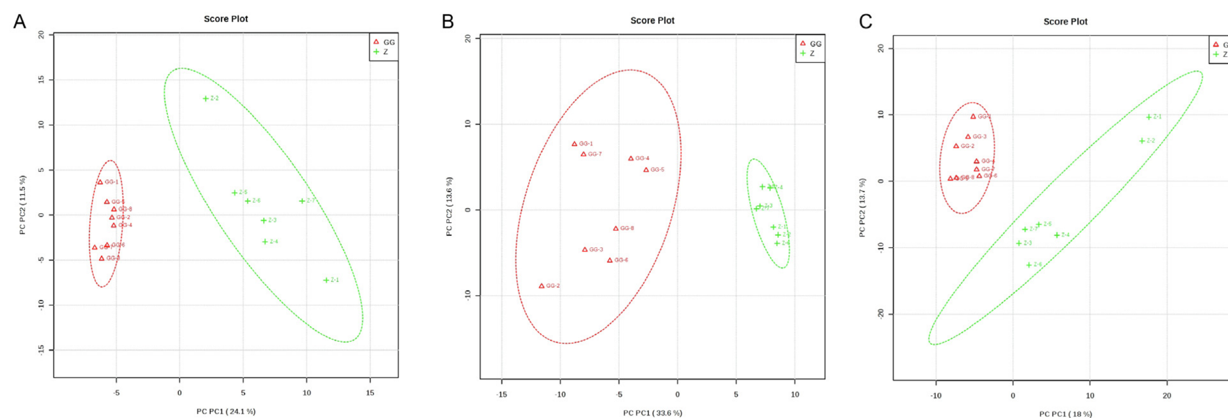


**Fig. 2.** PCA of metabolites in serum (A), liver (B) and kidney (C).

showed that GG could induce certain changes the metabolites of serum, liver and kidney metabolites in rats (Fig. 4).

#### 3.2.2. PLS-DA analysis results

The PLS-DA analysis method can distinguish the difference between groups more efficiently. Compared with the Z, the metabolites in serum, liver and kidney of rats in GG group can be completely separated, indicating a significant difference in metabolite spectrum. The PLS-DA model was verified by parameter test, which has a good explanatory and predictive degree (parameters R2Y and Q2 are both greater than 0.5).

### 3.3. Screening of differential metabolites

#### 3.3.1. Screening of serum differential metabolites

To search for potential differential metabolites, the criteria of VIP > 1, $P < 0.05$ and fold change > 1.5 (or < 0.5) in OPLS-DA were used for screening, and retrieval analysis through MassBank, Respect and GNPS databases. Fifty-two differential metabolites were screened from serum samples of GG group. Compared with Z, GG group L-norvaline, Glu-Gln, glutamine (D), L-pipecolic acid, fructose, kaempferol-3-*O*-rutinoside, L-pyroglutamic acid, L-leucine, DL-pipecolinic acid, DL-3-aminoisobutyric acid, L-(+)-lysine, 3-indoxyl sulfate, glutamic acid, deoxycanitine, 1-imidazoleacetic acid, adipic acid, 3-(4-hydroxyphenyl)lactic acid, glycine-betaine, pyridoxamine, gentisinic acid, fumaric acid, *N*-acetylneuraminic acid, 4-hydroxyphenyllactic acid, phytosphingosine, 3-hydroxyvaleric acid, xanthine, methionine, histidine, *trans*-vaccenic acid, *N-epsilon*-acetyllysine, 1-amino-1-cyclopentanecarboxylic acid, L-arginine, sebacic acid, butyryl carnitine (isomer of 920), 4-hydroxypyridine, $\gamma$-aminobutyric acid and glutathione decreased significantly; Methionine sulfoxide, *N*-$\alpha$-acetyl-L-ornithine, dimethylphthalate, 5-aminovaleric acid, L-citrulline, indole-3-carbinol, trimethylamine *N*-oxide, thiamine monophosphate, glucose, 4-methyl-5-thiazoleethanol, spermidine, hydroxy butyric acid, *N*-acetylhistamine, cytidine and D-alloisoleucine increased significantly, as shown in Table 1.

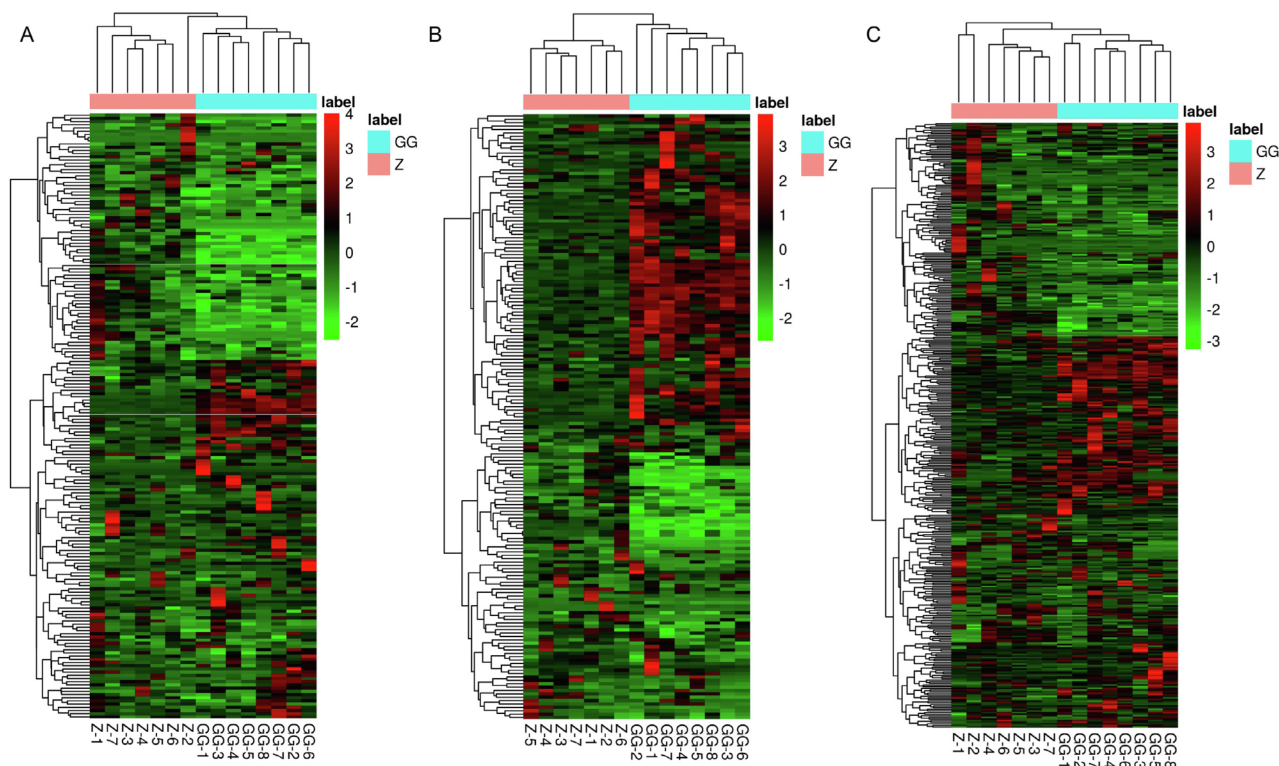


**Fig. 3.** Heatmap of metabolites in serum (A), liver (B) and kidney (C).

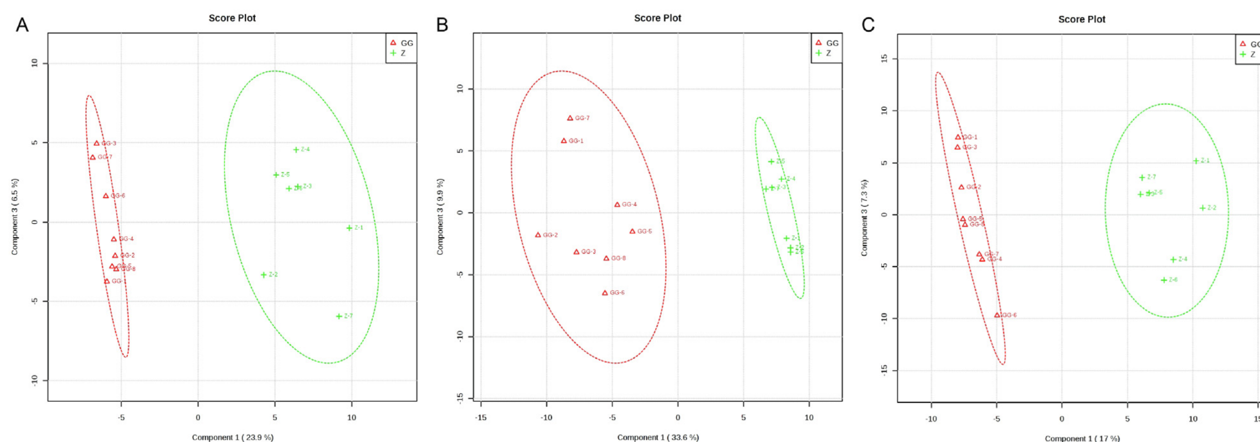


**Fig. 4.** PLS-DA score plot (A: serum; B: liver; C: kidney).

*3.3.2. Screening of liver differential metabolites*

Sixty-four differential metabolites were identified in liver samples. Compared with Z, GG group, tyrosine, creatine, $\gamma$-glutamylleucine, D-2-aminoadipic acid, glutamine, 3-pyridylacetic acid, deoxycarnitine, *N*-acetylneuraminic acid, glutamic acid, asparagine, methionine sulfoxide, Gly-Leu, alanine betaine, 1-methylnicotinamide, aspartate, trimethylamine *N*-oxide, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxyoxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one, morpholine, sebacic acid, (3*S*)-5-[(1*S*,8a*R*)-2,5,5,8a-tetramethyl-4-oxo-4a,6,7,8-tetrahydro-1*H*-naphthalen-1-yl]-3-methylpentanoic acid, urea, butyryl carnitine (isomer of 920), glutathione, $\beta$-nicotinamide adenine dinucleotide, protoporphyrin IX, dehydroisoandrosterone sulfate, glutamic acid, DL-pipecolinic acid, α-methylhistidine, *N*-acetylglutamate, 2-amino-3-[hydroxy-[3-octadecanoyloxy-2-[octadec-11-enoyl]oxypropoxy]phosphoryl]oxypropanoic acid, $\beta$-nicotinamide mononucleotide, L-carnosine, (2*S*)-2-[(2*R*,3*S*,7*R*,8*R*,8a*S*)-2,3,4′-trihydroxy-4,4,7,8a-tetramethyl-6′-oxospiro[2,3,4a,5,6,7-hexahydro-1*H*-naphthalene-8,2′-3,8-dihydrofuro[2,3-*e*]isoindole]-7′-yl]-3-methylbutanoic acid, *N*-acetylmethionine, L-pyroglutamic acid, anserine, glycodeoxycholic acid, glycochenodeoxycholic acid, uridine-5-monophosphate and dimethyl sulfoxide increased significantly; Thiazolidine-4-carboxylic acid, DL-3-aminoisobutyric acid, ndoleacetic acid, 2-hydroxyisocaproic acid, L-(−)-phenylalanine, L-leucine, pyrrole-2-carboxylic acid, cytosine, (*S*)-3-amino-4-phenylbutyric acid, propionic acid, *rac*-glycerol 3-phosphoate, 5-aminovaleric acid, D-arabinose-5-phosphate disodium salt, adipic acid, (*S*)-3-amino-5-methylhexanoic acid, 3-indoxyl sulfate, L-5-oxoproline, 2-hydroxy-4-methylpentanoate, (3*R*,4*S*)-

**Table 1**
Identification of serum differential metabolites.

| tR (min) | Metabolite names | Formula | Reference (*m*/*z*) | Adduct types | Trends (compared with normal) | GG *vs* Z | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | VIP | Log2FC | *P* |
| 6.777 | L-Norvaline | $C_5H_{11}NO_2$ | 116.0717 | $[M-H]^-$ | ↓ | 2.05 | −3.63 | 0.000 |
| 9.603 | Glu-Gln | $C_{10}H_{17}N_3O_6$ | 276.1190 | [M+H]+ | ↓ | 2.04 | −3.46 | 0.000 |
| 7.844 | Methionine sulfoxide | $C_5H_{11}NO_3S$ | 166.0532 | [M+H]+ | ↑ | 2.04 | 2.62 | 0.000 |
| 7.859 | *N*-α-acetyl-L-ornithine | $C_7H_{14}N_2O_3$ | 173.0931 | $[M-H]^-$ | ↑ | 1.99 | 2.76 | 0.000 |
| 1.208 | Dimethyl phthalate | $C_{10}H_{10}O_4$ | 195.0651 | [M+H]+ | ↑ | 1.97 | 3.16 | 0.000 |
| 6.670 | 5-Aminovaleric acid | $C_5H_{11}NO_2$ | 116.0717 | $[M-H]^-$ | ↑ | 1.93 | 3.60 | 0.000 |
| 8.056 | L-Citrulline | $C_6H_{13}N_3O_3$ | 174.0884 | $[M-H]^-$ | ↑ | 1.93 | 3.82 | 0.000 |
| 7.559 | Glutamine (D) | $C_5H_{10}N_2O_3$ | 145.0618 | $[M-H]^-$ | ↓ | 1.86 | −4.05 | 0.000 |
| 10.36 | L-pipecolic acid | $C_6H_{11}NO_2$ | 130.0862 | [M+H]+ | ↓ | 1.76 | −1.85 | 0.000 |
| 2.878 | Fructose | $C_6H_{12}O_6$ | 179.0561 | $[M-H]^-$ | ↓ | 1.75 | −0.85 | 0.000 |
| 0.526 | Kaempferol-3-*O*-rutinoside | $C_{27}H_{30}O_{15}$ | 593.1511 | $[M-H]^-$ | ↓ | 1.74 | −1.03 | 0.000 |
| 7.439 | L-pyroglutamic acid | $C_5H_7NO_3$ | 130.0498 | [M+H]+ | ↓ | 1.71 | −1.10 | 0.000 |
| 6.571 | L-leucine | $C_6H_{13}NO_2$ | 130.0873 | $[M-H]^-$ | ↓ | 1.69 | −3.81 | 0.000 |
| 10.162 | DL-pipecolinic acid | $C_6H_{11}NO_2$ | 130.0862 | [M+H]+ | ↓ | 1.67 | −1.28 | 0.000 |
| 8.027 | DL-3-aminoisobutyric acid | $C_4H_9NO_2$ | 102.0560 | $[M-H]^-$ | ↓ | 1.66 | −7.19 | 0.000 |
| 1.256 | Indole-3-carbinol | $C_9H_9NO$ | 130.0651 | [M+H-$H_2O$]+ | ↑ | 1.66 | 1.23 | 0.000 |
| 10.373 | *L*-(+)-lysine | $C_6H_{14}N_2O_2$ | 147.1128 | [M+H]+ | ↓ | 1.59 | −3.11 | 0.000 |
| 0.647 | 3-Indoxyl sulfate | $C_8H_7NO_4S$ | 212.0023 | $[M-H]^-$ | ↓ | 1.58 | −0.72 | 0.000 |
| 8.001 | Glutamic acid | $C_5H_9NO_4$ | 148.0604 | [M+H]+ | ↓ | 1.58 | −1.39 | 0.000 |
| 9.246 | Deoxycanitine | $C_7H_{15}NO_2$ | 146.1175 | [M+H]+ | ↓ | 1.58 | −1.42 | 0.000 |
| 7.627 | 1-Imidazoleacetic acid | $C_5H_6N_2O_2$ | 127.0502 | [M+H]+ | ↓ | 1.57 | −2.03 | 0.000 |
| 8.133 | Trimethylamine *N*-oxide | $C_3H_9NO$ | 76.0757 | [M+H]+ | ↑ | 1.56 | 1.48 | 0.000 |
| 12.826 | Thiamine monophosphate | $C_{12}H_{18}N_4O_4PS$ | 345.0775 | [M]+ | ↑ | 1.55 | 2.16 | 0.000 |
| 2.099 | Adipic acid | $C_6H_{10}O_4$ | 145.0506 | $[M-H]^-$ | ↓ | 1.54 | −3.02 | 0.000 |
| 8.254 | 4-Methyl-5-thiazoleethanol | $C_6H_9NOS$ | 144.0477 | [M+H]+ | ↑ | 1.53 | 2.38 | 0.000 |
| 2.326 | Glucose | $C_6H_{12}O_6$ | 179.0561 | $[M-H]^-$ | ↑ | 1.53 | 3.02 | 0.000 |
| 5.623 | Spermidine | $C_7H_{19}N_3$ | 146.1651 | [M+H]+ | ↑ | 1.52 | 3.04 | 0.000 |
| 3.058 | 3-(4-Hydroxyphenyl)lactic acid | $C_9H_{10}O_4$ | 181.0506 | $[M-H]^-$ | ↓ | 1.50 | −4.40 | 0.000 |
| 7.482 | Glycine-betaine | $C_5H_{11}NO_2$ | 117.0784 | [M]+ | ↓ | 1.50 | −1.34 | 0.000 |
| 10.154 | Pyridoxamine | $C_8H_{12}N_2O_2$ | 169.0971 | [M+H]+ | ↓ | 1.48 | −1.06 | 0.000 |
| 7.203 | Gentisinic acid | $C_7H_6O_4$ | 155.0338 | [M+H]+ | ↓ | 1.48 | −1.11 | 0.000 |
| 2.508 | Fumaric acid | $C_4H_4O_4$ | 115.0036 | $[M-H]^-$ | ↓ | 1.46 | −3.47 | 0.000 |
| 2.960 | Hydroxy butyric acid | $C_4H_8O_3$ | 103.0400 | $[M-H]^-$ | ↑ | 1.46 | 3.22 | 0.004 |
| 6.675 | *N*-acetylneuraminic acid | $C_{11}H_{19}NO_9$ | 310.1132 | [M+H]+ | ↓ | 1.45 | −1.09 | 0.000 |
| 2.719 | 4-Hydroxyphenyllactic acid | $C_9H_{10}O_4$ | 181.0506 | $[M-H]^-$ | ↓ | 1.43 | −1.97 | 0.000 |
| 3.049 | Phytosphingosine | $C_{18}H_{39}NO_3$ | 318.2994 | [M+H]+ | ↓ | 1.41 | −3.61 | 0.006 |
| 2.922 | 3-Hydroxyvaleric acid | $C_5H_{10}O_3$ | 117.0557 | $[M-H]^-$ | ↓ | 1.39 | −4.37 | 0.000 |
| 1.090 | Xanthine | $C_5H_4N_4O_2$ | 153.0407 | [M+H]+ | ↓ | 1.39 | −1.62 | 0.000 |
| 6.475 | Methionine | $C_5H_{11}NO_2S$ | 150.0580 | [M+H]+ | ↓ | 1.37 | −2.65 | 0.000 |
| 10.152 | Histidine | $C_6H_9N_3O_2$ | 156.0767 | [M+H]+ | ↓ | 1.37 | −1.99 | 0.000 |
| 0.972 | *trans*-Vaccenic acid | $C_{18}H_{34}O_2$ | 281.2480 | $[M-H]^-$ | ↓ | 1.35 | −0.65 | 0.000 |
| 7.820 | *N*-*epsilon*-acetyllysine | $C_8H_{16}N_2O_3$ | 189.1233 | [M+H]+ | ↓ | 1.35 | −1.53 | 0.000 |
| 10.321 | 1-Amino-1-cyclopentanecarboxylic acid | $C_6H_{11}NO_2$ | 130.0862 | [M+H]+ | ↓ | 1.33 | −1.11 | 0.000 |
| 8.103 | L-arginine | $C_6H_{14}N_4O_2$ | 173.1044 | $[M-H]^-$ | ↓ | 1.30 | −4.06 | 0.000 |
| 5.868 | *N*-acetylhistamine | $C_7H_{11}N_3O$ | 154.0974 | [M+H]+ | ↑ | 1.28 | 1.71 | 0.000 |
| 1.490 | Sebacic acid | $C_{10}H_{18}O_4$ | 201.1132 | $[M-H]^-$ | ↓ | 1.24 | −0.96 | 0.000 |
| 7.925 | Butyryl carnitine (isomer of 920) | $C_{11}H_{21}NO_4$ | 232.1553 | [M+H]+ | ↓ | 1.23 | −0.94 | 0.000 |
| 5.930 | 4-Hydroxypyridine | $C_5H_5NO$ | 96.0443 | [M+H]+ | ↓ | 1.21 | −0.96 | 0.000 |
| 6.061 | D-alloisoleucine | $C_6H_{13}NO_2$ | 132.1019 | [M+H]+ | ↑ | 1.21 | 1.88 | 0.000 |
| 7.590 | γ-Aminobutyric acid | $C_4H_9NO_2$ | 104.0706 | [M+H]+ | ↓ | 1.11 | −0.87 | 0.000 |
| 10.367 | Glutathione | $C_{10}H_{17}N_3O_6S$ | 308.0910 | [M+H]+ | ↓ | 1.11 | −1.42 | 0.000 |
| 3.744 | Cytidine | $C_9H_{13}N_3O_5$ | 244.0928 | [M+H]+ | ↑ | 1.08 | 1.49 | 0.000 |

3-amino-4-methylhexanoic acid, phytosphingosine, D-(+)-malic acid, FA 18:2 + 2O and glutathione (oxidized) decreased significantly, as shown in Table 2.

### 3.3.3. Screening of renal differential metabolites

Fifty-four differential metabolites were found in kidney samples. Compared with Z, GG group L-pipecolic acid, ethanolamine, trimethylamine *N*-oxide, DL-3-aminoisobutyric acid, D-(+)-malic acid, pyroglutamic acid, 4-methyl-5-thiazoleethanol, kynurenic acid, glycohyodeoxycholic acid, pantothenate, (*E*,6*S*)-7-hydroxy-2-methyl-6-[(10*S*,13*S*,14*S*,17*S*)-4,4,10,13,14-pentamethyl-3-oxo-1,2,5,6,7,11,12,15,16,17-decahydrocyclopenta[*a*]phenanthren-17-yl]hept-2-enoic acid, 2-piperidone, phenylacetylglycine, uridine 5′-diphospho-*N*-acetylglucosamine, biliverdin, *rac*-glycerol 3-phosphoate, 4-methyldaphnetin, *N*-methyl-DL-alanine increased significantly; *S*-adenosyl-L-methionine, salicylic acid, 5-fluorouracil, methyl heptadecanoic acid, argininosuccinic acid, 5-methylcytidine, targinine, (3*S*)-5-[(1*S*,8a*R*)-2,5,5,8a-tetramethyl-4-oxo-4a,6,7,8-tetrahydro-1*H*-naphthalen-1-yl]-3-methylpentanoic acid, L(+)-cystathionine, cinnamic acid, arachidonic acid, neoandrographolide, gluconic acid, Tyr, carnosine, citric acid, 2-hydroxyisocaproic acid, 9-nitro-20(*S*)-camptothecin, (4*S*,5*Z*,6*S*)-4-(2-methoxy-2-oxoethyl)-5-[2-[(*E*)-3-phenylprop-2-enoyl]oxyethylidene]-6-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4*H*-pyran-3-carboxylic acid, quinaldic acid, proline, ectoine, L-pyroglutamic acid, (*S*)-3-amino-4-phenylbutyric acid, stearic acid, threonic acid, 2-hydroxyisobutyric acid, ascorbic acid, palmitoylcarnitine,

**Table 2**
Identification of liver differential metabolites.

| tR (min) | Metabolite name | Formula | Reference (*m/z*) | Adduct type | Trend (compared with normal) | GG *vs* Z | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | VIP | Log2FC | P |
| 5.684 | Thiazolidine-4-carboxylic acid | $C_4H_7NO_2S$ | 134.0270 | $[M+H]^+$ | ↓ | 1.73 | −6.32 | 0.000 |
| 7.794 | DL-3-aminoisobutyric acid | $C_4H_9NO_2$ | 102.0560 | $[M-H]^-$ | ↓ | 1.70 | −1.30 | 0.000 |
| 6.202 | Tyrosine | $C_9H_{11}NO_3$ | 182.0811 | $[M+H]^+$ | ↑ | 1.67 | 2.09 | 0.000 |
| 6.108 | Ndoleacetic acid | $C_{10}H_9NO_2$ | 176.0706 | $[M+H]^+$ | ↓ | 1.66 | −4.63 | 0.000 |
| 10.413 | 2-Hydroxyisocaproic acid | $C_6H_{12}O_3$ | 131.0713 | $[M-H]^-$ | ↓ | 1.65 | −1.81 | 0.000 |
| 7.702 | Creatine | $C_4H_9N_3O_2$ | 132.0767 | $[M+H]^+$ | ↑ | 1.65 | 1.53 | 0.000 |
| 7.563 | γ-Glutamylleucine | $C_{11}H_{20}N_2O_5$ | 261.1445 | $[M+H]^+$ | ↑ | 1.61 | 1.61 | 0.000 |
| 7.570 | *D*-2-Aminoadipic acid | $C_6H_{11}NO_4$ | 162.0760 | $[M+H]^+$ | ↑ | 1.61 | 1.28 | 0.000 |
| 7.255 | Glutamine | $C_5H_{10}N_2O_3$ | 147.0764 | $[M+H]^+$ | ↑ | 1.60 | 1.57 | 0.000 |
| 7.665 | 3-Pyridylacetic acid | $C_7H_7NO_2$ | 138.0549 | $[M+H]^+$ | ↑ | 1.59 | 1.40 | 0.000 |
| 9.237 | Deoxycarnitine | $C_7H_{15}NO_2$ | 146.1175 | $[M+H]^+$ | ↑ | 1.59 | 1.17 | 0.000 |
| 6.169 | L-(−)-phenylalanine | $C_9H_{11}NO_2$ | 164.0717 | $[M-H]^-$ | ↓ | 1.57 | −0.80 | 0.000 |
| 6.694 | *N*-acetylneuraminic acid | $C_{11}H_{19}NO_9$ | 310.1132 | $[M+H]^+$ | ↑ | 1.57 | 1.27 | 0.000 |
| 7.681 | L-glutamic acid | $C_5H_9NO_4$ | 148.0604 | $[M+H]^+$ | ↑ | 1.57 | 1.64 | 0.000 |
| 7.117 | Asparagine | $C_4H_8N_2O_3$ | 133.0607 | $[M+H]^+$ | ↑ | 1.55 | 1.31 | 0.000 |
| 7.845 | Methionine sulfoxide | $C_5H_{11}NO_3S$ | 166.0532 | $[M+H]^+$ | ↑ | 1.53 | 2.01 | 0.000 |
| 7.222 | Gly-Leu | $C_8H_{16}N_2O_3$ | 189.1233 | $[M+H]^+$ | ↑ | 1.50 | 2.44 | 0.000 |
| 6.145 | L-leucine | $C_6H_{13}NO_2$ | 130.0873 | $[M-H]^-$ | ↓ | 1.48 | −4.34 | 0.000 |
| 1.325 | Pyrrole-2-carboxylic acid | $C_5H_5NO_2$ | 112.0393 | $[M+H]^+$ | ↓ | 1.47 | −6.01 | 0.000 |
| 9.822 | Alanine betaine | $C_6H_{13}NO_2$ | 132.1019 | $[M+H]^+$ | ↑ | 1.47 | 1.44 | 0.000 |
| 6.841 | 1-Methylnicotinamide | $C_7H_9N_{2O}$ | 137.0703 | $[M+H]^+$ | ↑ | 1.39 | 2.08 | 0.000 |
| 1.972 | Cytosine | $C_4H_5N_3O$ | 112.0505 | $[M+H]^+$ | ↓ | 1.38 | −4.81 | 0.000 |
| 1.368 | (S)-3-amino-4-phenylbutyric acid | $C_{10}H_{13}NO_2$ | 180.1019 | $[M+H]^+$ | ↓ | 1.38 | −1.29 | 0.000 |
| 2.116 | Propionic acid | $C_3H_6O_2$ | 73.0295 | $[M-H]^-$ | ↓ | 1.37 | −2.89 | 0.000 |
| 5.790 | Aspartate | $C_4H_7NO_4$ | 132.0302 | $[M-H]^-$ | ↑ | 1.36 | 3.80 | 0.000 |
| 8.173 | Trimethylamine *N*-oxide | $C_3H_9NO$ | 76.0757 | $[M+H]^+$ | ↑ | 1.35 | 1.99 | 0.001 |
| 7.170 | Rac-glycerol 3-phosphoate | $C_3H_9O_6P$ | 171.0063 | $[M-H]^-$ | ↓ | 1.34 | −0.94 | 0.001 |
| 7.654 | (3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-[(2*S*,3*R*,4*S*,5S,6*R*)-3,4,5-tri-hydroxy-6-[[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxyoxan-2-yl]oxymethyl] oxan-2-yl]oxychromen-4-one | $C_{26}H_{28}O_{16}$ | 595.1304 | $[M-H]^-$ | ↑ | 1.34 | 1.94 | 0.001 |
| 7.956 | Morpholine | $C_4H_9NO$ | 88.0756 | $[M+H]^+$ | ↑ | 1.33 | 1.68 | 0.001 |
| 1.432 | Sebacic acid | $C_{10}H_{18}O_4$ | 201.1132 | $[M-H]^-$ | ↑ | 1.32 | 2.52 | 0.001 |
| 1.203 | (3*S*)-5-[(1*S*,8a*R*)-2,5,5,8a-tetramethyl-4-oxo-4a,6,7,8-etrahydro-1*H*-naphthalen-1-yl]-t3-methylpentanoic acid | $C_{20}H_{32}O_3$ | 321.2424 | $[M+H]^+$ | ↑ | 1.32 | 1.44 | 0.001 |
| 6.587 | 5-Aminovaleric acid | $C_5H_{11}NO_2$ | 116.0717 | $[M-H]^-$ | ↓ | 1.30 | −0.58 | 0.001 |
| 1.832 | Urea | $CH_4N_2O$ | 61.0396 | [M+H]+ | ↑ | 1.26 | 2.36 | 0.001 |
| 7.921 | Butyryl carnitine (isomer of 920) | $C_{11}H_{21}NO_4$ | 232.1553 | $[M+H]^+$ | ↑ | 1.26 | 1.02 | 0.003 |
| 10.378 | Glutathione | $C_{10}H_{17}N_3O_6S$ | 308.0910 | $[M+H]^+$ | ↑ | 1.25 | 1.01 | 0.003 |
| 9.980 | β-Nicotinamide adenine dinucleotide | $C_{21}H_{27}N_7O_{14}P_2$ | 664.1163 | $[M+H]^+$ | ↑ | 1.25 | 2.28 | 0.003 |
| 6.965 | D-arabinose-5-phosphate disodium salt | $C_5H_{11}O_8P$ | 229.0118 | $[M-H]^-$ | ↓ | 1.22 | −0.85 | 0.004 |
| 2.231 | Adipic acid | $C_6H_{10}O_4$ | 145.0506 | $[M-H]^-$ | ↓ | 1.22 | −3.22 | 0.004 |
| 1.721 | (*S*)-3-amino-5-methylhexanoic acid | $C_7H_{15}NO_2$ | 146.1175 | $[M+H]^+$ | ↓ | 1.20 | −2.51 | 0.005 |
| 1.770 | Protoporphyrin IX | $C_{34}H_{34}N_4O_4$ | 563.2636 | $[M+H]^+$ | ↑ | 1.19 | 3.35 | 0.006 |
| 0.697 | Dehydroisoandrosterone sulfate | $C_{19}H_{28}O_5S$ | 367.1584 | $[M-H]^-$ | ↑ | 1.18 | 4.04 | 0.006 |
| 7.759 | Glutamic acid | $C_5H_9NO_4$ | 148.0604 | $[M+H]^+$ | ↑ | 1.18 | 1.17 | 0.006 |
| 0.632 | 3-Indoxyl sulfate | $C_8H_7NO_4S$ | 212.0023 | $[M-H]^-$ | ↓ | 1.17 | −1.07 | 0.007 |
| 11.437 | DL-pipecolinic acid | $C_6H_{11}NO_2$ | 130.0862 | $[M+H]^+$ | ↑ | 1.16 | 1.01 | 0.007 |
| 9.182 | α-Methylhistidine | $C_7H_{11}N_3O_2$ | 170.0924 | $[M+H]^+$ | ↑ | 1.16 | 1.79 | 0.008 |
| 11.502 | *L*-5-oxoproline | $C_5H_7NO_3$ | 130.0498 | $[M+H]^+$ | ↓ | 1.12 | −1.78 | 0.011 |
| 6.652 | *N*-acetylglutamate | $C_7H_{11}NO_5$ | 188.0564 | $[M-H]^-$ | ↑ | 1.10 | 1.07 | 0.013 |
| 5.770 | 2-Amino-3-[hydroxy-[3-octadecanoyloxy-2-[octadec-11-enoyl] oxypropoxy]phosphoryl]oxypropanoic acid | $C_{42}H_{80}NO_{10}P$ | 788.5440 | $[M-H]^-$ | ↑ | 1.09 | 5.64 | 0.014 |
| 9.607 | β-Nicotinamide mononucleotide | $C_{11}H_{15}N_2O_8P$ | 335.0638 | $[M+H]^+$ | ↑ | 1.08 | 3.77 | 0.015 |
| 10.660 | L-carnosine | $C_9H_{14}N_4O_3$ | 227.1138 | $[M+H]^+$ | ↑ | 1.08 | 1.89 | 0.015 |
| 8.134 | (2*S*)-2-[(2*R*,3*S*,7*R*,8*R*,8a*S*)-2,3,4′-trihydroxy-4,4,7,8a-tetramethyl-6′-oxospiro[2,3,4a,5,6,7-hexahydro-1*H*-naphthalene-8,2′-3,8-dihydrofuro [2,3-e]isoindole]-7′-yl]-3-methylbutanoic acid | $C_{28}H_{39}NO_7$ | 502.2773 | $[M+H]^+$ | ↑ | 1.07 | 1.13 | 0.017 |
| 5.168 | *N*-acetylmethionine | $C_7H_{13}NO_3S$ | 192.0688 | $[M+H]^+$ | ↑ | 1.07 | 1.61 | 0.017 |
| 5.949 | L-pyroglutamic acid | $C_5H_7NO_3$ | 30.0498 | $[M+H]^+$ | ↑ | 1.06 | 1.06 | 0.017 |
| 9.863 | 2-Hydroxy-4-methylpentanoate | $C_6H_{12}O_3$ | 131.0713 | $[M-H]^-$ | ↓ | 1.06 | −4.35 | 0.018 |
| 11.353 | Anserine | $C_{10}H_{16}N_4O_3$ | 239.1149 | $[M-H]^-$ | ↑ | 1.06 | 1.00 | 0.018 |
| 5.639 | Glycodeoxycholic acid | $C_{26}H_{43}NO_5$ | 472.3030 | $[M+Na]^+$ | ↑ | 1.05 | 4.27 | 0.019 |
| 1.775 | (3*R*,4*S*)-3-amino-4-methylhexanoic acid | $C_7H_{15}NO_2$ | 146.1175 | $[M+H]^+$ | ↓ | 1.04 | −1.45 | 0.021 |
| 5.634 | Glycochenodeoxycholic acid | $C_{26}H_{43}NO_5$ | 450.3210 | $[M+H]^+$ | ↑ | 1.04 | 2.04 | 0.022 |
| 6.744 | Uridine-5-monophosphate | $C_9H_{13}N_2O_9P$ | 323.0280 | $[M-H]^-$ | ↑ | 1.03 | 1.92 | 0.022 |
| 2.015 | Dimethyl sulfoxide | $C_2H_6OS$ | 79.0212 | $[M+H]^+$ | ↑ | 1.02 | 1.93 | 0.025 |
| 5.214 | Phytosphingosine | $C_{18}H_{39}NO_3$ | 318.2994 | $[M+H]^+$ | ↓ | 1.02 | −1.40 | 0.025 |
| 1.258 | D-(+)-malic acid | $C_4H_6O_5$ | 133.0142 | $[M-H]^-$ | ↓ | 1.01 | −1.91 | 0.027 |
| 0.999 | FA 18:2+2O | $C_{18}H_{32}O_4$ | 311.2221 | $[M-H]^-$ | ↓ | 1.00 | −0.58 | 0.027 |
| 11.461 | Glutathione (oxidized) | $C_{20}H_{32}N_6O_{12}S_2$ | 613.1592 | $[M+H]^+$ | ↓ | 1.00 | −1.46 | 0.027 |

resibufogenin, *N*-acetyl-L-leucine, mefenamic acid, *p*-coumaraldehyde, cysteine *S*-sulfate, spermidine decreased significantly, as shown in Table 3.

### 3.4. Pathway enrichment analysis

The differential metabolites screened above were introduced into MBRole 2.0 to analyze its metabolic pathway. Serum differential metabolites involve ABC transporters, arginine and proline metabolism, central carbon metabolism in cancer, nicotinate and nicotinamide metabolism, glutathione metabolism, phenylalanine metabolism, cAMP signaling pathway, metabolic pathways, GABAergic synapse, β-alanine metabolism (Fig. 5A).

Liver differential metabolites are mainly related to ABC transporters, arginine and proline metabolism, central carbon metabolism in cancer, arginine biosynthesis, nicotinate and nicotinamide

**Table 3**
Identification of renal differential metabolites.

| tR (min) | Metabolite names | Formula | Reference (*m*/*z*) | Adduct type | Trend (compared with normal) | GG *vs* Z | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | VIP | Log2FC | P |
| 11.421 | L-pipecolic acid | $C_6H_{11}NO_2$ | 130.0862 | $[M+H]^+$ | ↓ | 2.39 | −2.18 | 0.000 |
| 6.517 | Ethanolamine | $C_2H_7NO$ | 62.0600 | $[M+H]^+$ | ↑ | 2.28 | 0.91 | 0.000 |
| 7.096 | Trimethylamine *N*-oxide | $C_3H_9NO$ | 76.0757 | $[M+H]^+$ | ↑ | 2.15 | 0.99 | 0.000 |
| 11.061 | *S*-adenosyl-L-methionine | $C_{15}H_{22}N_6O_5S$ | 399.1445 | $[M+H]^+$ | ↓ | 1.98 | −1.82 | 0.000 |
| 7.777 | DL-3-aminoisobutyric acid | $C_4H_9NO_2$ | 102.0560 | $[M-H]^-$ | ↑ | 1.95 | 1.74 | 0.000 |
| 7.211 | D-(+)-malic acid | $C_4H_6O_5$ | 133.0142 | $[M-H]^-$ | ↑ | 1.92 | 1.80 | 0.000 |
| 8.677 | Salicylic acid | $C_7H_6O_3$ | 137.0244 | $[M-H]^-$ | ↓ | 1.88 | −6.87 | 0.000 |
| 4.881 | 5-fluorouracil | $C_4H_3FN_2O_2$ | 129.0105 | $[M-H]^-$ | ↓ | 1.81 | −3.00 | 0.000 |
| 1.034 | Methyl heptadecanoic acid | $C_{18}H_{36}O_2$ | 283.2642 | $[M-H]^-$ | ↓ | 1.79 | −0.75 | 0.000 |
| 10.993 | Argininosuccinic acid | $C_{10}H_{18}N_4O_6$ | 291.1299 | $[M+H]^+$ | ↓ | 1.74 | −0.81 | 0.000 |
| 5.958 | Pyroglutamic acid | $C_5H_7NO_3$ | 128.0353 | $[M-H]^-$ | ↑ | 1.74 | 1.55 | 0.000 |
| 9.718 | Targinine | $C_7H_{16}N_4O_2$ | 189.1346 | $[M+H]^+$ | ↓ | 1.69 | −0.73 | 0.000 |
| 4.305 | 5-Methylcytidine | $C_{10}H_{15}N_3O_5$ | 258.1084 | $[M+H]^+$ | ↓ | 1.69 | −0.88 | 0.000 |
| 1.377 | 4-Methyl-5-thiazoleethanol | $C_6H_9NOS$ | 144.0477 | $[M+H]^+$ | ↑ | 1.68 | 1.62 | 0.000 |
| 1.165 | (3*S*)-5-[(1*S*,8a*R*)-2,5,5,8a-tetramethyl-4-oxo-4a,6,7,8-tetrahydro-1*H*-naphthalen-1-yl]-3-methylpentanoic acid | $C_{20}H_{32}O_3$ | 321.2424 | $[M+H]^+$ | ↓ | 1.67 | −1.03 | 0.000 |
| 9.747 | L(+)-cystathionine | $C_7H_{14}N_2O_4S$ | 221.0601 | $[M-H]^-$ | ↓ | 1.67 | −3.55 | 0.000 |
| 4.931 | Kynurenic acid | $C_{10}H_7NO_3$ | 190.0498 | $[M+H]^+$ | ↑ | 1.65 | 2.05 | 0.000 |
| 6.174 | Cinnamic acid | $C_9H_8O_2$ | 147.0451 | $[M-H]^-$ | ↓ | 1.65 | −0.70 | 0.000 |
| 1.039 | Arachidonic acid | $C_{20}H_{32}O_2$ | 303.2329 | $[M-H]^-$ | ↓ | 1.65 | −1.00 | 0.000 |
| 5.770 | Glycohyodeoxycholic acid | $C_{26}H_{43}NO_5$ | 448.3070 | $[M-H]^-$ | ↑ | 1.65 | 2.03 | 0.000 |
| 3.041 | Pantothenate | $C_9H_{17}NO_5$ | 220.1179 | $[M+H]^+$ | ↑ | 1.63 | 1.90 | 0.000 |
| 4.722 | Gluconic acid | $C_6H_{12}O_7$ | 195.0510 | $[M-H]^-$ | ↓ | 1.62 | −4.62 | 0.000 |
| 8.095 | (*E*,6*S*)-7-hydroxy-2-methyl-6-[(10*S*,13*S*,14*S*,17*S*)-4,4,10,13,14-pentamethyl-3-oxo-1,2,5,6,7,11,12,15,16,17-decahydrocyclopenta[a] phenanthren-17-yl]hept-2-enoic acid | $C_{30}H_{46}O_4$ | 471.3468 | $[M+H]^+$ | ↑ | 1.57 | 5.04 | 0.010 |
| 10.592 | Neoandrographolide | $C_{26}H_{40}O_8$ | 503.2615 | $[M+Na]^+$ | ↓ | 1.57 | −2.35 | 0.010 |
| 6.009 | Tyr | $C_9H_{11}NO_3$ | 180.0666 | $[M-H]^-$ | ↓ | 1.55 | −2.20 | 0.010 |
| 1.628 | 2-piperidone | $C_5H_9NO$ | 100.0757 | $[M+H]^+$ | ↑ | 1.54 | 1.67 | 0.010 |
| 10.782 | Carnosine | $C_9H_{14}N_4O_3$ | 225.0993 | $[M-H]^-$ | ↓ | 1.53 | −1.62 | 0.010 |
| 8.392 | Citric acid | $C_6H_8O_7$ | 191.0197 | $[M-H]^-$ | ↓ | 1.53 | −2.68 | 0.010 |
| 9.896 | 2-Hydroxyisocaproic acid | $C_6H_{12}O_3$ | 131.0713 | $[M-H]^-$ | ↓ | 1.49 | −1.74 | 0.010 |
| 11.648 | 9-Nitro-20(*S*)-camptothecin | $C_{20}H_{15}N_3O_6$ | 394.1033 | $[M+H]^+$ | ↓ | 1.48 | −1.34 | 0.010 |
| 11.428 | (4*S*,5*Z*,6*S*)-4-(2-methoxy-2-oxoethyl)-5-[2-[(*E*)-3-phenylprop-2-enoyl] oxyethylidene]-6-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy-4*H*-pyran-3-carboxylic acid | $C_{15}H_{22}O_2$ | 233.1546 | $[M-H]^-$ | ↓ | 1.47 | −1.37 | 0.020 |
| 3.544 | Quinaldic acid | $C_{10}H_7NO_2$ | 174.0549 | $[M+H]^+$ | ↓ | 1.46 | −2.70 | 0.020 |
| 9.820 | Proline | $C_5H_9NO_2$ | 116.0706 | $[M+H]^+$ | ↓ | 1.44 | −1.57 | 0.020 |
| 4.345 | Phenylacetylglycine | $C_{10}H_{11}NO_3$ | 194.0811 | $[M+H]^+$ | ↑ | 1.43 | 1.87 | 0.020 |
| 8.391 | Ectoine | $C_6H_{10}N_2O_2$ | 143.0820 | $[M+H]^+$ | ↓ | 1.42 | −3.92 | 0.020 |
| 7.279 | Uridine 5′-diphospho-*N*-acetylglucosamine | $C_{17}H_{27}N_3O_{17}P_2$ | 606.0742 | $[M-H]^-$ | ↑ | 1.42 | 2.55 | 0.020 |
| 7.164 | L-pyroglutamic acid | $C_5H_7NO_3$ | 130.0498 | $[M+H]^+$ | ↓ | 1.41 | −0.86 | 0.020 |
| 2.122 | Biliverdin | $C_{33}H_{34}N_4O_6$ | 583.2551 | $[M+H]^+$ | ↑ | 1.39 | 2.50 | 0.020 |
| 6.886 | Rac-glycerol 3-phosphoate | $C_{11}H_{15}N_5O_4$ | 282.1196 | $[M+H]^+$ | ↑ | 1.39 | 2.10 | 0.020 |
| 1.173 | (*S*)-3-amino-4-phenylbutyric acid | $C_{10}H_{13}NO_2$ | 180.1019 | $[M+H]^+$ | ↓ | 1.38 | −0.87 | 0.030 |
| 0.456 | Stearic acid | $C_{18}H_{36}O_2$ | 283.2642 | $[M-H]^-$ | ↓ | 1.37 | −1.27 | 0.030 |
| 4.363 | Threonic acid | $C_4H_8O_5$ | 135.0298 | $[M-H]^-$ | ↓ | 1.37 | −3.06 | 0.030 |
| 5.614 | 4-Methyldaphnetin | $C_{10}H_8O_4$ | 191.0351 | $[M-H]^-$ | ↑ | 1.36 | 2.07 | 0.030 |
| 2.408 | 2-Hydroxyisobutyric acid | $C_4H_8O_3$ | 103.0400 | $[M-H]^-$ | ↓ | 1.36 | −2.51 | 0.030 |
| 2.566 | *N*-methyl-DL-alanine | $C_4H_9NO_2$ | 104.0706 | $[M+H]^+$ | ↑ | 1.35 | 2.07 | 0.030 |
| 1.368 | Ascorbic acid | $C_6H_8O_6$ | 175.0248 | $[M-H]^-$ | ↓ | 1.33 | −2.37 | 0.030 |
| 11.757 | Resibufogenin | $C_{24}H_{32}O_4$ | 429.2282 | $[M+FA-H]^-$ | ↓ | 1.33 | −1.28 | 0.030 |
| 6.700 | Palmitoylcarnitine | $C_{23}H_{45}NO_4$ | 400.3421 | $[M+H]^+$ | ↓ | 1.32 | −0.59 | 0.040 |
| 4.324 | *N*-acetyl-L-leucine | $C_8H_{15}NO_3$ | 172.0979 | $[M-H]^-$ | ↓ | 1.32 | −2.43 | 0.040 |
| 3.300 | Mefenamic acid | $C_{15}H_{15}NO_2$ | 242.1175 | $[M+H]^+$ | ↓ | 1.30 | −2.89 | 0.040 |
| 5.946 | *p*-Coumaraldehyde | $C_9H_8O_2$ | 147.0451 | $[M-H]^-$ | ↓ | 1.30 | −1.54 | 0.040 |
| 3.505 | Cysteine *S*-sulfate | $C_3H_7NO_5S_2$ | 199.9692 | $[M-H]^-$ | ↓ | 1.29 | −1.71 | 0.040 |
| 5.774 | Spermidine | $C_7H_{19}N_3$ | 146.1651 | $[M+H]^+$ | ↓ | 1.29 | −1.05 | 0.040 |

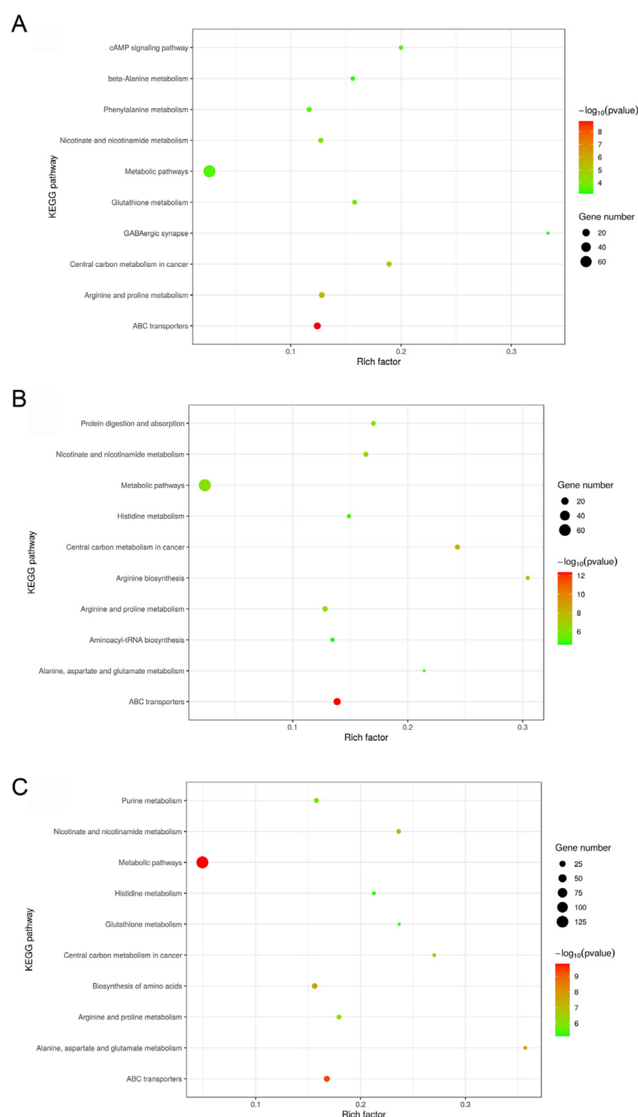


**Fig. 5.** Metabolic pathway (A: serum; B: hepatic; C: renal).

metabolism, protein digestion and absorption, metabolic pathways, alanine, aspartate and glutamate metabolism, histidine metabolism and aminoacyl-tRNA biosynthesis are significantly related, as shown in Fig. 5B.

Renal differential metabolites are related to metabolic pathways, ABC transporters, amino acid biosynthesis, arginine and proline metabolism, central carbon metabolism in cancer, nicotinate and nicotinamide metabolism, alanine, aspartate and glutamate metabolism, purine metabolism, histidine metabolism and glutathione metabolism pathways, as shown in Fig. 5C. It was found that serum, liver and kidney metabolites had the strongest correlation with ABC transporters, followed by arginine and proline metabolism, nicotinate and nicotinamide metabolism and central carbon metabolism in cancer.

## 4. Discussion

Drug-induced liver and kidney injury and acute and chronic liver and kidney failure are increasing year by year, which has become another thorn seriously restricting clinical safe drug use (Liu et al., 2010). Garidi-5 is the first prescription for eliminate viscosity and dry yellow water disease in Mongolian medicine clinical of past dynasties. But the composition of the prescription of five drugs have a certain toxicity, is listed as the category of drugs with caution. Liver and kidney are not only important places for drug metabolism and excretion, but also susceptible target organs for drug toxicity. The metabolic disorder of liver and kidney has become the main cause of liver and kidney toxicity. In this study, take garidi-5 as an example, detected its endogenous metabolites 28 d after intragastric administration, screened and identified the abnormal metabolic changes caused by garidi-5, and provided experimental basis for exploring its liver and kidney toxicity and mechanism.

### 4.1. Garidi-5 disordered ABC transporters metabolic pathway

This study found that the metabolism of ABC transporters in serum, liver and kidney of rats after administration of garidi-5 was disordered, such as abnormal changes in the contents of lycine-betaine, xanthine, glutathione, L-leucine, L-arginine, cytidine, spermidine and urea. ABC transporters need to consume ATP and participate in the transmembrane transport of amino acids, glucose, metabolites, drugs and charged ions (Wu et al., 2020), which is extremely important in the disposal of small molecules (endogenous metabolites, uremic toxins and drugs) in blood, kidney, liver, intestine and other organs. In chronic kidney disease (CKD), ABC transporters in renal and non-renal tissues are directly or indirectly affected by various small molecular metabolites, leading to abnormal inter organ communication (Torres et al., 2021).

Studies have shown that glycine-betaine is a quaternary amine alkaloid, mainly metabolized in mitochondria of liver and kidney cells. It can provide active methyl for the body and participate in protein and lipid metabolism. It can protect liver and kidney, inhibit inflammation, antitumor and play a positive role in the prevention and treatment of obesity, diabetes, cardiovascular disease and non-alcoholic fatty liver disease (NAFLD) (Liao et al., 2014; An et al., 2021). Xanthine is a purine base widespread in the body. It is also the product of purine metabolism, which is catalyzed by xanthine oxidase and further oxidized to uric acid. Liver and kidney are the main sites of purine metabolism. When they are damaged, xanthine oxidase decreases, interferes with the metabolism of xanthine into uric acid, causes too little formation of blood uric acid, and affects the excretion of xanthine (Bao et al., 2020). Glutathione (GSH) is mainly synthesized in the liver and transported to other tissues and organs through blood and bile. It is involved in glucose and lipid metabolism in the body and can activate various metabolic enzymes, and plays an important role in improving liver and kidney damage and sepsis (Piecha et al., 2007; Hu et al., 2019). L-Leucine is the main amino acid for hydrolysis, conversion and synthesis of protein, as well as one of the branch chain amino acids, which plays an important role in liver diseases and is used for liver diseases with reduced bile secretion, poisoning, lipid metabolism and glucose metabolism disorders, etc. (Jin et al., 2019). L-Arginine (L-Arg) is one of the essential amino acids. It has antioxidation and immune regulation function. It promotes the synthesis of nitric oxide (NO) in the body and participates in intracellular signal transduction. It plays an important role in maintaining liver and kidney function, and has a certain antagonistic effect on the myocardial, liver and kidney injury and alcoholic fatty liver changes in diabetic rats (Zhang et al., 2020; Saka et al., 2021). Cytidine is an important component of ribonucleic acid, which plays a physiological role in cells mainly in the form of cytidylic acid. Spermidine is a polyamine compound that is critical in cell proliferation, autophagy and development, and its elevated level is related to many tumors and cancers (Novita et al., 2021). The catabolism of protein can determine the urea production. Urea is mainly synthesized by the liver and excreted from the kidney (He, 2014). Decreased renal function can lead to accumulation of urea (Feng et al., 2019). It is also found that the level of urea

increases after administration of garidi-5. The results showed that garidi-5 could down-regulate the expression of glycine-betaine, xanthine, glutathione, L-leucine and L-arginine, and up-regulate the expression of spermidine, cytidine and urea.

It is speculated that garidi-5 may affect the expression of the above metabolites and disorder the metabolic pathway of ABC transporters in serum, liver and kidney of rats, leading to abnormal protein metabolism and pathological changes of liver and kidney to produce toxicity in rats.

### 4.2. Garidi-5 disorder arginine and proline metabolism pathway

This study found that the metabolism of arginine and proline in serum, liver and kidney of rats after administration of garidi-5 was disordered, such as 5-aminovaleric acid, creatine, urea, L-glutamate acid, *S*-adenosine-L-methionine, spermidine etc. metabolites have abnormal changes. Arginine is one of the essential amino acids, participating in the urea cycle, mainly synthesizing proteins, polyamines and creatine. It can also enter the tricarboxylic acid cycle as a substrate to provide energy (Guo et al., 2019). Arginine is mainly synthesized in renal tubular epithelial cells, and abnormal can induce renal inflammation, fibrosis, CKD and the growth and proliferation of some tumor cells (Levillain, 2012; Xiao et al., 2013). Proline is synthesized from arginine and glutamate, and is an important functional amino acid participate in cell proliferation and differentiation, protein synthesis, metabolism, antioxidant and immune response (Chen et al., 2017). Proline is mainly metabolized in the small intestine, liver and kidney, and is catalyzed by ornithine in liver to generate polyamines, including spermidine, putrescine and spermidine, which are necessary metabolites for protein synthesis and cell growth, proliferation and differentiation (Tan et al., 2015). Studies have shown that 5-aminovaleric acid is an intermediate product produced by L-lysine catabolism, which can inhibit the oxidation of fatty acids (Olli KArkkAinen et al., 2020). Creatine mainly exists in skeletal muscle, brain, myocardium, kidney, liver and other organs. Long-term intake of high concentration of creatine can cause muscle spasm, gastrointestinal diseases, and have adverse effects on liver, kidney and cardiovascular system (Liu, 2017).

The increase of *urea* level is closely related to chronic kidney disease. L-glutamate acid is mainly involved in the metabolism of amino acids in the body, and a variety of metabolites such as proline, *arginine* and $\gamma$-aminobutyric acid are synthesized which are closely related to resistance (Galili et al., 2001). *S*-Adenosyl-L-methionine (SAM) is a key metabolite that regulates hepatocyte growth, death, and differentiation. The biosynthesis of SAM is inhibited, which will aggravate liver injury. SAM depletion in the liver is closely related to hepatocyte necrosis and liver fibrosis (Tao et al., 2017). Spermidine has anti-inflammatory, antioxidant and anti-atherosclerotic effects, and participates in lipid metabolism (Gao, 2019). The results showed that the expressions of creatine, urea and L-glutamate *acid* were up-regulated and the expression of *S*-adenosine-L-methionine was down-regulated after administration of garidi-5. The expression of spermidine contained in serum and kidney and 5-aminovaleric acid metabolite contained in serum and liver were disordered. It is suggested that garidi-5 can disorder the metabolic pathways of arginine and proline in serum, liver and kidney of rats by dysregulating the above metabolites, resulting in the imbalance of amino acid metabolism in rats, thus causing liver and kidney toxicity.

### 4.3. Garidi-5 disorder nicotinate and nicotinamide metabolism

Nicotinic acid (NA) is a component of dehydrogenase coenzyme I and coenzyme II, which is converted into nicotinamide (NAM) *in vivo*. It plays an important role in maintaining lipid metabolism, glucose glycolysis, protein, carbohydrate and amino acid metabolism in the body (Chen and Jiang, 2015). This study found that the metabolism of nicotinate and nicotinamide metabolism in serum, liver and kidney of rats were disordered after administration of garidi-5, such as the increase of the content of 1-methylnicotinamide. 1-methylnicotinamide (MNA) is the main metabolite of nicotinamide, which has antithrombosis and anti-inflammatory effects. Nicotinamide (NA) is transformed into MNA and *N*-methyltransferase (NNMT), and its up-regulated expression is closely related to liver cirrhosis (Magdalena et al., 2010).

### 4.4. Same differential metabolites in serum, liver and kidney

This study found that trimethylamine *N*-oxide, L-pyroglutamic acid and *DL*-3-aminoisobutyric acid were common differential metabolites in serum, liver and kidney of GG group. Elevated plasma trimethylamine *N*-oxide (TMAO) level can induce inflammatory response, increase intracellular calcium release, activate autonomic nervous system, aggravate ventricular remodeling, affect cardiac structure and function, and promote the occurrence of cardiovascular diseases (Tan et al., 2019). The increase of TMAO content can also cause dysfunction of vascular endothelial cells in rats with chronic kidney disease (Organ et al., 2016). The contents of TMAO in serum, liver and kidney of GG group were significantly increased, indicating that TMAO is a biomarker of liver and kidney toxicity of GG.

Pyroglutamic acid exists in free form in blood, tissue fluid and various organs. Pyroglutamic acid is an intermediate product of $\gamma$-glutamyl cycle, which can interconvert with glutamate and antagonize glutamate receptors *in vivo*, and is associated with a series of genetic diseases. When lipid metabolism is abnormal, the level of pyroglutamate decreases (Kumar & Bachhawat, 2012). The content of pyroglutamic acid in GG group was significantly decreased in serum, but significantly increased in liver and kidney, suggesting that the occurrence of liver and kidney toxicity in GG may be closely related to the abnormal metabolism of glutamine.

## 5. Conclusion

In conclusion, garidi-5 can change the expression of metabolites such as trimethylamine *N*-oxide, L-pyroglutamic acid, glycine-betaine, xanthine, glutathione, L-leucine, L-arginine, cytidine, spermidine, urea, 5-aminovaleric acid, creatine, L-glutamic acid, *S*-adenosyl-L-methionine and 1-methylnicotinamide in rats, and disorder ABC transporters, arginine and proline metabolism, nicotinate and nicotinamide metabolic pathways in serum, liver and kidney of rats, so as to aggravate the abnormal metabolism of protein and amino acid in rats, leading to liver and kidney toxicity.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was supported by the Mongolian Medicine Safety Evaluation and Innovation Team Project (No. MY20190003) and "Innovation Team Development Plan" Project of Institutions of Higher Learning in Inner Mongolia (No. NMGIRT2216).

## References

An, D., Yang, J. H., & Song, X. M. (2021). Research progress in the mechanism of betaine in reducing obesity and related metabolic disorders. *Central South Pharmacy, 19*(7), 1393–1398.

Bao, C. Y., Xiang, L. J., Liu, H., Wang, S. Y., & Wang, T. L. (2020). Screening of diagnostic markers of gastric cancer in salivary purine metabolites. *Acta Universitatis Medicinalis Anhui, 55*(5), 811–814.
Chen, Y. J., Zhong, Y. X., Dong, W., Li, C. J., Wang, L. X., Pu, Y. Z., ... Liu, P. (2014). Developmental toxicity of muscone on zebrafish embryos. *Chinese Journal of Pharmacology and Toxicology, 28*(2), 267–273.
Chen, J., & Jiang, Y. G. (2015). Effects of niacin on lipid metabolism in rats model of non-alcoholic fatty liver disease. *Journal of Clinical Hepatology, 31*(2), 261–265.
Chen, Q. P., Xu, S. X., Liu, Q. Y., & Shi, D. S. (2017). Research progress on effects of Proline on reproductive performance of sows. *China Animal Husbandry & Veterinary Medicine, 44*(3), 767–772.
Feng, Q., Shang, X. L., Li, Z., & Hu, F. (2019). Feature analysis of urinary metabolism in advanced chronic kidney disease. *Journal of Modern Laboratory Medicine, 34*(1), 93–96.
Galili, G., Tang, G. L., Zhu, X. H., & Gakiere, B. (2001). Lysine catabolism: A stress and development super-regulated metabolic pathway. *Current Opinion in Plant Biology, 4*(3), 261–266.
Gao, M. Y. (2019). *Effects of spermidine on lipid metabolism in liver*. Beijing: Peking Union Medical College.
Guo, C. Z., Xue, Y. F., Seddik, H. E., Yin, Y. Y., Hu, F., & Mao, S. Y. (2019). Dynamic changes of plasma metabolome in response to severe feed restriction in pregnant ewes. *Metabolites, 9*(6), 112.
He, B. X., Yang, Y. T., He, Y. L., Peng, C., & Cao, Z. X. (2016). Preliminary study on the volatile oil of common *aucklandia root* in embryotoxicity of zebrafish. *China Journal of Traditional Chinese Medicine and Pharmacy, 31*(11), 4714–4716.
He, X. H. (2014). *Establishment of renal injury induced by hydroxyproline and renal calculi model in rats*. Guangxi: Medical College of Guilin.
Hu, G. X., Peng, J., Cui, W. J., Yang, G. H., Zhang, Z. K., Zhao, L. Z., ... Qiao, L. J. (2019). Effects of glutathione on serum tPA and PAI-1 factors in patients with septic acute kidney injury patients with metastasizing septicemia. *Chinese Journal of Nosocomiology, 29*(24), 3777-3780+3785.
Inner Mongolia Health Department (1984). *Inner Mongolia Mongolian Patent Medicine Standard* (p. 348). Huhhot: Inner Mongolia People's Publishing House.
Jin, S. S., Yao, K., Yin, Y. L., Kang, B. J., Li, Y. Y., & Wang, P. (2019). Effects of leucine and α-ketoglutarate acid on the weight, serum, viscera organ of mice. *Food & Machinery, 35*(8), 172–176.
Kumar, A., & Bachhawat, A. K. (2012). Pyroglutamic acid: Hrowing light on a lightly studied metabolite. *Special Section: Chemistry and Biology, 102*(2), 288–297.
Levillain, O. (2012). Expression and function of arginine-producing and consuming-enzymes in the kidney. *Amino Acids, 42*(4), 1237–1252.
Liao, H. X., Liu, H. X., Liang, J. Q., Landan, W. QT., & Pu, Q. Y. (2014). Effects of betaine on chronic alcoholic fatty liver in SD rats. *Journal of Chinese Medicinal Materials, 37*(9), 1647–1650.
Liu, J. (2017). Side effects and safety of creatine as a sports nutrition supplement. *Science and Technology Economic Guide, 21*, 187.
Liu, R. Y., Ye, H., Xie, T., Feng, L. L., & Li, F. R. (2010). Metabolomics and its application in the evaluation of drug toxicity. *Chemistry of Life, 30*(5), 774–778.
Magdalena, S., Tamara, I. K., Andrzej, J., Małgorzata, S., Wojciech, S., Magdalena, B., ... Stefan, C. (2010). Nicotinamide *N*-methyltransferase (NNMT) and 1-methylnicotinamide (MNA) in experimental hepatitis induced by concanavalin A in the mouse. *Pharmacological Reports, 62*(3), 483–493.
Novita, S. I., Setiawan, T., Seock, K. K., Toni, Y. W., Kae, W. C., & Young, H. K. (2021). Metabolism and function of polyamines in cancer progression. *Cancer Letters, 519*(10), 91–104.
Organ, C. L., Otsuka, H., Bhushan, S., Wang, Z. N., Bradley, J., Trivedi, R., ... Lefer, D. J. (2016). Choline diet and its gut microbe-derived metabolite, trimethylamine *N*-oxide, exacerbate pressure overload-induced heart failure. *Circulation: Heart Failure, 9*(1), e002314.
Piecha, G., Koleganova, N., & Geldyyev, A. (2007). Glutathione peroxidase mimic ebselen prevents atherogenesis and cardiac remodeling in renal impairment in the ApoE knockout mouse. *Pathology, Research and Practice, 203*(5), 326.
Saka, W. A., Akhigbe, R. E., Abidoye, A. O., Dare, O. S., & Adekunle, A. O. (2021). Suppression of uric acid generation and blockade of glutathione dysregulation by *L*-arginine ameliorates dichlorvos-induced oxidative hepatorenal damage in rats. *Biomedicine & Pharmacotherapy, 138*. https://doi.org/10.1016/J.BIOPHA.2021.111443.
Tan, M. J., Kong, X. F., Liu, Q. Y., & Shi, D. S. (2015). Polyamines and conceptus development in mammals. *Chinese Journal of Animal Nutrition, 27*(1), 43–48.
Tao, G., Lei, C., Yu, S. X., & Liu, Q. Y. (2017). *S*-adenosyl-*L*-methionine for the treatment of chronic liver disease: A systematic review and meta-analysis. *Published Online, 10*(3), 0122124.
Torres, A. M., Dnyanmote, A. V., Granados, J. C., & Nigam, S. K. (2021). Renal and non-renal response of ABC and SLC transporters in chronic kidney disease. *Expert Opinion on Drug Metabolism & Toxicology, 17*(5), 21–28.
Tan, Y., Sheng, Z. X., Zhou, P., Liu, C., Zhao, H. J., Song, L., ... Yan, H. B. (2019). Plasma trimethylamine *N*-oxide as a novel biomarker for plaque rupture in patients with ST-segment-elevation myocardial infarction. *Circulation: Cardiovascular Interventions, 12*(1), e007281.
Wurihan, M. XH., Baolor, H. XJ., Tergel, M., & Bai, M. R. (2021). Research review on toxicity of Mongolian medicine *Aconiti Kusnezoffii Radix*. *China Journal of Traditional Chinese Medicine and Pharmacy, 36*(7), 4159–4162.
Wang, L. N., Zheng, H. Z., Wang, Z. B., Jin, J. J., Gao, Y., Cheng, Y., & Hu, Y. C. (2016). Study on the mechanism of hepatotoxicity in mice induced by *Terminalia chebula* extract with proteomics. *China Journal of Traditional Chinese Medicine and Pharmacy, 31*(4), 1475–1478.
Wu, Y. F., Zhang, J. G., & Li, Q. (2020). Progress in the pharmacokinetic mechanism of drug resistance in hepatocellular carcinoma. *Chinese Pharmaceutical Journal, 55*(19), 1578–1584.
Xiao, F., Shao, J. G., Qian, Z., Sun, Y., & Gu, C. Y. (2013). Expression of arginase 2 in hepatocellular carcinoma tissues and its clinicopathological significance. *Academic Journal of Second Military Medical University, 34*(2), 155–159.
Yang, L. (2018). Study on the cardiotoxicity and mechanism of *Acorus tatarinowii schott* from the pathway of apoptosis. *Guangzhou University of Chinese Medicine, 2018*, 000161.
Zhang, H., Zhang, Z. H., Zhang, C. S., & Wang, Z. Z. (2020). Effects of *L*-arginine and α 1-adrenergic receptor on the regulation of renal functions. *Acta Academiae Medicinae Sinicae, 42*(6), 815–819.