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Research Article

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The trajectory analysis for the outline of *Fructus Cnidii* metabolite profiles after the regulation to its toxicity by *Glycyrrhiza uralensis*

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ABSTRACT

Glycyrrhiza uralensis is a common plant in many Asian and European countries, the most widely-used function in clinical is regulating property of other drugs, but the mechanism is not explicit. Fructus Cnidii (FC) is a traditional Chinese medicine, but it has mild toxicity. In order to reveal the outline of metabolite profiles for Glycyrrhiza uralensis to regulate FC toxicity, we carried out metabonomics study in rats model based on Ultra Performance Liquid Chromatography-Quadrupole-Time of Flight Mass Spectrometry (UPLC-Q-TOF/MS). All rats were randomly divided into 4 groups: W, G, S, SG. The rats of every group were orally administered on days 1 to 13. All rats urine samples were collected over night by metabolism cages. The exported LC/MS data were processed by mixOmics package of R. Pattern recognition including PCA, PLS and PLSDA function were used to analysis the trajectory for the outline of metabolite profiles. Ability of different functions was evaluated. The results showed PLSDA has more powerful discriminative ability of pattern recognition. And with the accumulation of time, the effects of licorice regulate the toxicity of FC become more and more obvious.

Keywords: *Fructus Cnidii*, *Glycyrrhiza uralensis*, regulation, toxicity, R language

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INTRODUCTION

Glycyrrhiza uralensis (licorice) is a traditional Chinese medicine that has been used clinical for centuries in Asian and European countries. Its effects include regulating property of other drugs, improving the function of spleen, circulating blood, moisturizing lung, ceasing coughing and so on. One of the most important functions of *Glycyrrhiza uralensis* is regulating property of other drugs, which is the most widely-used function in clinical. *Fructus Cnidii* (FC) is also a traditional Chinese medicine, it has the function of warming kidney to invigorate yang, eliminating dampness and insect disinfestations. But it has mild toxicity that was recorded in the classical pharmacopeia.

Metabonomics is a valuable technology for measuring relative concentrations of endogenous small molecules in biofluids and characterizing the changes of the metabolites occurring in organisms [1]. The data were exported from UPLC-Q-TOF/MS system and processed with R language. R is a language and environment for statistical computing and graphics. R provides an Open Source route to participation in that activity [2]. In order to differentiate the two groups of rats model more effectively in FC toxicity metabonomics study, we processed exported mass data with these packages to evaluate ability of pattern recognition.

EXPERIMENTAL SECTION

2.1. Chemicals and drugs Acetonitriles (chromatographic pure, TEDIA, USA), formic acid (chromatographic pure, DIMA, USA), ultrapure water (Sartorius, GER). Extract of FC and licorice were provided by Pharmaceutical teaching and research section, School of Pharmacy, Shenyang Pharmaceutical University. Alanine Aminotransferase

Kit (ALT), Aspartate Aminotransferase Kit (AST), Total Bilirubin Kit (TBI), Alkaline phosphatase Kit (ALP), Urea Kit (Urea) and Creatinine Kit (Cre) were purchased from BIOSINO BIOTECHNOLGY AND SCIENCE INC.

2.2. Experimental method SPF male Sprague-Dawley rats (weighing 200±20 g) were commercially obtained from the medical laboratory animal center of Guangdong province. All rats were randomly divided into 4 groups (each 8 rats): Control (W), licorice (G, 0.15 g/kg/day), FC (S, 0.45 g/kg/day), licorice + FC (SG, 0.15+0.45 g/kg/day). All rats urine samples were collected over night by metabolism cages.

The rats of each group were orally administered on days 1 to 13, the control group received water. Fasted for 12 h with free access to water after the last administration. Approximately 1mL of blood samples were collected in clean tubes and standing half an hour, then each blood sample was centrifuged for 10 min under 3000 rpm, separated 400μl of serum to test ALT, AST, TBI, ALP, BUN and CRE by Automatic biochemistry analyzer.

After centrifugation at 3000rmp for 10 min to collect the supernatants, samples were stored at -20℃ until UPLC-Q-TOF/MS analysis.The analytical column was a Waters C_{18} column (1.7μm, 50×2.1 mm) with ACQUITY UPLC BEH C_{18} 1.7μm VanGuard Pre-Column 3/PK 2.1×5 mm Column (Waters Made in Ireland). LC/MS data were recorded with a micromass oa-Q-Tof (Water, USA) equipped with ESI ionization source. Both positive and negative ion mode was applied for all urine samples [3].

2.3. Data preprocessing The mass spectrometer and UPLC system were operated under Micromass MassLynx 4.1 software. The resulting three-dimensional matrix involving peak index (RT-m/z pair), sample names (observations), and normalized peak area percent were introduced into R software through RandFriends[4] to be processed. Package of mixOmics[5] was loaded to be used.

RESULTS AND DISCUSSION

3.1. PCA In package mixOmics, there are several PCA methods. PCA is a technique which, quite literally, takes a different viewpoint of multivariate data. The PCA result is shown as Fig. 1. Compared with in negative mass mode, in positive mass mode the three groups (G, S and SG) are discriminative more significantly. It shown the results from 10th day began to change.

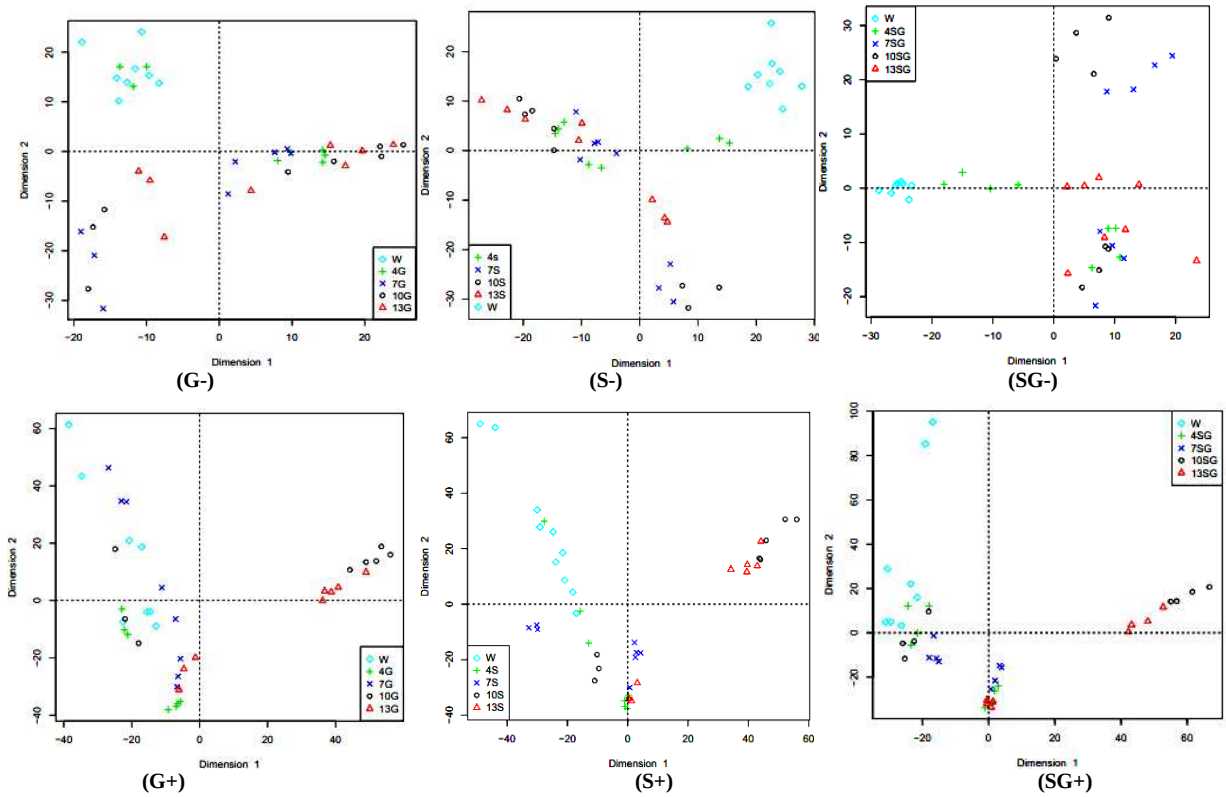

Fig. 1: PCA scores of rats model groups - (G-), (S-), (SG-) negative mass mode; (G+), (S+), (SG+) positive mass mode

3.2. PLSDA Function to perform standard Partial Least Squares regression to classify samples. The PLSDA and

valid results are shown as Fig. 3. From below figure, pattern recognition ability of PLSDA much better than PCA, the development tendency are very clear.

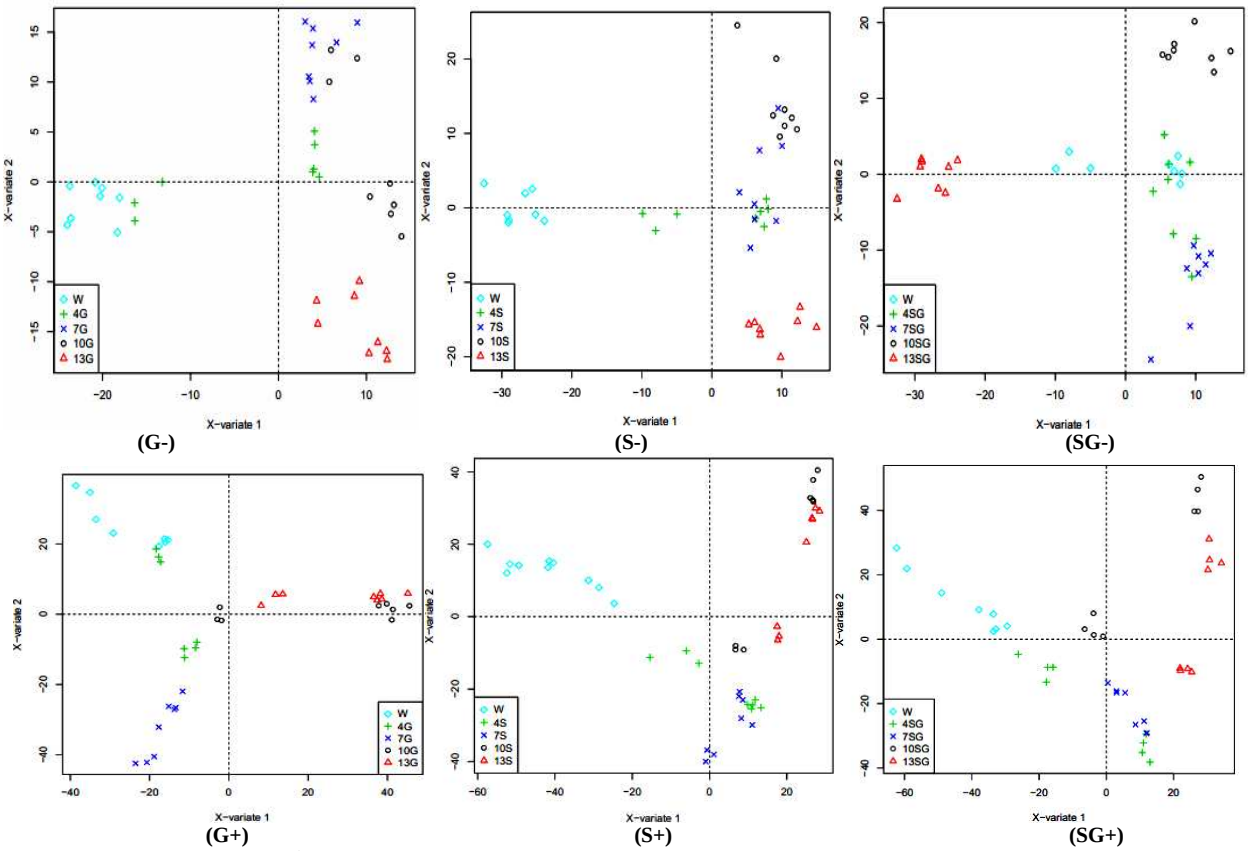

Fig. 2: PLSDA scores of rats model groups - (G-), (S-), (SG-) negative mass mode; (G+), (S+), (SG+) positive mass mode

3.3. PLS Function to perform Partial Least Squares regression. PLS employs takes the dependent variable into account when defining scores and loadings, whereas PCR concentrates on capturing variance in X only. The PLS results are shown as Fig. 3.

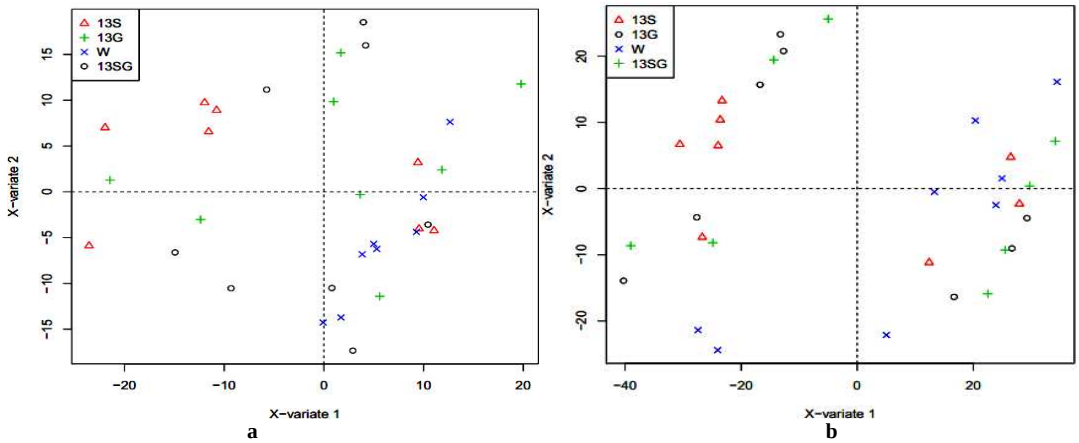

Fig. 3: PLS scores of rats model groups - (a) negative mass mode; (b) positive mass mode

From above figure, both in NM and PM, we found that 13S mostly gathered at the left side of the longitudinal axis, and other three groups at the right side. It shown that rats after feeding FC for 13 days, ALT, AST, TBI, ALP, BUN and CRE of serum biochemical indices have great change that can distinguish with other groups.

CONCLUSION

In this work, we carried out study of effect of licorice on SD rats by metabonomics method and used mixOmics package of R to analysis the trajectory of metabolite profiles of outline for *Glycyrrhiza uralensis* to regulation

Fructus Cnidii toxicity. We evaluated ability of pattern recognition of different function in the package, and PLSDA has powerful discrimination ability. More importantly, with the increase of time, the outline of FC metabolite profiles after the regulation to its toxicity by licorice presents a certain trajectory. Especially from 10th days, the effect of licorice on SD rats is obviously. And the mixOmics package is a very useful tool for pattern recognition in data processing. This work laid the foundation for selection of biomarkers of regulating drugs' property of licorice.

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