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Original Article

Metabolomics analysis in rats after administration of *Datura stramonium*

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Abstract: This study aimed to evaluate the effect of *Datura stramonium* on rats by examining the differences in urine and serum metabolites between *Datura stramonium* groups and control group. SIMCA-P+12.0.1.0 software was used for partial least-squares discriminant analysis (PLS-DA) to screen for the differential metabolites. Fifteen metabolites in urine including malonic acid, pentanedioic acid, D-xylose, D-ribose, xylulose, azelaic acid, threitol, glycine, butanoic acid, D-mannose, D-gluconic acid, galactonic acid, myo-inositol, octadecanoic acid, pseudouridine and ten metabolites in serum including alanine, butanedioic acid, L-methionine, propanedioic acid, hexadecanoic acid, D-fructose, tetradecanoic acid, D-glucose, D-galactose, oleic acid were selected as the characteristic metabolites. The PLS-DA scores plot indicated that serum and urine metabolites have a variety of changes among low dose group, high dose group and control group. These metabolites were related with amino metabolism, lipid metabolism and energy metabolism. The result reflected the relationship between metabolites in rat fluid and *Datura stramonium* spectra. Potential differences in metabolites and metabolic pathway analysis showed that the establishment of urine and serum metabolomics methods for further evaluating drug has great significance.

Keywords: Metabolomics, GC-MS, *Datura stramonium*, PLS-DA

Introduction

Datura stramonium belongs to Solanaceae family that causes hallucinogenic. The plant is probably originated in the Caspian Sea territories and spread to Europe in the first century. At present it grows in Europe, Asia, America and South Africa [1]. As a potential medicinal tree, the pharmacological properties of *Datura stramonium* is well known for its anti-inflammatory, anticholinergic, antihistaminic, acaricidal, antimicrobial, and anticancer activities [2]. Mueser KT reported that *Datura stramonium* was used to treat depression, madness and epilepsy [3]. However, the various parts of *Datura stramonium* including leaves and seeds cause serious poisoning. The main toxic components of *Datura stramonium* are scopolamine, atropine and other alkaloids which are classified as deliriants, or anticholinergics [4]. *Datura stramonium* overdose produces a classic anticholinergic syndrome that can lead to severe and sometimes fatal complications [5]. *Datura stramonium* intoxication typically produces delirium, hyperthermia, tachycardia, bizarre behavior, and severe mydriasis with resultant painful photophobia that can last several days [6, 7]. So efficacy and toxicity are dual characteristics of *Datura stramonium*. There were a lot of cases to poisoning from *Datura stramonium* [8-12]. In order to provide scientific guidance for this plant, exploring the behavior of toxic ingredients in *vivo* metabolism becomes important.

Metabolomics is a holistic way how various factors and/or stimuli cause perturbations in the overall composition of metabolites in an organism or biofluid [13]. Toxicology biological signals in the side effects of plant drug are manifested by changed metabolites in body. The character-

Table 1. Summary of the changes in relative levels of metabolites in rat urine indicated by the PLS-DA loading plots and statistical analysis

ID	Retention time	Metabolites compound	Group		Metabolic pathways
			Low	High	
1	8.03	Malonic acid	↓	↓*	Amino metabolism
2	14.598	Pentanedioic acid	-	↑*	Amino metabolism
3	15.684	D-xylose/d-fructose	↓*	-	Energy metabolism
4	15.878	D-ribose	↓*	↓**	Energy metabolism
5	15.92	Xylulose	↓*	-	Energy metabolism
6	16.988	Azelaic acid	↓*	-	Amino metabolism
7	17.366	Threitol	-	↓*	-
8	17.497	Glycine	↑**	-	Amino metabolism
9	17.865	Butanoic acid	↑*	-	Amino metabolism
10	18.727	D-mannose	↑*	↑*	Energy metabolism
11	19.012	D-gluconic acid	↑**	↑*	Energy metabolism
12	19.313	Galactonic acid	↑*	↑*	Energy metabolism
13	20.157	Myo-inositol	↓*	-	-
14	21.221	Octadecanoic acid	↓*	-	Lipid metabolism
15	22.293	Pseudo eridine	↑*	-	-

Note: Marks indicate the direction of the change, i.e. ↓ for decrease, ↑ for increase, - for no change. Compared control group with *Datura stramonium* group (Low dosage and High dosage), *P<0.05 and **P<0.01, as indicated by the statistical analysis T-test.

istics of metabolomics are integrity, dynamic and non-targeted which are suitable for studying its dynamic process of compound toxicity. In recent years, the metabolomics approach, which is based on the systematic strategy, provides a new platform for illuminating the toxicity mechanisms of exogenous compounds or drugs [13-18]. Chan W *et al* described a metabonomic study characterizing the nephrotoxicity induced by aristolochic acid using LC-MS and pattern recognition methods [19]. Lu F founded the metabolic changes in response of Xanthii Fructus were observed in urinary samples, which were revealed by orthogonal projection to latent structures discriminate analysis (OPLS-DA), and 10 metabolites could be served as the potential toxicity biomarkers [20]. This research based on systems biology approach of GC-MS metabonomics to study the changes of metabolites in rats after administration of *Datura stramonium*.

Material and methods

Chemicals and reagents

All chemicals and reagents were of the highest available purity. Methylhydroxylamine hydrochloride, n-heptane and pyridine were purchased from Aladdin Industrial, Inc. (China). N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Sigma-Aldrich in Germany. Acetonitrile was purchased from Tedia Reagent Company in the USA.

Instrumentation and conditions

Analyses were performed using an Agilent 6890N gas chromatograph (GC) (Agilent Technologies Inc.) coupled with a 5975B series mass selective detector (MSD) and a 7683B series autosampler. The analytical conditions were: temperature of injector 230°C; temperature of ion source 150°C; temperature of transfer line 230°C; The column was HP-5MS (30 m × 0.25 μm × 0.25 mm) and flow rate was 1 mL/min with high purity helium (99.999%) as carrier gas. The column temperature was held at 85°C for 5 min, and then increased by 10°C/min to 300°C, and hold there for 5 min. To minimize cross-contamination between runs, the needle on the injector was washed for six times with heptane before each injection. Ions were generated at an electron impact (EI) energy of 70 eV, and 20 scans/s were recorded over the mass range of 30-600 m/z. The solvent delay was always 5 min. 1 μL sample was injected by splitless mode.

Animal treatment and sample collection

Sprague-Dawley rats (male, 220±20 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All 21 rats were housed at the Animal Research Center of Wenzhou Medical University Laboratory. All experimental procedures were approved ethically by the Wenzhou Medical University Administration Committee of Experimental Animals. Rats were randomly divided into three groups: control group, low dose group, high dose group.

Table 2. Summary of the changes in relative levels of metabolites in rat serum indicated by the PLS-DA loading plots and statistical analysis

ID	Retention time	Metabolites compound	Group		Metabolic pathways
			Low	High	
1	6.985	Alanine	↓*	↓**	Amino metabolism
2	10.888	Butanedioic acid	↑*	↑*	Amino metabolism
3	13.822	L-methionine	↓*	-	Amino metabolism
4	14.986	Propanedioic acid	↑	↑**	Amino metabolism
5	15.924	D-fructose	↑	↑**	Energy metabolism
6	16.333	Tetradecanoic acid	↑*	↑*	Lipid metabolism
7	18.105	D-glucose	↓	↑**	Energy metabolism
8	18.162	D-galactose	↓	↑**	Energy metabolism
9	19.453	Hexadecanoic acid	↓	↓*	Lipid metabolism
10	20.959	Oleic acid	↓	↓*	Lipid metabolism

Note: Marks indicate the direction of the change, i.e. ↓ for decrease, ↑ for increase, - for no change. Compared control group with *Datura stramonium* group (Low dosage and High dosage), *P<0.05 and **P<0.01, as indicated by the statistical analysis T-test.

The dry crude *Datura stramonium* was ground into powder and then boiled in distilled water (100 g/L). The rest filtrate was replenished with distilled water to make the final concentration 0.1 g/mL. Low dose (0.3 g/kg) and high dose (0.6 g/kg) of *Datura stramonium* decoction were intragastric administered by a single dose.

Blood samples (0.25 mL) were collected from the tail vein of rats into 1.5 mL eppendorf tubes. The serum were separated after centrifugation at 3000 g for 15 min at 4°C and then stored at -80°C. Urine samples were collected over ice into 0.1 mL of 1% sodium azide from the rats for 12 hours (from 8 pm until 8 am the following day) and then centrifuged for 10 minutes at 4°C. The supernatant was stored at -80°C until use.

Sample collection

The procedure of sample pretreatment was carried out according to our previous method [21, 22]. 250 μL of acetonitrile was added to 100 μL of serum or urine. The mixture was then vortex for 3 min to extract metabolites and precipitate proteins. The precipitated proteins were separated out by centrifugation at 10,000 g for 10 min. To concentrate and derivatize the metabolites in the solution, 250 μL of the supernatant was extracted from each sample and lyophilized for about 24 h in freeze dryer. Derivatization of dried samples was performed in two steps: methylation was carried out at 70°C for 24 hours after adding 50 μL of methylhydroxylamine hydrochloride (15 mg/mL in pyridine), followed by the addition of 50 μL of MSTFA (with 1% TMCS as the catalyst) was added and then vortexed after adding 150 μL n-heptane to end derivatization. This derivatization system helps in reducing silylation artifacts, and is among the most commonly used for the derivatization of samples for GC-MS metabolomics studies.

Data processing and pattern recognition

The Agilent chromatography work station was used to integrate the peak areas corresponding to various metabolites in the total ion chromatography using GC-MS, and the data was exported into Microsoft Excel, with the peaks normalized to the total sum of spectrum prior to multivariate analyses. The concentrations of the metabolites were expressed as relative peak areas. After removal of overloaded metabolite peaks in GC-MS analysis, the metabolite data were imported into SIMCA-P 12 software (Umetrics, Umea, Sweden) for partial least squares discriminant analysis (PLS-DA) processing. The endogenous metabolites in the serum and urine were identified using the NIST 2008 mass spectrometry database.

Statistical analysis

Experimental values were carried out as mean ± S.D. Statistical analysis was performed using SPSS software (Version13.0, SPSS). Independent samples T-test was used to evaluate the significance of differences between the groups where necessary. The difference with a *P* value of <0.05 was considered statistically significant.

Results and discussion

GC-MS metabolite spectrum analysis

More than 100 metabolites were detected in the urine, 15 representative metabolites of which are listed in **Table 1**. At the same time, more than 80 metabolites were detected in the

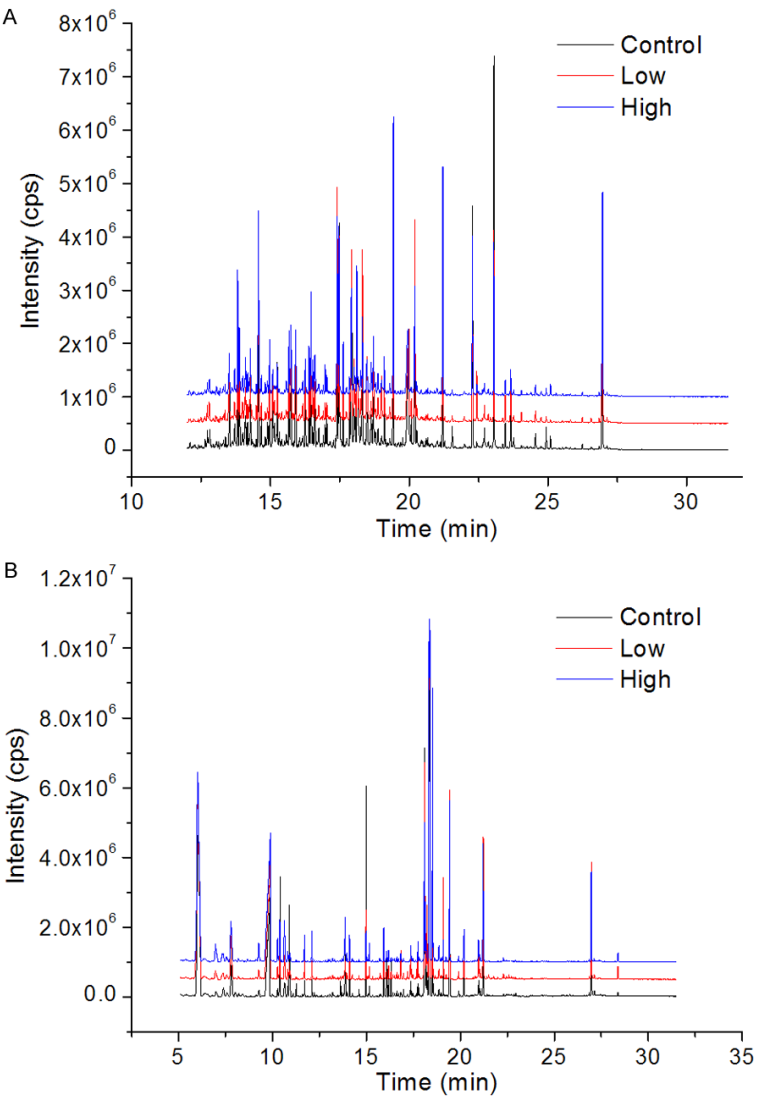

Figure 1. Typical GC-MS total ion chromatogram of rat urine (A) and serum (B) after intragastric administration of *Datura stramonium*.

serum, 10 representative metabolites of which are listed in **Table 2** (with a degree of matching above 80%). Typical GC-MS total ion chromatogram of rat urine and serum after intragastric administration of *Datura stramonium* were shown in **Figure 1**.

PLS-DA was used to analyze *Datura stramonium* group and control group. The scores and loading plots were shown in **Figure 2**. Data were visualized with the scores plot of the two principal components (t1 and t2), in which each point represented an individual peak of a sample. The metabolites associated with the groups separation were indicated by the corresponding loading plots, in which each point stood for a metabolite, The farther away from the center of the metabolite, the greater the contribution to separate [23].

The PLS-DA scores plot (**Figure 2A**) in urine demonstrates that the spectral characteristics of the three groups were different. The loading plot shows that malonic acid, pentanedioic acid, D-xylose, D-ribose, xylulose, azelaic acid, threitol, glycine, butanoic acid, D-mannose, D-gluconic acid, galactonic acid, myo-inositol, octadecanoic acid and pseudouridine are among the major contributors to the separation (**Figure 2B**). The PLS-DA scores plot (**Figure 2C**) in serum demonstrated that the spectral characteristics of the three groups are different. The loading plot for the first two principal components shows that alanine, butanedioic acid, L-methionine, propanedioic acid, hexadecanoic acid, D-fructose, tetradecanoic acid, D-glucose, D-galactose and oleic acid are among the major contributors to the separation (**Figure 2D**).

Changes in metabolite

The changes of metabolites among low dose group, high dose group and control group were shown in **Tables 1** and **2**. Compared to the control group, the level of malonic acid and D-ribose in rat urine of the low dose group and high dose group decreased, while the level of D-mannose, D-gluconic acid, galactonic acid increased. These metabolites may be as the potential biomarkers in rats after administration of *Datura stramonium*. Malonic acid is metabolite of fatty acids; propionic acid coenzyme A constitutes the active form of fatty acid synthesis. In addition, malonic acid could competitive inhibition respiratory electron transport chain with succinate dehydrogenase, it also plays an important role in aerobic respiration and energy produc-

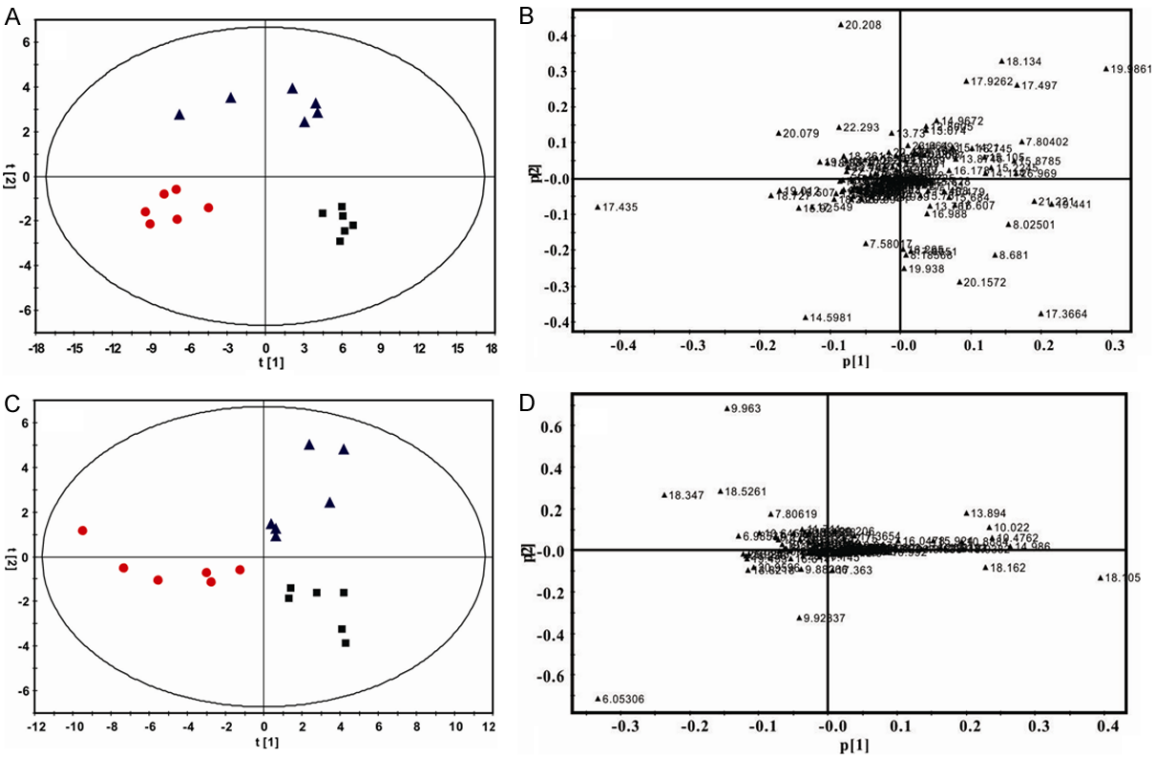

Figure 2. PLS-DA score plot of rat urine (A) and serum (C), (B, D) were the corresponding load plot of (A, C). (■ Control group, ▲ Low dose group, ● High dose group).

tion process [24]. The drop of its content demonstrates the effect on aerobic respiration in rats after administration of *Datura stramonium*, thus affecting the energy transfer.

Some metabolites in urine such as D-xylose, xylulose, Azelaic acid, myo-inositol and octadecanoic acid decreased in low dose group, while there are no change in high dose group. Other metabolites in urine such as glycine, butanoic acid and pseudouridine increased in low dose group, while there is no change in high dose group in **Table 1**. This change may be related to the dual characteristics of *Datura stramonium*.

In **Table 2**, however, compared to the control group, the level of alanine, oleic acid and hexadecanoic acid in rat serum of the low dose group and high dose group decreased, while the level of butanedioic acid, D-fructose and tetradecanoic acid increased. The level of D-glucose and D-galactose in low dose group decreased while increased in high group in **Table 2**. Both D-glucose and D-galactose are intermediate of energy metabolism; the unusual demonstrates the disorder of metabolic pathways. Alanine plays a key role in glucose-alanine cycle between tissues and liver [25]. Butanedioic acid is the intermediates of citric acid cycle (TCA), but it is also one of the fermentation products of anaerobic metabolism, Stetsura reported have the effect on neurological and emotional disturbances in patients with dorsopathy, which consistence with *Datura stramonium* was used to treat depression, madness and epilepsy [26]. Oleic acid, hexadecanoic acid and tetradecanoic acid are fatty acid, the changes of the content demonstrates disorder of lipid metabolism.

Conclusion

The current study was the first report of the perturbations in several metabolic pathways associated with rat urine and serum extracts after administration of *Datura stramonium* by GC-MS. The results showed that urine and serum metabolites groups were quite different. The PLS-DA scores plot can clearly significant serum and urine metabolites have a variety of changes among low dose group, high dose

group and control group. These metabolites were related with amino metabolism, lipid metabolism and energy metabolism. In addition, urine and serum metabolomics joint pattern characteristics could provide more comprehensive and reliable information to understand body's metabolic response after administration of *Datura stramonium*. In conclusion, GC-MS metabonomics based on systems biology approach could be applied to evaluate the efficacy of *Datura stramonium*.

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Disclosure of conflict of interest

None.

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