KXS Balances the Tryptophan Metabolism in Mild to Moderate Depressed Patients and Chronic Restraint Stress Induced Depressive Rats

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Abstract

Objective: Tryptophan metabolism is involved in the etiology and exacerbation of depressive disorders. Kai-Xin-San (KXS) has been widely used to treat depression, but how it regulates depressive-like behavior by shifting the balance of the tryptophan-serotonin metabolism and kynurenine pathway remains vague.

Methods: Ten participants with mild to moderate depression treated with KXS (KXS preparation) were analyzed in this study. Depression rating scale score, the concentration of serum tryptophan, 5-hydroxytryptophan and kynurenine was measured at baseline and the endpoint of KXS treatment. To explore the specific regulatory mechanism of KXS in tryptophan metabolism, chronic restraint stress (CRS) induced depressive rats were conducted and the hippocampus level of tryptophan metabolites and key enzymes were analyzed respectively.

Results: KXS significantly decreased depression rating scale scores and increased the serum tryptophan and kynurenine concentration in depressive patients. Also, it alleviated the depressive behavior in CRS rats obviously. Comparing with CRS group, KXS increased tryptophan, 5-hydroxytryptophan, kynurenine level in hippocampus. Furthermore, KXS decreased the expression of indoleamine 2,3-dioxygenase, increased kynurenic acid by upregulating the expression of kynurenine aminotransferase while decreased quinolinic acid level in hippocampus, which suggested that KXS more favored improving serotonin pathway and neuroprotective kynurenic acid branch in tryptophan metabolism.

Conclusion: This is the first tryptophan metabolomic study of patients with depression undergoing KXS treatment. Combining these clinical results with CRS induced rat model studies, it verified that KXS achieves an excellent antidepressant effect and balances tryptophan-kynurenine metabolic pathways by regulating some key metabolic products and enzymes.

Introduction

Depression is a deleterious mental disorder, ranking the single largest contributor to non-fatal health loss worldwide (7.5% of all Years Lived with Disability)(Baldessarini, Forte et al. 2017, World Health 2017). The current relevant studies range from serotonergic neurotransmitter deficiency hypothesis to immune and inflammation system(van Praag and Korf 1971, Schildkraut 1995, Delgado 2000, Ménard, Hodes et al. 2016). Although monoamine transmitter deficiency in the brain played a vital role for over the past few decades, more than 30% of depressive patients do not response to typical monoaminergic antidepressant treatments(Berlim and Turecki 2007). The urge need for more efficient antidepressant strategies is calling for further exploration in monoamine transmitter mechanism. As the sole precursor of 5-hydroxytryptamine (serotonin), tryptophan has been paid attention in mental disorders research(Shaw, Turner et al. 2001, Wigner, Czarny et al. 2018). The main catabolic route of tryptophan is the kynurenine pathway, which is responsible for approximately 95–99% of tryptophan metabolism in the body. Kynurenine final metabolic product, including neurotoxic quinolinic acid and neuroprotective kynurenic acid, have impacts on central nervous system partly by affecting N-methyl-D-aspartic acid receptor

(NMDAR) function and inducing neural functional disturbance subsequently. Thus, kynurenine pathway is hypothesized to be another key link between tryptophan metabolism and emotional disorders, which may hold promise as a novel treatment target(Réus, Jansen et al. 2015).

KXS, a TCM formula, has been used to treat depression for a considerably long history with unique advantage(Feng, Tang et al. 2016, Fu, Xu et al. 2019). KXS's anti-depressive efficacy and safety in the treatment of mild to moderate depression has been proved in a randomized, double-blind, and positive-drug-controlled clinical trial in recent years(Chen, Hu et al. 2018, Hu, Wang et al. 2021). Pharmacology studies indicate that the therapeutic effects of KXS may be associated with the function of neurotransmitter system, especially 5-HT system. KXS could significantly increase the expression of tryptophan hydroxylase, the key enzyme during the 5-HT synthesis process in the hippocampus and prefrontal cortex of the depressed rats and suppress the expression of 5-HT transporter(Dong, Li et al. 2013). A metabolic study of Alzheimer rats indicated that KXS exerted regulatory effects in tryptophan metabolism by reducing indole sulfate, xanthurenic acid, and kynurenic acid(He, Wang et al. 2020). But there is no systematic study elaborates the mechanism of tryptophan-serotonin pathway and kynurenine pathway involved in the anti-depressive effects of KXS.

Here, it is hypothesized that KXS ameliorates depressive-like behavior by modifying tryptophan metabolism. First, we measured the serum concentration of tryptophan as well as 5-hydroxytryptophan and kynurenine in patients with mild to moderate depression treated with KXS. Then, to make the underlying mechanism clearer, we conducted chronic restraint stress (CRS) induced depressive rats and measured the concentrations of tryptophan, 5-hydroxytryptophan, kynurenine, kynurenic acid, quinolinic acid levels and the expressions of indoleamine 2,3-dioxygenase (IDO), kynurenine aminotransferase (KAT), kynurenine 3-monooxygenase (KMO) in rat hippocampus to clarify the metabolic characteristic alternations after KXS treatment.

Materials And Methods

Sample collection

Ten participants with mild to moderate depression treated with KXS and ten participants treated with fluoxetine were recruited randomly among the 156 patients treated in the Department of Psychosomatic Xijing Hospital of the Fourth Military Medical University and the 261 Hospital of the Chinese People's Liberation Army (PLA Mental Health Center)(Hu, Wang et al. 2021). The blood samples collected at baseline and after eight-week treatment were stored at -80°C for further measurement.

The serum parameters of human blood samples were quantified by a targeted ultra-high performance liquid chromatography multiple reaction monitoring mass spectrometry (UHPLC-MRM-MS/MS) approach. An aliquot of each serum sample was precisely weighted and transferred into an Eppendorf tube. Triple-volume of precooled acetonitrile was added to the centrifuge tube. The samples were vortexed for 5 min and then incubated for 30 min at 4°C, followed by centrifugation for 15 min at 12,000 rpm at 4°C. After

that, 300 μL filtered supernatant was transferred to an auto-sampler vial. After vacuum drying, 500 μL methanol solvent was added into each vial for reconstitution. The mobile phase A consists of 1% formic acid and 10mM ammonium acetate in water. The mobile phase B was 0.1% formic acid in acetonitrile. The column temperature was set at 40°C and the injection volume was 2 μL, delivered at the speed of 0.4 mL/min. An AB SCIEX QTRAP 5500 ion trap mass spectrometer equipped with LC system (SHIMADZU 30AD) was used for assay development. Analyst TF 1.7 and Master View 1.0 software were used for data collection and processing. Multi Quant 3.0.2 software was applied for quantification.

Drug preparation

KXS preparation was administrated to patients as KXS pills as previously reported(Hu, Wang et al. 2021). *Ginseng Radix*, *Polygala Radix*, *Acorus Rhizoma* and *Poria* (Lyye Pharmaceutical Co., Ltd., Beijing, China) were mixed at the ratio of 3:3:2:2 and processed as described formerly(Dong, Wang et al. 2020). KXS powder was obtained after soaking, circumfluence extraction and evaporation. 1g yield powder is equivalent to 4.83g total crude herbs.

Chemicals and reagents

L-tryptophan, 5-hydroxy-L-tryptophan and L-kynurenine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (AR grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. Potassium dihydrogen phosphate (AR grade) was purchased from XiLONG SCIENTIFIC. Perchloric acid (AR grade) was obtained from Shanghai Jinlu Chemical Co., Ltd. All solutions were prepared with ultrapure water. IDO ELISA kit and KMO ELISA kit were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd.

Animal ethics evaluation

Male Sprague-Dawley rats (4 weeks old, weighing 100 ± 15 g) purchased from Animal Breeding Center of PLA General Hospital were housed under standard controlled conditions (lights on 08:00–20:00, room temperature 22 ± 1 ℃, humidity 65%) with food and water *ad libitum*. All the procedures were approved by the Animal Experimentation Ethics Committee of PLA General Hospital. Rats were weighted and anesthetized with pentobarbital sodium (40 mg/kg) before sacrifice.

Modeling and drug administration

Rats were screened by sucrose preference test before recruitment. Eligible individuals were kept for follow-up management. The animals were placed individually into a stainless cylindrical tube (5cm in internal diameter and 20cm in length) with adjustable length for 4 hours every day for consecutive 28 days. A groove on the trunk of the tube assured the respiration and excretion of rats. Animals distributed to control group (n = 12) were housed in cages undisturbed. Each cage contains three rats with free access to chow and tap water. The rats were screened by sucrose preference test, tail suspension test and open-field test when the modeling procedure accomplished to assure successful modeling.

The model rats were randomly distributed into CRS group (n = 12) and KXS treated group (n = 12). Rats in CRS group were administrated with distilled water orally and individuals in KXS group were administrated

with KXS at the dose of 365.4 mg/kg/day orally between 8:00 and 9:00 AM daily for consecutive 14 days. Restraint stress was continuously applied during the treatment. Sucrose preference test, tail suspension test and open-field test were carried out once the drug administration phase was accomplished.

Sucrose preference test

The SPT was conducted to assess the anhedonia behavior of rats over a 4-day period. After the foreshadowing procedures finished, two bottles filled with 1% sucrose solution and distilled water separately were presented to animals on the fourth day. The positions of two bottles were exchanged in the middle of the 2-hour duration and then the bottles were removed. The consumption of sucrose solution and distilled water was recorded.

Tail suspension test

The TST was conducted to appraise the desperation extent of rats. Animals were taken into testing room 30 min before the test for adaptation. Experimenters suspended the rats by adhibiting the tails to a metal rack with adhesive tape. Each test last for 5 min and the accumulated immobility time was calculated by behavioral test software automatically.

Open-field test

The OFT was carried out in an open squared arena (100 cm×100 m) to assess the locomotor activity of rats. The open field square was divided into 25 sections (20 cm in length and 20 cm in width). The behavior of animals during 5 min was recorded digitally by tracking software for further analysis. Between each assessment, the floor would be cleaned by 75% ethanol to get rid of olfactory interference. The travelled distance, times of crossing central areas and movement tracking of rats were recorded and calculated automatically.

Tissue preparation

Rats were anesthetized and decapitated quickly after behavioral tests. The hippocampus tissues were stripped off with sterilized pre-cooled tools on ice, placed in cryopreservation tubes and preserved in a -80°C refrigerator for later use. The hippocampus tissue was rinsed with pre-chilled PBS (0.01M, pH = 7.4) to remove residual blood and chopped after weighing. The shredded tissue was mixed with the corresponding volume of PBS at a weight-to-volume ratio of 1:9 and transferred into a glass homogenizer and triturated well on ice. The homogenate was centrifuged at 5000 × g for 10 minutes, and the supernatant was collected for detection.

Tryptophan, 5-hydroxytryptophan and kynurenine determination in rat hippocampus by high performance liquid chromatography

The HPLC system consists of an Agilent 1200 separation module equipped with an Agilent G1322A degasser, an Agilent G1329A Automatic temperature control sampler, an Agilent G1316A column oven, an Agilent G1315B diode array detector. The system was run isocratically using a C18 column (Agilent 5μm,

250 × 4.6 mm). A mobile phase with 0.05mol/L potassium dihydrogen phosphate buffer: methanol (80:20, v/v) was used at a flow rate of 1.0 ml/min at 25°C. Rat hippocampus sample supernatants (20 μL) were autoinjected into the column. The detection was performed at 225 nm wavelength for tryptophan and kynurenine and 278 nm wavelength for 5-hydroxytryptophan.

Indoleamine 2,3-dioxygenase, kynurenine aminotransferase, kynurenine 3-monooxygenase, kynurenic acid and quinolinic acid determination in rat hippocampus by ELISA

With commercial ELISA kits, the level of indoleamine 2,3-dioxygenase (MLBIO, Shanghai, China), kynurenine aminotransferase (MLBIO, Shanghai, China), kynurenine 3-monooxygenase (MLBIO, Shanghai, China), kynurenic acid (MLBIO, Shanghai, China) and quinolinic acid (MLBIO, Shanghai, China) in rat hippocampus supernatants was performed according to manufacturer's instructions. The mean optical density was detected at a wavelength of 450 nm and the content was calculated according to the standard curves and relative regression equations.

Data analysis and statistical methods

All statistical analyses were performed with GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA, USA). Results were reported as mean ± standard deviation. One-way analysis of variance (ANOVA) was employed to compare the differences among groups. A value of $p < 0.05$ was considered as statistically significant.

Results

Effects of KXS on depressive symptoms and tryptophan metabolism

Patients in KXS group showed a statistically significant improvement in HAM-D17 total score and SDS standard score after 8 weeks of treatment (19.20 ± 1.48 *vs*. 6.20 ± 1.32, $p < 0.001$; 60.50 ± 4.95 *vs*. 41 ± 2.91, $p < 0.001$) (Fig. 1a&1b). The reductions in HAM-D17 score at 8 weeks in KXS and FLX groups were comparable, which suggested improvement in the clinical features. Compared to baseline, eight-week treatment with KXS induced increase in serum tryptophan (1401.00 ± 217.60 *vs*. 1103.00 ± 190.20, $p = 0.0149$), and kynurenine (38.78 ± 8.84 *vs*. 30.10 ± 5.72, $p = 0.0352$), but without significant change in 5-hydroxytryptophan serum level (1.98 ± 0.72 *vs*. 2.28 ± 1.23, $p = 0.56$). Fluoxetine group displayed similar results as it increased serum tryptophan and kynurenine concentration (1257.00 ± 239.00 *vs*. 1157.00 ± 246.90, $p = 0.4237$; 37.51 ± 7.97 *vs*. 31.55 ± 8.20, $p = 0.1496$), while decreased serum 5-hydroxytryptophan level (1.14 ± 0.53 *vs*. 1.53 ± 0.66, $p = 0.16$) after an eight-week treatment.

The effects of KXS on depressive-like behavior in rats exposed CRS

Comparing with control group, CRS rats showed lower sucrose consumption (74.56 ± 9.43 *vs*. 91.87 ± 2.92, $p < 0.001$) (Fig. 2c), prolonged TST immobility time (69.92 ± 19.80 *vs*. 28.25 ± 10.52, $p < 0.001$) (Fig. 2d), but less body weight gain (Fig. 2b). CRS induced weakened locomotor activity reflected by less total distance (30864 ± 3753 *vs*. 44606 ± 2251, $p < 0.001$) and central area crossing times in open-field test (Fig. 2e & 2f). KXS reversed all the abnormal behaviors induced by CRS, as the rats showed increased sucrose consumption (89.33 ± 3.43 *vs*.74.56 ± 9.43, $p < 0.01$), increased total moving distance (37721 ± 5503 *vs*. 30864 ± 3753, $p < 0.05$), increased central area crossing times and decreased TST immobility time (50.42 ± 14.02 *vs*. 69.92 ± 19.80, $p = 0.01$), approving that KXS exerted efficacy in anti-depression as it significantly alleviated the anhedonia symptoms.

The regulatory effects of KXS on tryptophan metabolism in CRS rats

The hippocampus concentrations of tryptophan and 5-hydroxytryptophan were detected to profile the changes in tryptophan metabolism. As shown in Fig. 3 (Part 1), CRS rats presented decreased tryptophan level (15.40 ± 3.431 *vs*.23.33 ± 1.643, $p < 0.001$) (Fig. 3a) and decreased 5-hydroxytryptophan level (25.66 ± 2.917 *vs*. 38.12 ± 6.685, $p = 0.0015$) (Fig. 3b). Two-week treatment with KXS significantly increased tryptophan concentration (23.97 ± 3.363 *vs*. 15.40 ± 3.431, $p < 0.001$) and 5-hydroxytryptophan concentration (33.46 ± 6.767 *vs*. 25.66 ± 2.917, $p = 0.0455$).

The effects of KXS on kynurenine pathway

The levels of kynurenine, kynurenic acid, quinolinic acid, IDO, KAT and KMO were detected with ELISA kits. As shown in Fig. 3 (Part 2), comparing with control group, CRS rats displayed decreased kynurenine level (0.656 ± 0.278 *vs*. 1.239 ± 0.343, $p = 0.0272$) (Fig. 3c), decreased kynurenic acid level (1.479 ± 0.157 *vs*. 1.702 ± 0.054, $p = 0.0145$) (Fig. 3d), and upregulated IDO level in hippocampus (245.8 ± 29 *vs*. 166.5 ± 55.31, $p = 0.03$) (Fig. 3d). There was not obvious change in quinolinic acid level (Fig. 3e). We observed a tendency of increase for KMO, but not statistically significant (Fig. 3g). CRS also induced the decrease of KAT concentration in model rat hippocampus (1246 ± 76.48 *vs*. 1337 ± 36.56, $p = 0.0492$) (Fig. 3h). Two-week treatment with KXS increased the level of kynurenine (1.243 ± 0.383 *vs*. 0.656 ± 0.278, $p = 0.0314$) and kynurenic acid (1.737 ± 0.094 *vs*. 1.479 ± 0.157, $p = 0.0052$). The level of quinolinic acid in hippocampus was downregulated after KXS treatment (2.595 ± 0.191 *vs*. 2.852 ± 0.124, $p = 0.0239$). KXS reversed the alternations of IDO and KAT levels (182.6 ± 50.61 *vs*. 245.8 ± 29, $p = 0.0368$; 1334 ± 66.67 *vs*. 1246 ± 76.48, $p = 0.0454$) significantly. There was a trend of decrease in KMO level, but not statistically significant.

Discussion

To our knowledge, this is the first metabolomic study that systematically assessed the tryptophan metabolic abnormalities in the hippocampus in CRS induced rodent depression model combining with human serum analysis. Eight-week treatment with KXS preparation reduce patients' depression scale

scores, alleviate depressive symptoms and regulate the key indicators in tryptophan metabolism simultaneously. Two-week treatment with KXS improves the depressive behaviors of CRS rats, more clearly balances the tryptophan metabolism and the kynurenine pathway.

Tryptophan exerts important biological functions as the sole precursor of serotonin and the substrate for numerous bioactive metabolites with two metabolic pathways, the tryptophan-serotonin metabolism and the kynurenine pathways (Fig. 4). 5-hydroxytryptophan is derived from tryptophan by the catalysis of tryptophan hydroxylase (TPH). As the intermediate in tryptophan-serotonin metabolism, 5-hydroxytryptophan is degraded into serotonin by the catalysis of aromatic L-amino acid decarboxylase (AADC), which is on behalf of the rate-limiting pivotal in serotonin biosynthesis. Thus, disturbed tryptophan availability can influence peripheral serotonin synthesis(Matthes and Bader 2018). Under physiological conditions, only a small proportion of tryptophan was utilized for serotonin synthesis. The majority flux of tryptophan is converted into kynurenine by TDO in liver tissue and IDO in extrahepatic tissue. In kynurenine pathway, kynurenine converting into quinolinic acid and kynurenic acid by different enzymes KMO and KAT have been widely researched in the aspects of their impacts on CNS function(Schwarcz and Du 1991, Heyes 1993). Quinolinic acid is characterized by neurotoxicity by interacting with glutamatergic system and kynurenic acid exerts neuroprotective effects as an antagonist of NMDA receptors(Cervenka, Agudelo et al. 2017).

Many studies have put insight into the alternations of tryptophan metabolites profiles in depression patients. It’s demonstrated that the plasm concentrations of tryptophan and kynurenine are significantly lower in major depressive disorder patients than those in healthy controls(Kuwano, Kato et al. 2018). Metabolic factors of kynurenine play a crucial role in the occurrence and development of major depressive disorder. Kynurenine or tryptophan would be a potential biomarker in diagnosing depression patients and a crucial role in depression remission(Benatti, Blom et al. 2016, Wigner, Czarny et al. 2017, Liu, Ding et al. 2018). A systematic review suggested that treatment-resistant depression (TRD) was also associated with the activation of kynurenine pathway(Wigner, Czarny et al. 2017). Only relatively few studies have investigated the impact of antidepressant treatment on the tryptophan metabolism and kynurenine pathway without entirely consistent conclusions. In a study by Halaris et al.(Halaris, Myint et al. 2015), escitalopram treatment in 20 depressed individuals showed a reduction of kynurenine pathway neurotoxic metabolites as well as in the kynurenine/tryptophan ratio. While interferon-alpha treatment in patients was associated with decreased tryptophan levels and increased levels of kynurenine and kynurenine/tryptophan ratio(Hunt, Macedo et al. 2020). Based on the previously randomized, double-blind, parallel-group study in 156 patients with mild to moderate depression, we investigated the serum changes in tryptophan metabolism in 10 patients, it was found that tryptophan and kynurenine level increased after KXS treatment. The consistent results shown by CRS induced depressive rat model suggested KXS also improved the tryptophan and kynurenine level, without increased IDO. Both preclinical and clinical studies indicated that KXS might favor the tryptophan-serotonin metabolism, by higher 5-HT catalyzed.

Over-activation of the kynurenine pathway can not only result in less tryptophan abundance for serotonin synthesis, but also cause imbalance of neuroactive metabolites like kynurenic acid and quinolinic acid, the dysregulation of which have hence been linked to depressive disorders(Müller and Schwarz 2007, Müller and Schwarz 2008, Chen and Guillemin 2009). Both preclinical and clinical studies have reported that the dysfunction in tryptophan metabolism and kynurenine pathway is likely to be normalized by antidepressant treatment(Ogyu, Kubo et al. 2018). Eskelund et al. found that vortioxetine reduced quinolinic acid levels in various brain regions in both Flinders Sensitive Line rats, a genetic rat model, and lupus mice, a model of increased depression-like behavior associated with inflammation(Eskelund, Li et al. 2017). As a model used to evaluate the efficacy of antidepressants in rodents, chronic unpredictable mild stress (CUMS) induces long-term behavioral disturbances that resemble symptoms of clinical depression. Tryptophan metabolism and kynurenine pathway may have an associated abnormality with changes in kynurenine, kynurenic acid, quinolinic acid, especially metabolized into neurotoxic 3-hydroxycaninuric acid (3-HK, the precursor of quinolinic acid) branch in the cortex and hippocampus under CUMS(Wang, Li et al. 2018, Li, Jiang et al. 2020). We observed the process of depression is accompanied by changes in the concentrations of kynurenine, kynurenic acid and quinolinic acid, and the kynurenine pathway is over-activated by induction of CRS with or without IDO, KAT and KMO in rat experiments.

In recent two decades, studies have demonstrated KXS's pleiotropic anti-depression effects *in vitro* and *in vivo*. Two-week treatment with KXS balanced kynurenine pathway by upregulating the expression of KAT and modulating the level of kynurenic acid and quinolinic acid. A possible explanation of this might be that KXS possesses the ability of modifying the hypothalamic-pituitary-adrenal (HPA) axis(Dang, Sun et al. 2009, Zhang, Wang et al. 2016), regulating the neurogenesis and astrocytes dysfunction(Zhu, Xu et al. 2013, Yan, Hu et al. 2016), impacting the expression of proinflammatory cytokines(Dong, Wang et al. 2017), which might all influences the kynurenine metabolism differently in the periphery and CNS.

Conclusion

Targeting tryptophan metabolism offers a wide range of potential therapeutic options which are particularly applicable for the treatment of depression. The present study has demonstrated that KXS shed light on new targets for the treatment of depressive disorder by modulating the tryptophan metabolism and kynurenine pathway. It helps increase the serotonin synthesis by enhancing the tryptophan-serotonin metabolism, Meanwhile, it reversed the abnormalities of the kynurenine pathway by shifting the degradation of kynurenine into kynurenic acid branch.

Limitations

Although our study provided some proof of KXS's effects on regulating tryptophan metabolism and kynurenine pathway in the treatment of depression, there also have several defects. First, in the clinical trial, it was shown that KXS induced increase in serum tryptophan level but no change in 5-hydroxytryptophan, and fluoxetine treatment induced decrease in 5-hydroxytryptophan level but no

obvious change in tryptophan. In CRS rats, it was displayed that KXS upregulated the level of 5-hydroxytryptophan in hippocampus. This discrepancy may be partially explained by the distribution difference of 5-hydroxytryptophan in peripheral and central. Secondly, the human sample size limited the present study to a certain extend. More evidence is needed to clarify the exact mechanism of KXS's effects on modulating tryptophan metabolism and kynurenine pathway.

Statements And Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Yuan Hu and Yuanbo Wang designed the study; Yuanbo Wang carried out the animal experiments and drafted the manuscript; Xia Li conducted the data analyses; Rui Jing contributed to HPLC analyses. Wenshan Yang and Yichen Wang participated in the experimental design; Chaochen Wang and Lei Yao participated in sample collecting and preparation; Xiaoming Cui was involved in drafts of the manuscript. All author performed the experiments and approved the final manuscript.

Ethics Approval

Experimental protocols relating human subjects have been reviewed and approved by the Ethics Committee of Xijing Hospital of The Fourth Military Medical University of the Chinese People's Liberation Army (Xi'an China, Ethical approval No. YS201505074).

Consent to Participate

All the participants have signed written inform consent.

References

1. Baldessarini, R., A. Forte, V. Selle, K. Sim, L. Tondo, J. Undurraga and G. Vázquez (2017). "Morbidity in Depressive Disorders." Psychotherapy and psychosomatics **86**(2): 65–72.**doi**:**https**://**doi**.**org**/**10**.**1159**/**000448661**
2. Benatti, C., J. M. Blom, G. Rigillo, S. Alboni, F. Zizzi, R. Torta, N. Brunello and F. Tascedda (2016). "Disease-Induced Neuroinflammation and Depression." CNS Neurol Disord Drug Targets **15**(4): 414–433.**doi**:**https**://**doi**.**org**/**10**.**2174**/**1871527315666160321104749**

3. Berlim, M. and G. Turecki (2007). "What is the meaning of treatment resistant/refractory major depression (TRD)? A systematic review of current randomized trials." European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology **17**(11): 696–707.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**euroneuro**.**2007**.**03**.**009**
4. Cervenka, I., L. Z. Agudelo and J. L. Ruas (2017). "Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health." Science **357**(6349).**doi**:**https**://**doi**.**org**/**10**.**1126**/**science**.**aaf9794**
5. Chen, C., Y. Hu, X. Dong, X. Zhou, L. Mu and P. Liu (2018). "Proteomic Analysis of the Antidepressant Effects of Shen-Zhi-Ling in Depressed Patients: Identification of Proteins Associated with Platelet Activation and Lipid Metabolism." Cellular and molecular neurobiology **38**(5): 1123–1135.**doi**:**https**://**doi**.**org**/**10**.**1007**/**s10571**-**018**-**0582**-**9**
6. Chen, Y. and G. J. Guillemin (2009). "Kynurenine pathway metabolites in humans: disease and healthy States." Int J Tryptophan Res **2**: 1–19.**doi**:**https**://**doi**.**org**/**10**.**4137**/**ijtr**.**s2097**
7. Dang, H., L. Sun, X. Liu, B. Peng, Q. Wang, W. Jia, Y. Chen, A. Pan and P. Xiao (2009). "Preventive action of Kai Xin San aqueous extract on depressive-like symptoms and cognition deficit induced by chronic mild stress." Exp Biol Med (Maywood) **234**(7): 785–793.**doi**:**https**://**doi**.**org**/**10**.**3181**/**0812**-**rm**-**354**
8. Delgado, P. L. (2000). "Depression: the case for a monoamine deficiency." J Clin Psychiatry **61 Suppl 6**: 7–11.**doi**:
9. Dong, X. Z., Z. L. Li, X. L. Zheng, L. H. Mu, G. Q. Zhang and P. Liu (2013). "A representative prescription for emotional disease, Ding-Zhi-Xiao-Wan restores 5-HT system deficit through interfering the synthesis and transshipment in chronic mild stress-induced depressive rats." J Ethnopharmacol **150**(3): 1053–1061.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**jep**.**2013**.**10**.**018**
10. Dong, X. Z., D. X. Wang, Y. P. Lu, S. Yuan, P. Liu and Y. Hu (2017). "Antidepressant effects of Kai-Xin-San in fluoxetine-resistant depression rats." Braz J Med Biol Res **50**(10): e6161.**doi**:**https**://**doi**.**org**/**10**.**1590**/**1414**-**431x20176161**
11. Dong, X. Z., D. X. Wang, T. Y. Zhang, X. Liu, P. Liu and Y. Hu (2020). "Identification of protein targets for the antidepressant effects of Kai-Xin-San in Chinese medicine using isobaric tags for relative and absolute quantitation." Neural Regen Res **15**(2): 302–310.**doi**:**https**://**doi**.**org**/**10**.**4103**/**1673**-**5374**.**265555**
12. Eskelund, A., Y. Li, D. P. Budac, H. K. Müller, M. Gulinello, C. Sanchez and G. Wegener (2017). "Drugs with antidepressant properties affect tryptophan metabolites differently in rodent models with depression-like behavior." J Neurochem **142**(1): 118–131.**doi**:**https**://**doi**.**org**/**10**.**1111**/**jnc**.**14043**
13. Feng, D. D., T. Tang, X. P. Lin, Z. Y. Yang, S. Yang, Z. A. Xia, Y. Wang, P. Zheng, Y. Wang and C. H. Zhang (2016). "Nine traditional Chinese herbal formulas for the treatment of depression: an ethnopharmacology, phytochemistry, and pharmacology review." Neuropsychiatr Dis Treat **12**: 2387–2402.**doi**:**https**://**doi**.**org**/**10**.**2147**/**ndt**.**S114560**
14. Fu, H., Z. Xu, X. L. Zhang and G. Q. Zheng (2019). "Kaixinsan, a Well-Known Chinese Herbal Prescription, for Alzheimer's Disease and Depression: A Preclinical Systematic Review." Front

Neurosci **13**: 1421.**doi**:**https**://**doi**.**org**/**10**.**3389**/**fnins**.**2019**.**01421**

15. Halaris, A., A. M. Myint, V. Savant, E. Meresh, E. Lim, G. Guillemin, D. Hoppensteadt, J. Fareed and J. Sinacore (2015). "Does escitalopram reduce neurotoxicity in major depression?" J Psychiatr Res **66**–**67**: 118–126.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**jpsychires**.**2015**.**04**.**026**
16. He, Y., Y. Wang, S. Liu, Z. Pi, Z. Liu, J. Xing and H. Zhou (2020). "A metabolomic study of the urine of rats with Alzheimer's disease and the efficacy of Ding-Zhi-Xiao-Wan on the afflicted rats." J Sep Sci **43**(8): 1458–1465.**doi**:**https**://**doi**.**org**/**10**.**1002**/**jssc**.**201900944**
17. Heyes, M. P. (1993). "Metabolism and neuropathologic significance of quinolinic acid and kynurenic acid." Biochem Soc Trans **21**(1): 83–89.**doi**:**https**://**doi**.**org**/**10**.**1042**/**bst0210083**
18. Hu, Y., Y. Wang, C. Chen, W. Yang, W. Zhu, Y. Wang and P. Liu (2021). "A randomized, placebo-controlled, double-blind study on the effects of SZL on patients with mild to moderate depressive disorder with comparison to fluoxetine." J Ethnopharmacol **281**: 114549.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**jep**.**2021**.**114549**
19. Hunt, C., E. C. T. Macedo, R. Suchting, C. de Dios, V. A. Cuellar Leal, J. C. Soares, R. Dantzer, A. L. Teixeira and S. Selvaraj (2020). "Effect of immune activation on the kynurenine pathway and depression symptoms - A systematic review and meta-analysis." Neurosci Biobehav Rev **118**: 514–523.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**neubiorev**.**2020**.**08**.**010**
20. Kuwano, N., T. A. Kato, D. Setoyama, M. Sato-Kasai, N. Shimokawa, K. Hayakawa, M. Ohgidani, N. Sagata, H. Kubo, J. Kishimoto, D. Kang and S. Kanba (2018). "Tryptophan-kynurenine and lipid related metabolites as blood biomarkers for first-episode drug-naïve patients with major depressive disorder: An exploratory pilot case-control study." J Affect Disord **231**: 74–82.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**jad**.**2018**.**01**.**014**
21. Li, C. C., N. Jiang, L. Gan, M. J. Zhao, Q. Chang, X. M. Liu and R. L. Pan (2020). "Peripheral and cerebral abnormalities of the tryptophan metabolism in the depression-like rats induced by chronic unpredicted mild stress." Neurochem Int **138**: 104771.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**neuint**.**2020**.**104771**
22. Liu, H., L. Ding, H. Zhang, D. Mellor, H. Wu, D. Zhao, C. Wu, Z. Lin, J. Yuan and D. Peng (2018). "The Metabolic Factor Kynurenic Acid of Kynurenine Pathway Predicts Major Depressive Disorder." Front Psychiatry **9**: 552.**doi**:**https**://**doi**.**org**/**10**.**3389**/**fpsyt**.**2018**.**00552**
23. Matthes, S. and M. Bader (2018). "Peripheral Serotonin Synthesis as a New Drug Target." Trends Pharmacol Sci **39**(6): 560–572.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**tips**.**2018**.**03**.**004**
24. Ménard, C., G. E. Hodes and S. J. Russo (2016). "Pathogenesis of depression: Insights from human and rodent studies." Neuroscience **321**: 138–162.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**neuroscience**.**2015**.**05**.**053**
25. Müller, N. and M. J. Schwarz (2007). "The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression." Mol Psychiatry **12**(11): 988–1000.**doi**:**https**://**doi**.**org**/**10**.**1038**/**sj**.**mp**.**4002006**

26. Müller, N. and M. J. Schwarz (2008). "A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders." Eur Arch Psychiatry Clin Neurosci **258 Suppl 2**: 97–106.**doi**:**https**://**doi**.**org**/**10**.**1007**/**s00406-008-2012-3**

27. Ogyu, K., K. Kubo, Y. Noda, Y. Iwata, S. Tsugawa, Y. Omura, M. Wada, R. Tarumi, E. Plitman, S. Moriguchi, T. Miyazaki, H. Uchida, A. Graff-Guerrero, M. Mimura and S. Nakajima (2018). "Kynurenine pathway in depression: A systematic review and meta-analysis." Neurosci Biobehav Rev **90**: 16–25.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**neubiorev**.**2018**.**03**.**023**

28. Réus, G. Z., K. Jansen, S. Titus, A. F. Carvalho, V. Gabbay and J. Quevedo (2015). "Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: Evidences from animal and human studies." J Psychiatr Res **68**: 316–328.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**jpsychires**.**2015**.**05**.**007**

29. Schildkraut, J. J. (1995). "The catecholamine hypothesis of affective disorders: a review of supporting evidence. 1965." J Neuropsychiatry Clin Neurosci **7**(4): 524–533; discussion 523–524.**doi**:**https**://**doi**.**org**/**10**.**1176**/**jnp**.**7**.**4**.**524**

30. Schwarcz, R. and F. Du (1991). "Quinolinic acid and kynurenic acid in the mammalian brain." Adv Exp Med Biol **294**: 185–199.**doi**:**https**://**doi**.**org**/**10**.**1007**/**978-1-4684-5952-4_17**

31. Shaw, K., J. Turner and C. Del Mar (2001). "Tryptophan and 5-hydroxytryptophan for depression." Cochrane Database Syst Rev(3): Cd003198.**doi**:**https**://**doi**.**org**/**10**.**1002**/**14651858**.**Cd003198**

32. van Praag, H. M. and J. Korf (1971). "A pilot study of some kinetic aspects of the metabolism of 5-hydroxytryptamine in depressive patients." Biol Psychiatry **3**(2): 105–112.**doi**:

33. Wang, J., X. Li, S. He, L. Hu, J. Guo, X. Huang, J. Hu, Y. Qi, B. Chen, D. Shang and Y. Wen (2018). "Regulation of the kynurenine metabolism pathway by Xiaoyao San and the underlying effect in the hippocampus of the depressed rat." J Ethnopharmacol **214**: 13–21.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**jep**.**2017**.**11**.**037**

34. Wigner, P., P. Czarny, P. Galecki and T. Sliwinski (2017). "Oxidative and Nitrosative Stress as Well as the Tryptophan Catabolites Pathway in Depressive Disorders." Psychiatr Danub **29**(4): 394–400.**doi**:**https**://**doi**.**org**/**10**.**24869**/**psyd**.**2017**.**394**

35. Wigner, P., P. Czarny, P. Galecki, K. P. Su and T. Sliwinski (2018). "The molecular aspects of oxidative & nitrosative stress and the tryptophan catabolites pathway (TRYCATs) as potential causes of depression." Psychiatry Res **262**: 566–574.**doi**:**https**://**doi**.**org**/**10**.**1016**/**j**.**psychres**.**2017**.**09**.**045**

36. World Health, O. (2017). Depression and other common mental disorders: global health estimates. Geneva, World Health Organization.

37. Yan, L., Q. Hu, M. S. Mak, J. Lou, S. L. Xu, C. W. Bi, Y. Zhu, H. Wang, T. T. Dong and K. W. Tsim (2016). "A Chinese herbal decoction, reformulated from Kai-Xin-San, relieves the depression-like symptoms in stressed rats and induces neurogenesis in cultured neurons." Sci Rep **6**: 30014.**doi**:**https**://**doi**.**org**/**10**.**1038**/**srep30014**

38. Zhang, J., D. Wang, J. Zhou, L. I. Mao-Xing, Z. P. Jia and R. X. Zhang (2016). "Mechanism and effects of Kaixin Powder, Danggui Shaoyao Powder and Hypericum perforatum L. on the behavior of high

fat rats with chronic stress." China Journal of Traditional Chinese Medicine and Pharmacy.**doi**:

39. Zhu, K. Y., S. L. Xu, R. C. Choi, A. L. Yan, T. T. Dong and K. W. Tsim (2013). "Kai-xin-san, a chinese herbal decoction containing ginseng radix et rhizoma, polygalae radix, acori tatarinowii rhizoma, and poria, stimulates the expression and secretion of neurotrophic factors in cultured astrocytes." Evid Based Complement Alternat Med **2013**: 731385.**doi**:**https**://**doi**.**org**/**10**.**1155**/**2013**/**731385**

Figures

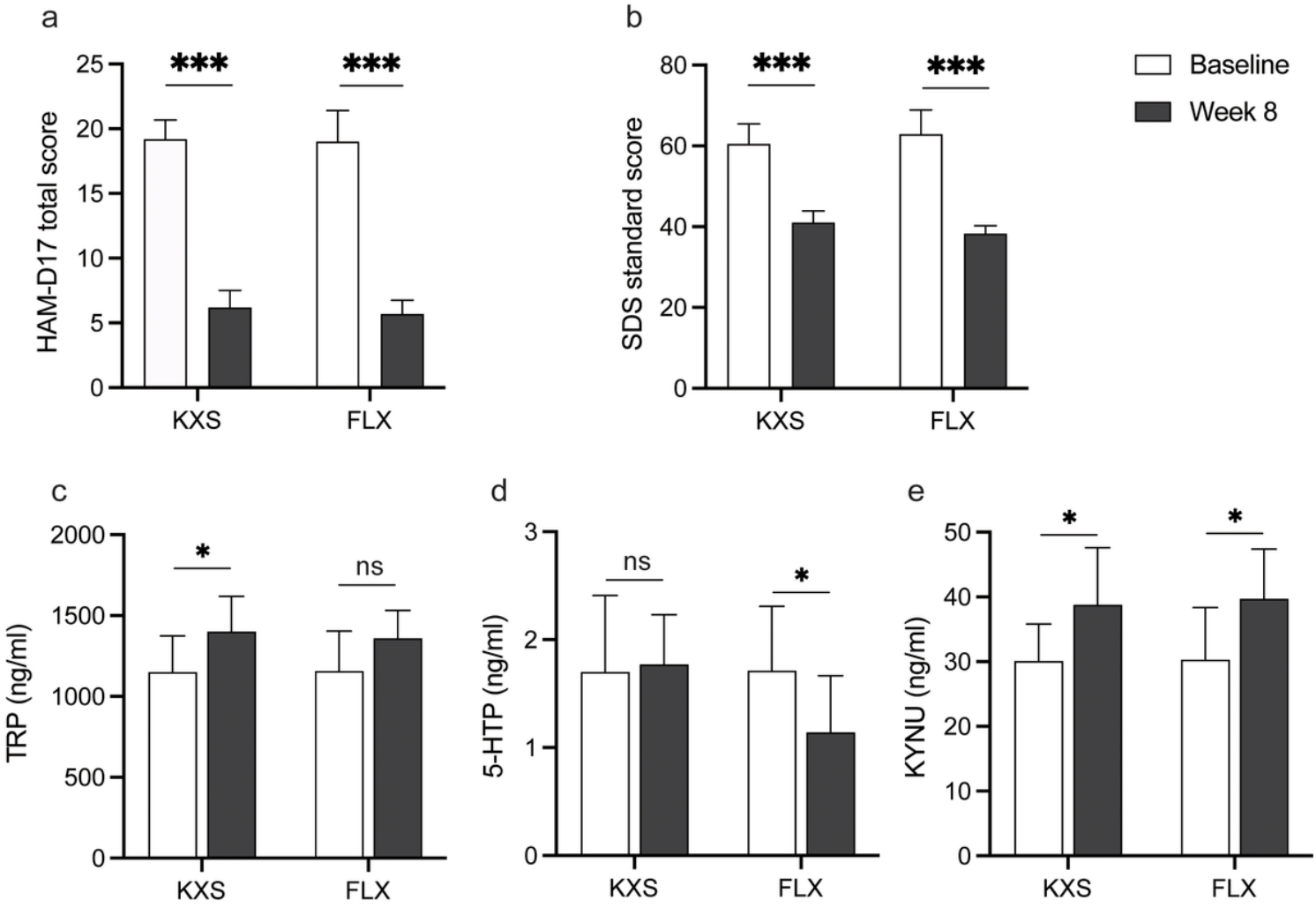

Figure 1

KXS's effects on depressive symptoms and tryptophan metabolism in mild to moderate depressed patients. The HAM-D17 total score (a) and SDS standard score (b) are significantly decreased after 8-week treatment with KXS compared to fluoxetine group. The serum concentration of tryptophan and kynurenine was upregulated after KXS treatment. Data are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = no significance compared to baseline.

Abbreviations: KXS, KXS group; FLX, fluoxetine group; HAM-D17, 17-item Hamilton Depression Rating Scale; SDS, Self-rating Depression Scale; TRP, tryptophan; 5-HTP, 5-hydroxytryptophan; KYNU, kynurenine.

Figure 2

The experimental procedure of present study (a). Body weight gain of rats in three groups (b). The sucrose preference test was displayed as the proportion (%) of sucrose consumed in two hours (c). The tail suspension test was displayed as the total immobility time in 5 min (d). The open-field test was displayed as the total moving distance (e) and the tracking in the open area (f). Data are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to CRS group.

Abbreviations: SPT, sucrose preference test; TST, tail suspension test; OFT, open-field test; CRS, chronic restraint stress.

Figure 3

KXS's effects on tryptophan-serotonin metabolism (Part 1) and kynurenine pathway (Part 2). KXS exerts regulatory effects on the concentration of tryptophan (a), 5-hydroxytryptophan (b), kynurenine (c), kynurenic acid (d), quinolinic acid (e), IDO (f), KMO (g) and KAT (h) in model rats. Data are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = no significance compared to CRS group.

Abbreviations: KYNA, kynurenic acid; QUIN, quinolinic acid; IDO, indoleamine 2,3-dioxygenase; KMO, kynurenine 3-monooxygenase; KAT, kynurenine aminotransferase.

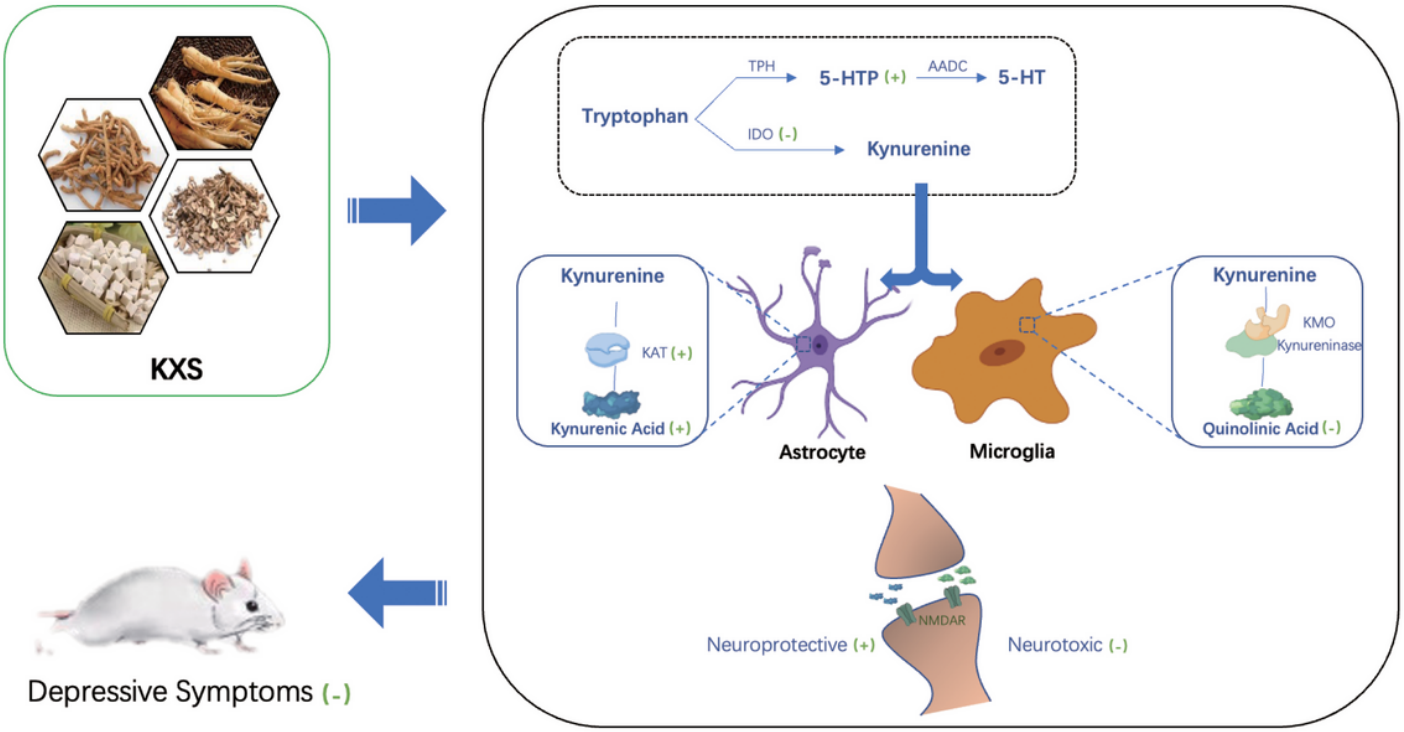

Figure 4

The relationship between tryptophan-serotonin metabolism, kynurenine-pathway and depression and the effects of KXS on tryptophan metabolism in CRS induced depressive rat hippocampus. The depression-like behaviors of CRS rats were obviously ameliorated by KXS treatment. CRS influenced contents of tryptophan, 5-hydroxytryptophan, kynurenine, kynurenic acid, quinolinic acid and expressions of IDO, KAT, KMO. KXS could effectively reverse the irregular changes.

Abbreviations: KXS, KXS preparation; TPH, tryptophan hydroxylase; 5-HT, 5-hydroxytryptamine.