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Antidepressant-like effects of the fractions of Xiaoyaosan on rat model of chronic unpredictable mild stress

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ABSTRACT

Ethnopharmacological relevance: Xiaoyaosan (XYS), composed of *Radix Bupleuri*, *Radix Angelicae Sinensis*, *Radix Paeoniae Alba*, *Rhizoma Atractylodis Macrocephalae*, *Poria*, *Herba Menthae*, *Rhizoma Zingiberis Recens* and *Radix Glycyrrhizae*, is a valuable traditional Chinese medicine (TCM) which is used for the treatment of depression in China. In the formula, *Radix Bupleuri* usually serves as the principal drug, *Radix Angelicae Sinensis* and *Radix Paeoniae Alba* serve as the ministerial drugs, *Rhizoma Atractylodis Macrocephalae*, *Poria*, *Herba Menthae* and *Rhizoma Zingiberis Recens* serve as adjunctive drugs, *Radix Glycyrrhizae* serves as messenger drug, they coordinate with each other and enhance the effect of the formula. In our previous experiments, the antidepressant effect of XYS was revealed. However, the antidepressant part (or component) of this prescription was still obscure. We divided the XYS into five different polar fractions, and explored the antidepressant activity of five different polar fractions to identify the active fraction.

Materials and methods: Behavior research and metabonomics method based on ^{1}H NMR were used for efficacy study of different fractions in chronic unpredictable mild stress (CUMS) model of depression. Rats were divided into 8 groups and drugs were administered during the 21 days model building period. The urine samples of rats were collected overnight (12 h) on 21 day and the metabolic profiling of the urine was measured using NMR. Multivariate analysis was also utilized to evaluate the active fraction of XYS.

Results: In the behavior research, there were significant difference between the lipophilic fraction group (XY-A) and the model group. In addition, with pattern recognition analysis of urinary metabolites, the results showed a clear separation of the model group and control group, while XY-A group was much closer to the control group in the OSC-PLS score plot. Seven endogenous metabolites contributing to the separation of the model group and control group were detected, while XY-A group regulated the 5 perturbed metabolites showing a tendency of recovering to control group.

Conclusions: The present work suggested that petroleum ether fraction was the most effective fraction, implying that lipophilic components contribute to the antidepressant effect of XYS.

1. Introduction

Depression is the complex psychiatric disorder characterized by anhedonia and feelings of sadness. Furthermore, the WHO revealed that depression is the fourth leading cause of disability worldwide, exceeded by lower respiratory infections, perinatal conditions and HIV/AIDS (The World Health Report, 2001). Many synthetic chemical antidepressants are not satisfactory, as there are often a variety of side effect. Therefore, searching for new products for the treatment of depression from traditional Chinese medicine is drawing ever-increasing attention worldwide.

XYS, a valuable traditional Chinese medicine, is composed of the following eight raw materials: *Radix Bupleuri* (*Bupleurum chinense* DC.), *Radix Angelicae Sinensis* (*Angelica sinensis* (Oliv.) Diel), *Radix Paeoniae Alba* (*Paeonia lactiflora* Pall.), *Rhizoma Atractylodis Macrocephalae* (*Atractylodes macrocephala* Koidz.), *Poria* (*Poria cocos*

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(Schw.) Wolf), *Herba Menthae* (*Mentha haplocalyx* Briq.), *Rhizoma Zingiberis Recens* (*Zingiber officinale* Rosc.) and *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch.). Even though it has been reported that XYS showed significant antidepressant effect (Xiong et al., 2007), the material basis for efficacy of XYS in producing significant antidepressant-like effect has not been reported.

CUMS, a well-validated animal model, plays a role in the evaluation of antidepressant drugs (Papp et al., 1991; Zhao et al., 2008). A lot of behavioral and biochemical changes induced by CUMS are reversible by antidepressant treatments (Zheng et al., 2010). Therefore, the CUMS model of depression makes it suitable for investigating the pathophysiology of depression and the antidepressant effects of diverse drugs (Willner, 1997).

Metabonomics, a new scientific platform for investigation of the metabolic response of living systems to any environmental stimuli (Nicholson et al., 1999), could be defined as an attempt to measure the variation of the metabolic profile in biofluid and tissues of an organism (Ma et al., 2010a,b). Global metabolites profiling of biofluids has been applied in the fields of therapy monitoring, pharmaceutical discovery and the evaluation of drug efficacy and toxicity (Nicholson et al., 2002; Griffin and Bollard, 2004; Chen et al., 2006; Yin et al., 2006; Ellis et al., 2007). NMR spectrometry can provide rapid, non-destructive and high-through methods, requiring minimal sample preparation (Li et al., 2008). And NMR spectra of a biofluid are rich in extremely complex information relating to the concentration of these metabolites in the sample (Liu et al., 2010). Based on the reasons above, the applications of metabonomics method based on NMR in this field are increasing.

In our previous study, the antidepressant effect of XYS was evaluated, and it was shown that XYS had apparently antidepressant effect (Dai et al., 2010). However, the active fraction of this prescription in producing antidepressant effect was still obscure. The objective of the present work was to evaluate the antidepressant activity of five different polar fractions of XYS to identify the active fraction using behavior research and metabonomics method-based on NMR.

2. Experimental

2.1. Plant materials

All the eight dried medicinal plants were purchased from Shanxi Huayang Pharmaceutical Company and authenticated by Prof. Xue-Mei Qin, Shanxi University, before preparation. Voucher specimens were deposited in the Modern Research Center for Traditional Chinese Medicine of Shanxi University.

2.2. Preparation of the extracts

A 3 kg amount of mixed herbs (XYS), i.e. *Radix Bupleuri*, *Radix Angelicae Sinensis*, *Radix Paeoniae Alba*, *Rhizoma Atractylodis Macrocephalae*, *Poria*, *Herba Menthae*, *Rhizoma Zingiberis Recens* and *Radix Glycyrrhizae* in the weight ratio of 6:6:6:6:6:2:2:3, were extracted twice with anhydrous ethanol (20 L) under reflux, every time for 2 h, and the combined extracts were filtrated and concentrated in vacuo to a syrup, which was suspended in H_2O (1 L). The suspension was extracted with equal volumes of petroleum ether, and the petroleum ether fraction (XY-A) was obtained (15.65 g). The H_2O fraction was further chromatographed over a D101 macroporous resin column eluted with 30%, 60% and 95% ethanol gradually and the fractions XY-B, XY-C and XY-D were obtained (60 g, 11.74 g, and 15 g) respectively. The residue of anhydrous ethanol extract was extracted with boiling water (24 L) for 2 h and this procedure was repeated twice, and the combined extracts were filtrated and concentrated in vacuo, and the fraction XY-E was obtained (160 g). Preparation of the extract and derived fractions is illustrated in Scheme 1.

According to the preparation of XYS formula, eight plant materials weighted about 3 kg were extracted with boiling water (24 L) for 2 h and this procedure was repeated twice, the combined decoction was dried in vacuo and grinded into powder (390 g) which served as the positive control.

2.3. Animals

A total of 64 male Sprague-Dawley rats, weighing 180 ± 20 g, were purchased from the Experimental Animal Center of Chinese Military Medical Sciences Academy. All the animals in the study were cared for and treated humanely according to the national legislations of China, as well as local guidelines. During the study, all animals were kept in standard animal houses at a temperature of 22 ± 2 °C and a relative humidity of 45 ± 10%, with a 12-h light/12-h dark cycle.

2.4. Drug administration

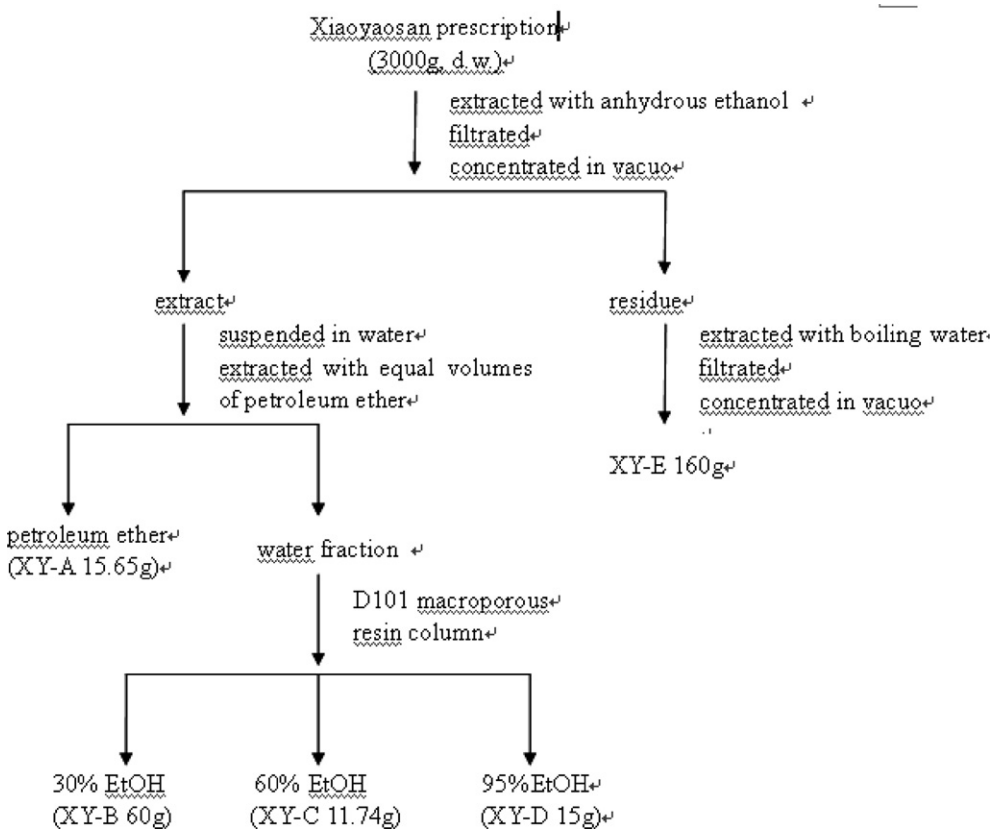
All drugs were dispersed in 1% CMC-Na added Tween 80 (0.5%, w/v). After one week of adaption, according to the body weights and behavior scores in open-field experiments, the rats were divided into eight groups (n = 8/group) randomly: (1) healthy control group (NS), (2) CUMS model group (MS), (3) XY-A group at the dose of 0.24 g/kg, (4) XY-B group at the dose of 0.92 g/kg, (5) XY-C group at the dose of 0.18 g/kg, (6) XY-D group at the dose of 0.23 g/kg, (7) XY-E group at the dose of 2.44 g/kg, and (8) XYS decoction at the dose of 5.98 g/kg. The healthy group of animals served as the negative control, while XYS decoction group of animals served as the positive control. Animals were given the calculated amounts of fractions by gastric intubation one time each day for continuous 21 days with the administration of volume of 10 mL/kg (rat body weight). Rats in control and model groups were given vehicle solvent (0.5%, w/v Tween 80 dissolved in 1% CMC-Na), and the amounts of XY-A to XY-E fractions and XYS administered to the groups were calculated according to effective dose of XYS obtained from previous experiment (Dai et al., 2010) and yield of extracts (effective dose: 46 g herb/kg, yields: XY-A 0.52%, XY-B 2.0%, XY-C 0.39%, XY-D 0.5%, XY-E 5.32%, XYS 13%). All drugs were given 30 min before the stress exposure.

2.5. Chronic unpredictable mild stress (CUMS) procedure

The CUMS procedure was performed as described (Harro et al., 1999) with a slight modification. The healthy control rats were housed together without disturbance, except for application of the necessary procedures. While the rats of other groups were housed individually and exposed to the following stressors in a random order every day between 9:30 and 12:00 a.m. for 3 weeks: cage tilting and damp sawdust for 24 h (200 mL of water per individual cage, which is enough to make the sawdust bedding wet), noises for 1 h (alternative periods of 60 dBA noise for 10 min and 10 min of silence), swimming in 4 °C cold water for 5 min, exposure to an experimental room at 50 °C for 5 min, 48 h of food deprivation and 24 h of water deprivation, respectively, tail clamp for 1 min, 15 unpredictable shocks (15 MA, one shock/5 s, 10 s duration) and restricted movement for 4 h.

2.6. Sample collection

Urine samples were collected overnight (12 h) in metabolism cages from all the rats on day 21. After centrifugation at 4000 rmp, 4 °C for 10 min to remove residues, urine samples were immediately stored in aliquots at −80 °C until analysis.



Scheme 1. Flow diagram of fractionation of the Xiaoyaosan prescription.

2.7. Behavior test

2.7.1. Open-field test

The open-field test was performed as previously described between 8:00 am to 12:00 am in a quiet room (≤60 dB) on day 21 (Wang et al., 2008). The open-field apparatus was a four-sided 100 cm × 100 cm × 40 cm wooden enclosure, with floor painted white and divided into 25 equal squares by black lines and side walls painted black. Tests were conducted in a darkened room lit by two 40-W light bulbs suspended above the center of the open field. Each rat was gently placed in the central square and observed for 5 min. Scores were calculated by counting the number of rearings (wall rears and free rears) and immobility time.

2.7.2. Sucrose preference test

The sucrose preference test was carried out at the end of 3-week period of CUMS exposure. The test was performed as described previously (Wang et al., 2008), with minor modifications. Briefly, 72 h before the test rats were trained to adapt to sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation procedure, the rats were deprived of water and food for 24 h, followed by the sucrose preference test, in which the rats were housed in individual cages and given free access to two bottles containing 100 mL of sucrose solution (1%, w/v) and 100 mL of water, respectively. After 60 min, the volume of both the consumed sucrose solution and water was recorded and 1% sucrose solution to that of total solution ingested within 1 h represented the parameter of hedonic behavior.

2.7.3. Statistical analysis

All data were expressed as mean ± S.E.M. The significance of variation between groups in data was determined using independent-sample *t* test by SPSS 11.5 (Chicago, IL, USA). The behavioral data of each group were compared with that of the model group. $p < 0.05$ was considered as statistically significant.

2.8. ^1H NMR experiment of urine samples

2.8.1. Samples preparation

The urine samples were thawed before use. 500 μL aliquots of each sample was diluted with 50 μL of phosphate buffer (0.2 M Na_2HPO_4/NaH_2PO_4, pH 7.4) containing D_2O as a field lock, and 0.1% sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)-1-propionate (TSP) as a chemical shift reference, centrifuged at 11,400 × *g* for 20 min. An aliquot of 500 μL was transferred into a 5 mm NMR tube for NMR analysis.

2.8.2. NMR spectrometry

^1H NMR spectra of urine were measured at 600.095 MHz on a Varian INOVA 600 spectrometer. Urine samples were analyzed using one-dimensional (1D) Nuclear Overhauser Effect Spectroscopy (NOESY, RD-90°-t_1-90°-t_m-90°-acquire). Water signal suppression was achieved with weak irradiation on the water peak during the recycling delay (RD = 2.0 s) and mixing time (tm = 0.10 s). A total of 64 transients were collected into 32k time domain data point, with a spectral width of 8000 Hz. Prior to Fourier transformation, the free induction decay (FID) was multiplied by an exponential weighting function corresponding to 0.5 Hz line broadening and zero-filled by a factor of 2. The resulting spectra were manually phased, baseline corrected, and referenced to TSP at δ 0.0.

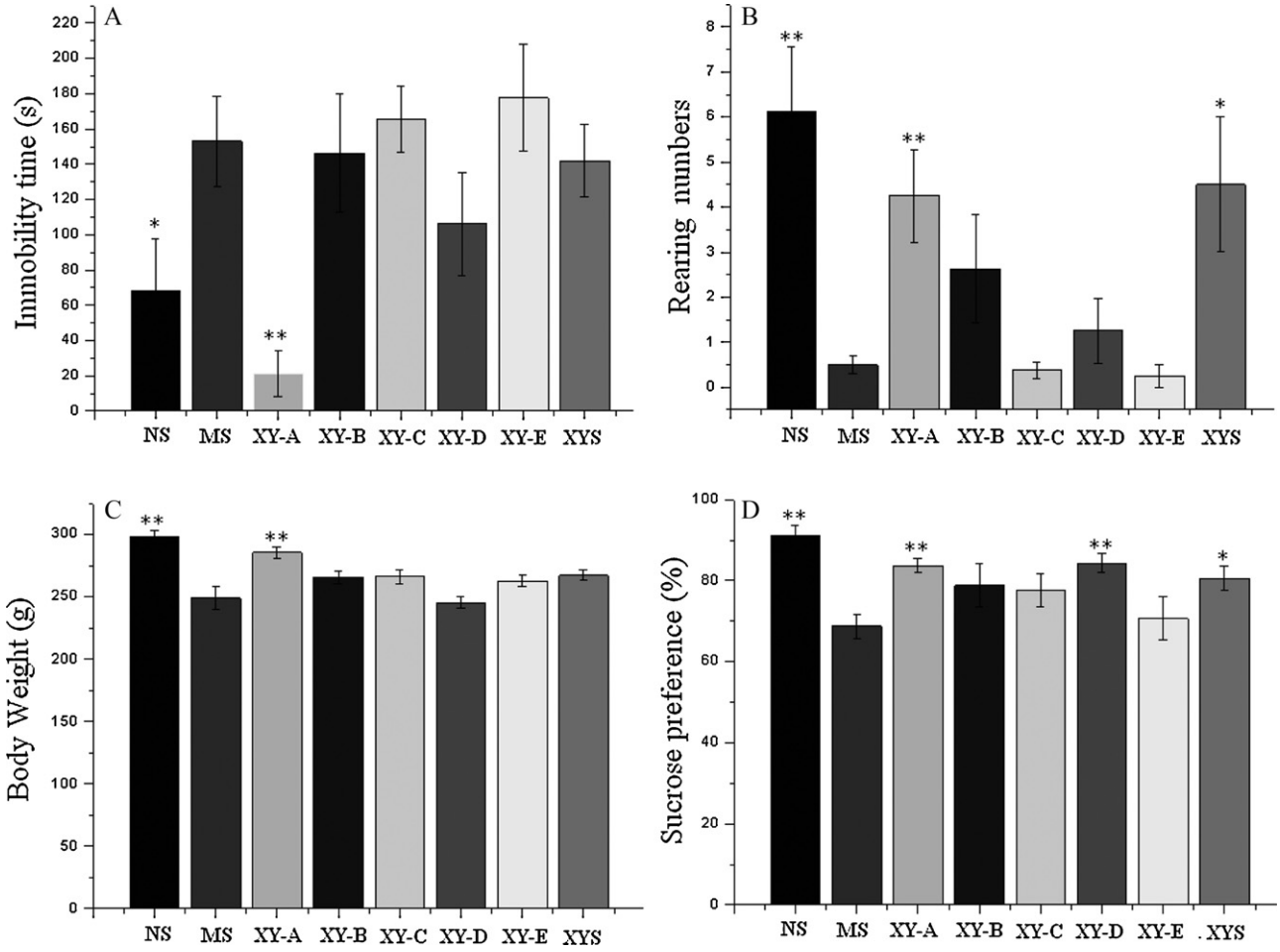


Fig. 1. The behavior scores in NS group, MS group, XY-A group, XY-B group, XY-C group, XY-D group, XY-E group, and XYS group were expressed as mean ± S.E.M. ($n = 8$). (A) Immobility time (s); (B) Rearing numbers; (C) Body weight (g); (D) Sucrose preference (%); compared with MS group: $^{*}p < 0.05$; $^{**}p < 0.01$.

2.8.3. Statistical analysis

All ^{1}H NMR spectra from urine samples were phased, baseline corrected and date-reduced to integrated regions 0.04 ppm wide corresponding to the region δ 10.0–0.52, using MestReC (version 4.9.9.6). The region of δ 6.0–4.5 in the spectra was removed to eliminate the effects of imperfect water saturation and the broad resonance from urea. Each data point was normalized to the sum of its row and then exported as text files for further multivariate statistical analysis.

Multivariate pattern recognition analysis was carried out by using SIMCA-P (Umetrics, Umea, Sweden). Partial least-squares discriminant analysis (PLS-DA) was performed for data from different groups to detect the distributions and separations among those groups. Prior to PLS-DA, all data variables were mean-centered, Pareto-scaled prior and preprocessed using orthogonal signal correction (OSC) to remove variations from noncorrelated factors. Independent-sample t test was further used to investigate the endogenous metabolites alteration using SPSS 11.5 (Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Analysis of behavior test

The effect of different fractions of XYS on locomotor activity in the CUMS-treated rats is shown in Fig. 1A and B. After 21 days of stress exposure, the CUMS rats significantly increased immobility time ($p < 0.05$) and decreased the number of rearings ($p < 0.01$) compared with the control rats in the open-field test. It was an indication that the locomotor activity was affected. After 21 days of treatment with five different fractions of XYS, the data showed that the differences of immobility time and the number of rearings between XY-A fraction group and the model group were all significant ($p < 0.01$). While there were no differences between the other fraction groups and the model group. Although XYS decoction had no effect on immobility time, long-term treatment with XYS decoction significantly increased the number of rearings ($p < 0.05$) in CUMS-treated rats, compared with the model group.

Fig. 1C and D shows the effects of different fractions of XYS on weight gain levels and the percentage of sucrose consumption in CUMS-treated rats. The decrease of body weight ($p < 0.01$) was observed in CUMS rats. This was similar to the loss of appetite experienced by patients with depression. Consumption of sucrose was significantly lower in CUMS rats than that in control rats ($p < 0.01$) after 21 days CUMS treatment, indicating impairment of hedonic reactivity in model rats. After 21 days of treatment with five different fractions, compared with the CUMS group, the XY-A fraction rats significantly increased the weight ($p < 0.01$) and the percentage of sucrose consumption ($p < 0.01$). And the XY-D fraction rats significantly increased the percentage of sucrose consumption ($p < 0.01$). The other fraction groups had no obvious effects in these same tests. In addition, the XYS decoction group rats also increased the percentage of sucrose consumption ($p < 0.05$).

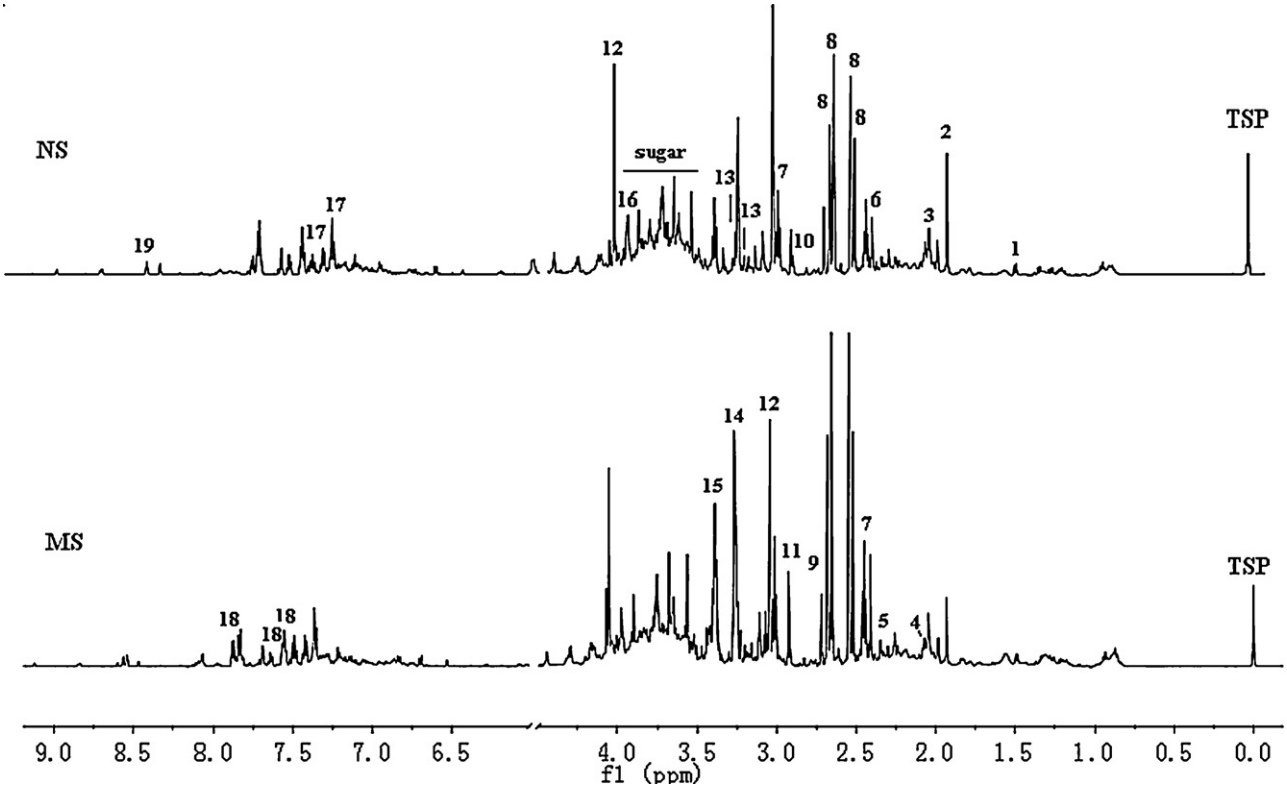


Fig. 2. Representative ^{1}H NMR spectras of urine from a control rat (NS) and CUMS rat (MS). Identified metabolites: 1, alanine; 2, acetate; 3 and 4, N-acetyl glycoprotein (NAc); 5, pyruvate; 6, succinate; 7, 2-oxoglutarate (2-OG); 8, citrate; 9, dimethylamine (DMA); 10, trimethylamine (TMA); 11, dimethylglycine (DMG); 12, creatinine; 13, taurine; 14, trimethylamine-N-oxide (TMAO); 15, glycine; 16, betaine; 17, phenylacetyl-glycine (PAG); 18, hippurate; 19, formate.

3.2. Analysis of urine samples from control group and model group by ^{1}H NMR

The typical ^{1}H NMR spectra of urine samples from the control group and the model group are shown in Fig. 2. The endogenous metabolites were identified according to published reports (Bollard et al., 2001; Nicholls et al., 2001; Wang et al., 2005; Li et al., 2008; Sun et al., 2009; An et al., 2010; Liu et al., 2010), and were labeled in the spectra. In this study, multivariate analysis technique was used to visualize and detect any possible subtle change due to CUMS treatment.

Further analysis by PLS-DA showed that the model group and control group can be clearly separated in the scores plot. A score plot of the first second components is shown in Fig. 3A. The profile of urine from CUMS rats and control rats along PC1 was clearly separated. The significant urinary metabolic variation between the two groups also suggested the CUMS depression model was achieved on day 21, which was in agreement with the results of behavioral tests. The metabolites responsible for these differences were identified from the loadings plot corresponding (Fig. 3B).

The chemical shifts which sit furthest from the origin contribute significantly to the clustering of different groups. Independent-

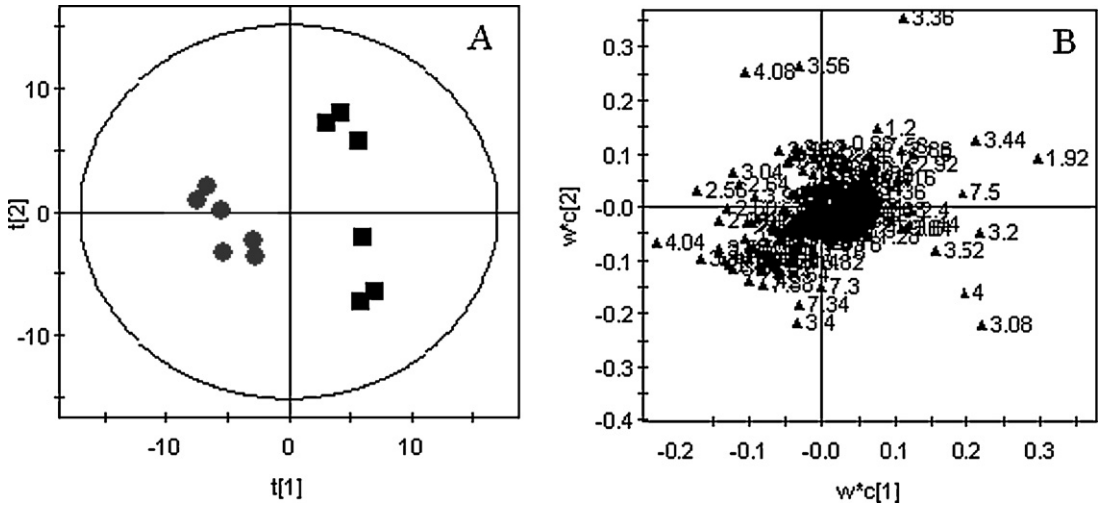


Fig. 3. PLS-DA analysis of NS and MS groups (A) score plot; (B) loading plot; (●) NS; (■) MS.

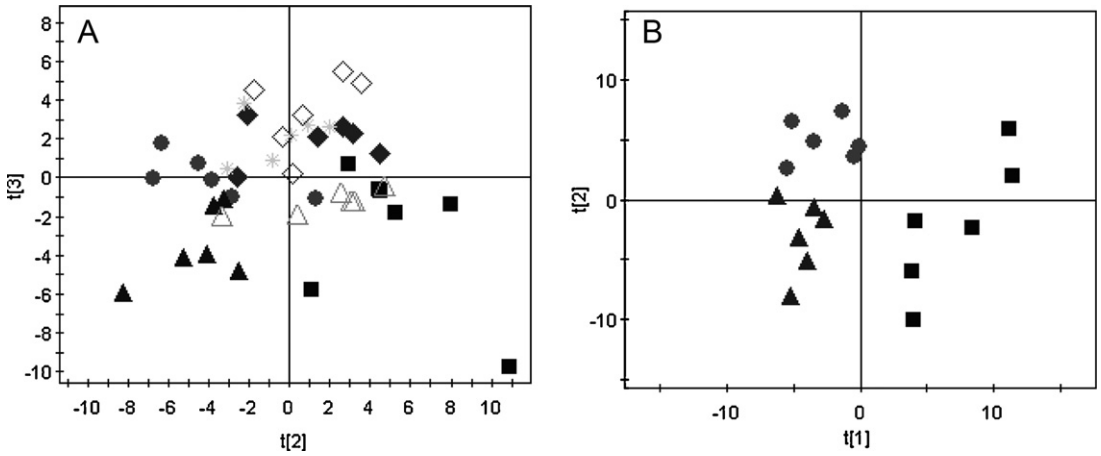


Fig. 4. (A) OSC-PLS score plots of different fractions of XYS treatment groups. (●) NS; (■) MS; (▲) XY-A group; (♦) XY-B group; (*) XY-C group; (□) XY-D group; (△) XY-E group. (B) PLS-DA score plots of XY-A treatment group. (●) NS; (■) MS; (▲) XY-A group.

sample *t* tests were also conducted on the normalized integral data of selected regions, which represented these metabolites, to detect the significance of these changes. According to the protocol detailed above, seven endogenous metabolites contributing to the separation of the model group and control group were detected in the urine samples. The increase of lactate (1.34d), creatinine (3.06s, 4.06s), taurine (3.24t, 3.40t) as well as the decrease of N-acetyl glycoprotein (NAc, 2.06s–2.10s), pyruvate (2.34s), phenylacetylglycine (PAG, 3.68s, 7.37m, 7.43m) and betaine (3.89s) were observed in the CUMS group compared with control group.

3.3. Analysis of urine samples from different administration groups by ^1H NMR

To determine whether different fractions of XYS were possible to influence metabolic pattern of the CUMS model subjects, a PLS-DA score plot of different fraction groups is shown in Fig. 4A. To maximize the group separation, OSC was applied to the data sets before PLS-DA. Score plots were used to visualize the separation of the groups, and the control group exhibited large differences with the model group along PC2. The subject of XY-A fraction group obtained better separation from those of the model group than those of other fraction groups; it is much closer to the control group. The change of urinary metabolic pattern showed the depression was being prevented and alleviated after taking XY-A fraction. In addition, it can be seen that from the PLS-DA score plot (Fig. 4B), the XY-A group was closed to the control group. The above evidence indicated that the XY-A fraction was the most active anti-depressive fraction in the five different polar fractions of XYS.

3.4. Analysis of representative metabolites

In this study, the concentration of 7 endogenous urinary metabolites was significantly affected by CUMS treatment. According to the Independent-sample *t* tests, we found that the peak areas of 5 metabolites (NAc, pyruvate, creatinine, taurine and betaine) could be reversed after taking the XY-A fraction. Fig. 5 shows that after taking different fractions, the concentrations of some metabolites had tendency to come back from the CUMS-treated group to the control group. The shift pattern suggested that different metabolic responses occurred due to the CUMS model interference and protective effect of the extracts of XYS against depression induced by CUMS. While comparing the anti-depression effect of the different polar fractions, the inhibition effect of XY-A fraction on increase or reduction of these metabolites induced by CUMS was better than other fractions.

4. Discussion

XYS was originated from the book of Taiping Huimin Heji Jufang in Song Dynasty (960-1127 AD) and has been used for centuries in China to treat mental diseases, including depression and schizophrenia. In our previous experiments, the antidepressant effect of XYS was also verified (Dai et al., 2010), but the active fraction of XYS that produce antidepressant-like effect is not yet clear. In the present tests, the XYS was therefore divided into five fractions: XY-A, XY-B, XY-C, XY-D and XY-E, to find the active fraction. Based on behavior research and metabonomics method, we ascertained that the active antidepressant fraction of XYS was XY-A fraction, which will supply help for illuminating material basis for efficacy of XYS in producing significant antidepressant-like effect.

In this behavioral assay, the changes induced by CUMS were showed. It is accepted that the depressive-like behavioral status was obviously developed in CUMS-treated rats after three weeks of stress exposure as seen in human depression. After 21 days of treatment with five different fractions of XYS, the data demonstrated that the XY-A fraction showed a reduction in the immobility time. In addition, the results also showed that XY-A fraction treatment increased the percentage of sucrose consumption, the number of rearing and body weight.

Metabonomic approaches provide a global overview of the integrated response of an organism to a stimulus. This feature is consistent with the principle of TCM, which is a kind of integrative medicine in which live organisms are seen as a whole. In order to fully reflect endogenous metabolite changes of depression and the protective effect of the extracts of XYS against depression to identify the active fraction, we used metabonomic approach based on NMR with multivariate analysis to monitor urinary metabolic profiles of rats. Tricarboxylic acid cycle (TCA) is a significant biological metabolic pathway in the body. In the CUMS group, elevation of creatinine and decrease of pyruvate implies impairment of energy metabolism in mitochondria. According to assessed metabolic pathway, creatinine is a waste product formed by the slow spontaneous degradation of creatine-phosphate (Wyss, 2000). While Creatine-phosphate functions as a “battery” that stores the energy of excess ATP (Ma et al., 2010a,b). When CUMS treatment caused energy decline, ATP insufficiency resulted in shifting the equilibrium and thus increased the total amount of free creatine which subsequently degraded to creatinine. While pyruvate associated with energy metabolism is the end product of glycolysis, and can be converted by oxidative decarboxylation to form acetyl CoA, which further enters the TCA cycle. We therefore infer that the decrease

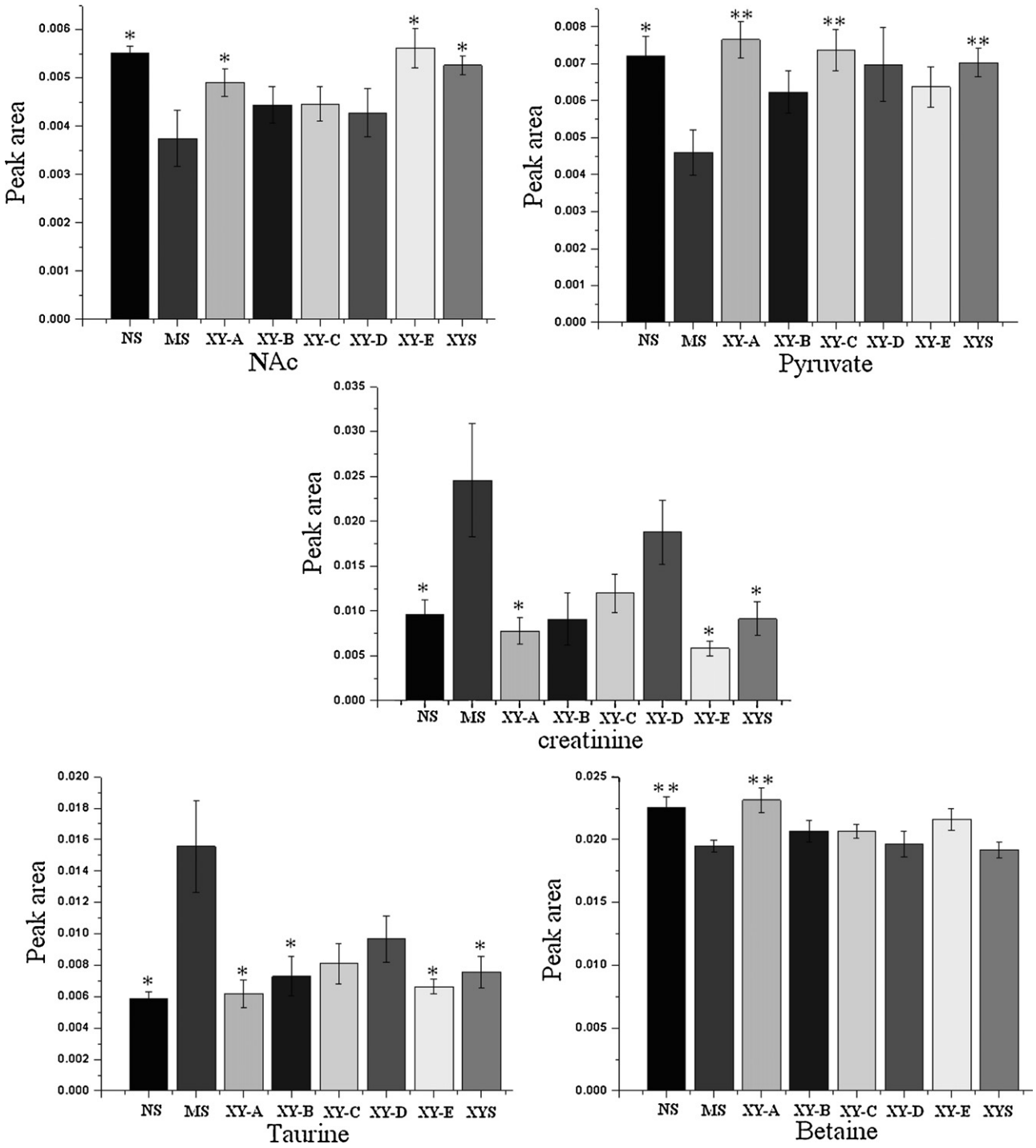


Fig. 5. Mean peak area (mean ± S.E.M.) of the representative metabolites in NS group, MS group, XY-A group, XY-B group, XY-C group, XY-D group, XY-E group, and XYS group. compared with MS group: $^{*}p < 0.05$; $^{**}p < 0.01$.

of pyruvate results in dysfunction of TCA. While the energy deficiency (according to the dysfunction of TCA cycle) or fatigue is one of the most frequently represented depressive symptoms in major depressive disorder (Serretti et al., 2004). In addition, according to the concepts of TCM, the liver is one of the most vulnerable organs to anger, stress and depression resulting in stagnation of liver-qi (gan-qi-yu-jie), which were the etiological factors of depressive disorders (Wang et al., 2009). Previous publication has shown that elevated taurine in response to the depletion of glutathione is caused by oxidative hepatic damage (Sattari and Mashayekhi, 2008; Bollard et al., 2009). In the present study, it is observed that CUMS treatment can increase the level of taurine. Betaine, an important precursor of S-adenosylmethionine (SAM), showed significant decrease in the CUMS rats due to increased activity of betaine-homocysteine-S-methyltransferase. Elevation of taurine and decrease of betaine indicated that the liver damage possibly has also occurred. In the dose groups, the observed changes in CUMS-treated rats after XY-A treatment are more likely to be due to the therapeutic effect of XY-A fraction. The above results showed XY-A fraction has a good effect on depression, suggesting that the active

constituents of XYS in producing antidepressant effect are mainly concentrated in this fraction.

We have found in previous reports that petroleum ether fraction of Yueju-Wan and lipophic parts of Banxia Houpu decoction exert antidepressant effects in experimental animal models (Luo et al., 2000; Wei et al., 2008), while our present result also suggested the low polar fraction play an important role in antidepressant formula. Using GC–MS analysis, we identified that the chemical components in XY-A fraction contained ligustilide and palmitic acid. It has been reported that ligustilide is primary component of *Angelicae Sinensis* essential oils (Zhou et al., 2007) and also is the main components of active fraction of Yueju-Wan (Wei et al., 2008), which could directly suppress the activity of CRF systems, or indirectly affect the GABAA receptor systems (both of the two effects may be related to the mechanism of depression) (Matsumoto et al., 1998). In addition, palmitic acid, one component of *Radix Bupleuri* essential oils (Li et al., 2005), has a peculiar position with respect to the biochemical characterization of Major Depression (Cocchi and Tonello, 2007). While *Radix Bupleuri* and *Radix Angelicae Sinensis* are the main ingredients of the XYS formula. However, as the components are very complicated, the analysis work are still carrying on and deserve further research, which will give the basis for new drug development for traditional Chinese medicine.

5. Conclusion

Recently, the usage of traditional herbs has been increasingly popular in the treatment of depression for its better compliance and lower side effects. Among them, Chinese traditional herbal prescriptions, representing the core of traditional Chinese medicine theory, are becoming more and more attractive, but they are still lack of wide scientific research. At the present time, the work in our laboratory has demonstrated that petroleum ether fraction (lipophilic components) is the active fraction of XYS, which may provide scientific evidence supporting XY-A fraction to become a novel potential antidepressant. In addition, our work also suggested that metabonomic approach is a useful tool in the investigation of active fraction of traditional Chinese medicine.

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