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Label-free quantitative proteomic analysis of reserpine-induced depression in mice intervened by berberine

Nianyun Yang*, Lisi Zou and Ye Wang
Jiangsu Plant Medicine Research and Development Center, Nanjing University of Chinese Medicine, Nanjing, China

Abstract: Proteomic analysis of reserpine-induced depression and the effects of berberine on this were investigated to delineate the possible underlying mechanism. Reserpine was used for the model of behavioral depression. Model mice were treated with berberine. Mice brain proteomic analysis was carried out by label-free nano LC-ESI-OrbiTrap MS/MS technology. The data were processed by Maxquant software. The differentially-expressed proteins were evaluated on GO and KEGG analysis, and key protein expression was validated by Western blot analysis. A total of 278 differentially-expressed proteins were identified. Reserpine could cause cerebral injury and depressive disorder in mice, the mechanism of which is related to steroid hormone biosynthesis, chemical carcinogenesis, nucleotide excision repair and the retinoic acid-inducible gene I-like (RIG-I-like) receptor signaling. Berberine treatments involve 3 distinct proteins in the RIG-I-like receptor signaling. RIG-I was validated, which was over-expressed in the model group and negative in the normal and administration groups. RIG-I mediated neuroinflammation could participate in the process of depression and RIG-I may become a target for berberine against depression.

Keywords: Depression, Proteome, berberine, retinoic acid-inducible gene I, neuroinflammation.

INTRODUCTION

Depression, a common spiritual disorder affecting more than 350 million people worldwide (Mutsatsa, 2016), is a major cause of disability around the world and dedicates greatly to the global burden of disease. The effects of depression can be long-lasting or recurrent and can dramatically influence a person's ability to function and live a meaningful life (Rosińczuk *et al*., 2017). A depressed mood may also be a symptom of some disorders such as dysthymia (Chen *et al*, 2020). Antidepressants, such as amitriptyline, may improve symptoms. Unfortunately, these drugs have a whole host of extremely unpleasant side-effects including sleep disorders, epileptic seizures, hepatic injury and tachyphylaxis (Miskovi, 2015). In recent years, research on the anti-depression effect of traditional Chinese medicines (TCM) has increased continually and achieved great progress. Treatments with TCM against depressive disorder apply the holistic concept and syndrome differentiation with multilevel and multitargets. Developing new antidepressant drugs from multiple targets has become a hotspot in recent years, so the research of anti-depression ingredients and mechanism of TCM should be strengthened. The current research mostly concentrates on the effects of TCM on monoamine neurotransmitters, brain neurotrophic factors, cytokines and the neuroendocrine system (Zhang, 2017). However, exploration of the antidepressant mechanism of TCM is still not thorough. It is necessary to strengthen the research of correlative Chinese herbs and take full advantage of genomics, proteomics and metabolomics to clarify the underlying mechanisms of TCM against depression (Chen, 2017; Wang *et al*, 2017). Coptidis Rhizoma, the rhizome of *Coptis chinensis* Franch, is one of the most widely used herbs in traditional Chinese medicine (Gai, 2018). This drug is used to clear away heart fire and tranquilize the mind and treat distress. Coptidis Rhizoma and its prescription products have been used to treat depressive illness clinically. Berberine in Coptidis Rhizoma displayed anti-depression effects in different animal model tests and also reversed the reserpine-induced behavioral despair (Huang, 2018), but the mechanism of function has not been fully clarified. The present study aims to examine the effects of berberine from Coptis on the depression-related behavior of mice induced by reserpine and proteomic changes and drug effects on brain tissues.

MATERIALS AND METHODS

Animals

Male SPF ICR mice (20-22g) were obtained from Jiangning District, Nanjing Qinglongshan Animal Center (Certificate No.201712610). The animal experiment protocols were permitted by the Animal Care Committee of Nanjing University of Chinese Medicine (Grant No.20170920110014). The animals were housed in groups of 10 under environmentally controlled conditions with free access to water and standard food. Food was withheld overnight prior to experiments while water was provided ad libitum.

Drugs and reagents

Reserpine (No. 20170106) was from Nanjing Liangwei Biotechnology Co. Ltd.; berberine (No. Z08A7H2686,

**Corresponding author:* e-mail: yny@njucm.edu.cn

purity greater than 99%) from Shanghaiyuanye Bio-Technology Co. Ltd.; and BCA protein quantitative detection kit (No.C503021-0500), dithiothreitol (No. A100281-0001), sodium dodecyl sulfate (No. A100227-0100) and trypsin (No. A100227-0100) were from Sangon Biotech (Shanghai) Co., Ltd. Milli-Q deionized water was used as the experimental water. All other chemicals used were of analytical grade.

Animal experimental design and protein sample pretreatment

This experiment was conducted in the Animal Research Center of Nanjing University of Chinese Medicine. All mice ate and drank freely. The mice were randomly divided into four groups (n=10) as follows: Normal group (I), which received 1mL nomad saline intraperitoneally; model group (II), which received 2.5mg/kg of reserpine dissolved in 1mL of nomad saline intraperitoneally; berberine high dosage group (III) and low dosage group (IV), which were individually treated with 20 and 5mg/kg of berberine dissolved in 1mL of nomad saline (IP). Groups III-IV also received 2.5mg/kg of reserpine dissolved in 1mL of nomad saline.

At the end of the experiment, the animals of group I-III were sacrificed by cervical dislocation. Mice brain tissues were newly collected, rinsed with phosphate buffer solution and wiped with filter paper. All ten samples per group were merged into 3 samples randomly. The tissues were precisely weighed immediately, added to precooling EP tubes, and quickly uniformized in an ice-water bath. Proteins were extracted using the SDT (4% (w/v) sodium dodecyl sulfate, 100mM Tris (hydroxymethyl) aminomethane/ hydrochloric acid, pH7.6, 0.1M dithiothrietol) lysis method. Each sample of 200μg of protein was digested with trypsinase according to the FASP (filter aided sample preparation) method. Peptides were desalinized and lyophilized (Sielaff *et al*, (2017).

NanoLC–MS/MS analysis

The peptides were dissolved in 0.1% HCOOH (FA) and 2% CH_3CN (ACN) and then centrifuged at 13,500*g* for 20 min. NanoLC-MS/MS was developed using an EASY-nLC system (Thermo Scientific). The peptide mixture was loaded onto a PepMap C18 trapping column (100μm x 3 cm) and then separated on an EASY column (3μm, 75 μm×100 mm) at a flow rate of 300nL/min. Peptides were eluted by application of a linear scale from 4% buffer B (0.1% FA, 84% ACN) to 50% buffer B for 40min, followed by ramping up to 90% buffer B in 5min. The eluted peptides were detected by Q Exactive and MS data were acquired using a data-dependent top20 method, dynamically choosing the most abundant precursor ions from the survey scan (300-1800 m/z) for HCD (high-energy collisional dissociation) fragmentation. Determination of the target value was based on Automatic Gain Control (AGC). Survey scans were acquired at a resolution of 70,000 at *m/z* 200 and resolution for HCD spectra was set to 17,500 at *m/z* 200. Normalized collision energy was 30 eV and the under-fill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum-fill time, was defined as 0.1%. The instrument was run with the peptide recognition mode enabled.

Protein identification and data analysis

The raw files were retrieved via Max Quant 1.5.3.17 (Thermo Fisher Scientific) with default settings for deep proteome analysis. The database was Swissprot_ mouse_ 16983_20180627. fasta for mouse species. The index parameters were as follows: Primary ions tolerance 6ppm, fragment ions mass tolerance 20ppm, proteins and peptides FDR≤ 0.01.

For further functional analysis, differential expression of proteins was analyzed for significant down-regulation or up-regulation. A change in expression was determined in comparison with the corresponding control. The proteins showed an average ratio-fold change ≥2.0 or ≤0.5 in the experiment and proteins with a minimum of two peptide matches in common were confidently considered as differential expression of proteins. GO analysis of differentially accumulated proteins was carried out using Blast2Go software, which can utilize authoritative databases in bioinformatics research together to generate the proteome biological process, molecular function and cellular component information. The KEGG database (http://www. genome.jp/kegg/pathway.html) was used to take advantage of the current knowledge of biochemical pathways and other types of molecular interactions.

Protein validation

The detected important differential protein expression was assessed using Western blotting. The protein concentration was gauged by the bicinchoninic acid method. Thirty μg proteins was loaded onto a gel, subjected to SDS-polyacrylamide gel electrophoresis, and then electro blotted onto PVDF membranes, which were hatched with retinoic acid-inducible gene I protein (RIG-I) and β-actin antibodies, in line with the manufacturer's protocol. Protein bands were visualized by enhanced chemiluminescence. Image J. provided tools for processing protein band images.

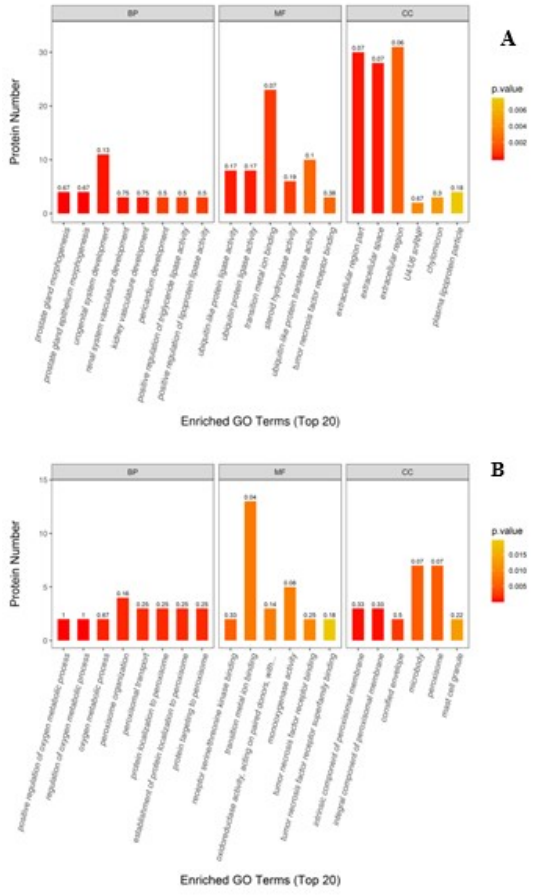
STATISTICAL ANALYSIS

The significance of differences of proteins expression between different groups was analyzed by one-way ANOVA with SPSS13.0 and the level of $P<0.05$ was taken as statistically significant. The significance of functional and pathway enrichment analysis of differential proteins was carried out by the Fisher exact test method.

RESULTS

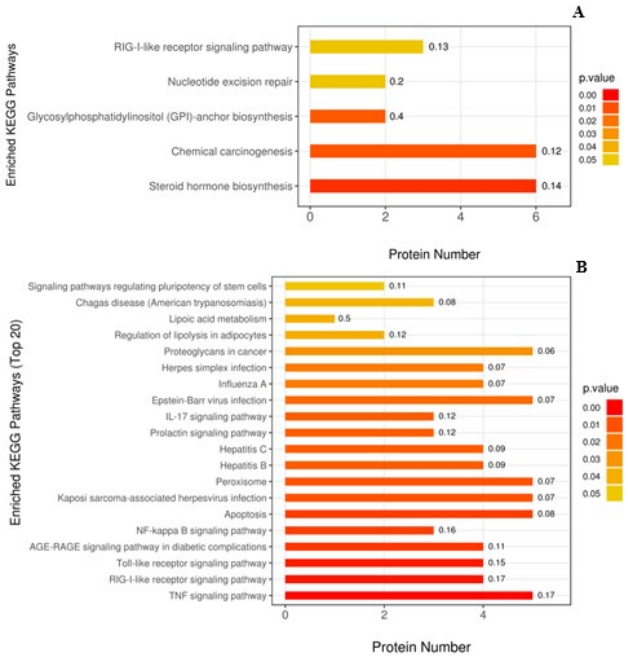
Primary data analysis and protein identification

Label-free was used to assess proteome changes of mice brain tissue in different groups. The samples were extracted by enzymolysis and then analyzed by nano LC-LTQ- Orbitrap-MS/MS technology. On the basis of analysis with MaxQuant, 5225 proteins were quantified. Changes in the protein profile were analyzed and 189 proteins showed a difference (*P*-values ≤0.05) with a FDR of less than 1%. Ninety-seven proteins were either increased by more than 2-fold or consistently expressed and the level of 92 proteins reduced to either less than 0.5-fold or lack of expression in group II compared with group I. Changes in the protein profile were analyzed and 89 proteins exhibited a difference (*P*-values ≤ 0.05) with a FDR of less than 1%. Thirty-five proteins raised by more than 2-foldand the level of 53 proteins decreased to less than 0.5-fold in group III compared with group II. Differential proteins could be effectively grouped, such as cytochrome P450 2B9 (Cyp2b9) and corticosteroid 11-β-dehydrogenase isozyme 1 (Hsd11b1), which are jointly involved in steroid hormone biosynthesis and chemical carcinogenesis in model mice.



Note: Abscissa represents GO functional classification, divided into Biological Process (BP), Molecular Function (MF) and Cellular Component (CC). Longitudinal coordinates represent the number of differentially expressed proteins. The bar chart color represents the significance of GO functional classification (*P* values). The label above the bar chart shows the enrichment factor (rich Factor≦1)

Fig. 1: GO-enriched analysis in group II compared with I (A) and group III compared with II (B)



Note: Longitudinal coordinates represent significantly enriched KEGG pathways. Abscissa represents the number of differentially expressed proteins in each pathway. The bar chart color represents the significance of pathway (*P* values). The label above the bar chart shows the enrichment factor (rich Factor≦1)

Fig. 2: KEGG analysis in group II compared with I (A) and in group III compared with II (B)

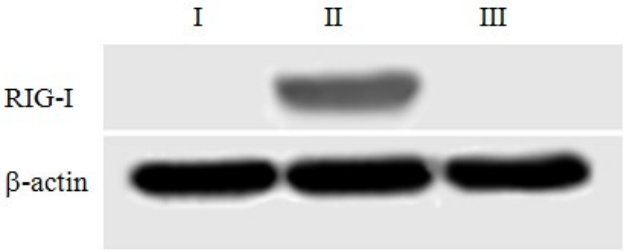


Fig. 3: Verification of RIG-I by Western blot

GO analysis was performed on differential proteins in II compared with I and III compared with II. Significant changes occurred on some molecular functions like peroxisome organization, transition metal ion binding, and tumor necrosis factor receptor binding (fig.1).

The 189 differential expressions of proteins in group II compared with group I was further surveyed using the KEGG database. These differential proteins involved several important signal channels, namely steroid

Table 1: Different protein expressions in group II compared with I and III compared with II

Accession no	Gene name	Protein name	Consistent presence or absence expression		
			II	I	III
RIG-I-like receptor signaling pathway					
Q6Q899	RIG-I	Retinoic acid-inducible gene I protein	presence	absence	absence
Q61160	Fadd	FAS-associated death domain protein	presence	absence	absence
Q60803	Traf3	TNF receptor-associated factor 3	absence	presence	presence

Note: Presence: High expression in II and no expression in I, Absence: no expression in II and high expression in I,

hormone biosynthesis, chemical carcinogenesis, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, nucleotide excision repair and RIG-I-like receptor signaling pathway (fig. 2).

The 89 differential expressions of proteins in group IV compared with group II were further investigated using the KEGG database. These proteins were associated with several important cell signaling or metabolic pathways, namely the TNF signaling pathway, RIG-I-like receptor signaling pathway, Toll-like receptor signaling pathway, AGE-RAGE (advanced glycation end products-the receptor for advanced glycation end products).

signaling pathway in diabetic complications, NF-κB signaling pathway, apoptosis, kaposi sarcoma-associated herpesvirus infection, peroxisome, hepatitis B, hepatitis C, prolactin signaling pathway, IL-17 signaling pathway, Epstein-Barr virus infection, influenza A, herpes simplex infection, proteoglycans in cancer, regulation of lipolysis in adipocytes, lipoic acid metabolism, chagas disease (American trypanosomiasis) and signaling pathways regulating pluripotency of stem cells (fig. 1).

KEGG enrichment analysis of differential proteins showed that the RIG-I-like receptor signaling pathway had changed significantly in group II compared with I (P=0.048467) and III compared with II (P=0.00098). There are 3 distinct proteins participating in this pathway, RIG-I, TNF receptor-associated factor 3 (TRAF3) and Fas-associated protein with death domain (FADD) (table 1). RIG-I and FADD was over-expressed in group II, while negative expression was observed in I and III. However, Traf3 presented the opposite case. Protein validation showed that RIG-I was expressed in model group II, but there was no expression of RIG-I in groups I and III (fig. 3).

DISCUSSION

As a traditional model of depression, the reserpine reversal test is ideal. It is time saving and can alleviate the suffering of animals compared with chronic unpredictable mild stress (CUMS), electrical stimulation and olfactory bulb removal (Fattahian, 2016) However, the etiological factors and protein expression differences are not fully clarified as yet (Bakhtiarpoor *et al*, 2018; Gao *et al*, 2016). Mice after an intraperitoneal injection of an acute attack dose of reserpine have depression-related behaviors. In this study, the proteomic changes were explored in brain tissues of model mice treated with berberine by a label-free quantification method. Protein is the specialist of life activities (Singh, 2017). Different physiological and pathological conditions may guide a change in gene expression, which may display as differential expression of proteins. The significant differential proteins were grouped into several categories on the results of GO and KEGG analysis. The results showed that brain protein expression changes of model mice were mainly involved in steroid hormone biosynthesis, chemical carcinogenesis, GPI-anchor biosynthesis, nucleotide excision repair and RIG-I-like receptor signaling pathways.

The RIG-I-like receptor signaling pathway changed significantly in group II compared with I and III compared with II. RIG-I signaling plays a vital role in microglia activation, neuroinflammation and related neurological diseases (Frank, 2015; Li, 2018). Depressive patients have a significant handicap in learning and memory and the mechanism is hyperfunction with hypothalamus-pituitary-adrenal (HPA). Neurosteroids such as allopregnanolone are positive allosteric modulators of GABAA receptors and exert an anxiolytic and antidepressant-like behavior (Gerritsen *et al*, 2019). It is believed that inflammatory cytokines could participate in the process of depression. Inflammatory factors can cause hormone abnormalities for depression, and the level of plasma IL-1 is associated with corticosterone level in depressed patients. Cytokines also play a role in the progress of depression by affecting the metabolism of neurotransmitters. IL-1 can stimulate norepinephrine release in the hypothalamus and enhance the inhibition of γ-GABA. (Farooq, 2017; Jiao, 2017; Singh, 2017; Xie, 2017). Berberine could reduce the level of RIG-I expression, leading to a decrease in inflammatory cytokines and influence the metabolism of neurosteroid hormone and neurotransmitters. In recent years, a large amount of evidence indicates that berberine has a good antidepressant effect in different animal models of depression. Coptis alkaloids also included coptisine, epiberberine, palmatine, columbamine, jatrorrhizine, berberrubine, magnoflorine and berberamine. The pharmacological activities of Coptis alkaloids have shown

some advantages, which need to be studied more (Liu, 2017; Pang, 2014).

CONCLUSIONS

The proteomic changes were explored in brain tissues of reserpine-induced depressive mice treated with berberine. Differential protein analysis revealed that reserpine might interfere with the biosynthesis and metabolism of neurosteroids and peroxisome function in nerve cells, inhibit nucleotide excision repair and promote inflammation, and thus cause cerebral injury and depressive disorder in mice. The RIG-I-like receptor signaling plays an important role during the development of reserpine injury. The pathway related proteins, RIG-I, FADD and TRAF3, has changed significantly. The antidepressant-like mechanism of berberine on depression induced by reserpine is related to the RIG-I-like receptor signaling. Berberine could improve RIG-I, FADD and TRAF3 expression and improve the depressed symptom efficiently. RIG-I may become a key target for berberine.

REFERENCES

Bakhtiarpoor M, Setorki M and Kaffashian MR (2018). Effects of essential oil of *Satureja bachtiarica* Bunge in a rat model of reserpine-induced depression. *Iran J. Med. Sci.,* **43**(4): 409-415.

Chen R, Cui Z, Capitao L, Wang G and Harmer C (2020). Precision biomarkers for mood disorders based on brain imaging. *British Med. J.,* **371**(m3618): 1-5.

Chen G and Guo X (2017). Neurobiology of Chinese herbal medicine on major depressive disorder. *Intern. Rev. Neurobiol.,* **135**: 77-95.

Frank JB, Juan CRV, Nancy HM, Alonso OF and Vaccari JPR (2015). RIG-I contributes to the innate immune response after cerebral ischemia. *J. Inflamm.,* **12**: 52.

Fattahian E, Hajhashemi V and Rabbani M (2016). Anti-inflammatory effect of amitriptyline on ulcerative colitis in normal and reserpine-induced depressed rats. *Iran J. Pharm. Res.,* **15**(Suppl): 125-137.

Farooq RK, Asghar K, Kanwal S and Zulqernain A (2017). Role of inflammatory cytokines in depression: Focus on interleukin-1β. *Biomed Rep.* **6**(1):15-20.

Gai XH, Liu SL, Ren T, Liu Y, Jin SU and Tian CW (2018). Research progress on chemical constituents of Coptidis Rhizoma and its pharmacological activities. *Chin Trad Herb Drugs,* **20**(10): 4919-4927.

Gao ZY, Yang P and Huang J (2016). The influence of dizocilpine on the reserpine-induced behavioral and neurobiological changes in rats. *Neurosci. Lett.,* **614**: 89-94.

Gerritsen L, Staufenbiel SM, Penninx B, Van Hemert AM, Noppe G, De Rijke YB and Van Rossum EFC (2019). Long-term glucocorticoid levels measured in hair in patients with depressive and anxiety disorders. *Psychoneuroendocrinology,* **101**: 246-252.

Huang WY and Dong H (2018). Clinical application and modern research progress of *Coptis chinensis* and its compound prescription in the treatment of depression. *Chin J. Hospit. Pharm.,* **38**(13): 102-106.

Jiao JT, Sun J, Ma JF, Dai MC, Huang J, Jiang C, Wang C, Cheng C and Shao JF (2017). Retraction note to: Relationship between inflammatory cytokines and risk of depression and effect of depression on the prognosis of high grade glioma patients.*J.Neurooncol.,* **124**(3): 475-484.

Li L, Yang R, Feng M, Wang YC and Jun YX (2018). Rig-I is involved in inflammation through the IPS-1/TRAF(6) pathway in astrocytes under chemical hypoxia. *Neurosci Lett.,* **672**(13): 46-52.

Liu YM, Niu L, Wang LL, Bai L, Fang XY, Li YC and Yi LT (2017). Berberine attenuates depressive-like behaviors by suppressing neuro-inflammation in stressed mice. *Brain Res. Bull.,* 220.

Miskovic M (2015). Comparison of Tolerance of Venlafaxine, Paroxetine and Amitriptyline in Depression Therapy. *Medical Archives*, **69**(2): 107-109.

Mutsatsa S (2016). A guide to medication adherence in depression. *Brit J. Mental Health Nurs.,* **11**(2): 259-261.

Rosińczuk and A Kołtuniuk (2017). The influence of depression, level of functioning in everyday life and illness acceptance on quality of life in patients with Parkinson's disease: A preliminary study. *Neuropsych. Dis. Treat.*, **13**: 881-887.

Wang Y, Li M, Liang Y, Yang Y and Zhai S (2017). Chinese herbal medicine for the treatment of depression: Applications, efficacies and mechanisms. *Curr. Pharm. Design,* **23**(34): 5180-5190.

Sielaff M, Kuharev J, Bohn T, J Hahlbrock, Bopp T, Tenzer S and Distler U (2017). Evaluation of FASP, SP3 and iST protocols for proteomic sample preparation in the low microgram range. *J. Proteome Res.,* **16**(11): 4060-4072.

Xie ZM, Wang XM, Xu N, Wang J, Pan W, Tang XH, Zhou ZQ, Hashimoto K and Yang JJ (2017). Alterations in the inflammatory cytokines and brain-derived neurotrophic factor contribute to depression-like phenotype after spared nerve injury: Improvement by ketamine. *Sci. Rep.,* **7**(1): 3124.

Singh T, Kaur T and Goel RK (2017). Ferulic Acid supplementation for management of depression in epilepsy. *Neurochem. Res.,* **42**(10): 1-9.

Singh P, Kesharwani RK and Keservani RK (2017). *Protein, Carbohydrates, and Fat*., P.103-115

Pang J, Zou ZR and Xia S (2014). Influence of 8-alkyl-berberine on ethology and neurotransmitter in brain tissue of anxiety model mice. *Chin Trad Herb Drugs.* **45**(20): 2953-2957.

Zhang LY, Jiang J and He M (2017). Research progress on pharmacology of Traditional Chinese Medicine against depression. *Chin. J. Exp. Tradit Med. Formul.,* **23**(24): 224-234.