The extract of Acanthopanacis Cortex relieves the depression-like behavior and modulates IL-17 signaling in chronic mild stress-induced depressive mice

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Abstract

Background: Acanthopanacis Cortex (AC) is a valuable Chinese herbal medicine in *Eleutherococcus* Maxim., which has the effects of immune inflammatory modulation, anti-stress, anti-fatigue, sedation and analgesia. The functions of herbs from *Eleutherococcus* Maxim. involve neuroactivity, anti-fatigue, anti-stress and immune inflammatory modulation, and the peripheral functions of which are consistent with AC. However, the central nervous system function of AC has not been clearly illustrated. Since immune inflammatory modulation plays a critical role in the treatment of depression. In this study, we investigated the effect of AC against depression through immune inflammatory modulation.

Methods: Chronic mild stress (CMS)-induced depressive mice were used to evaluate the effect of AC against depression. Behavior study and detection of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines were carried out in the study. Interleukin-17 (IL-17) signaling cascade was involved to further study the underlying mechanism of AC against depression.

Results: AC had a beneficial effect on CMS-induced depressive mice, including improvement in depressive behavior and modulation of the levels of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines. Moreover, AC was able to suppress IL-17-midiated signaling cascade and thereby alleviating neuroinflammation and depression.

Conclusions: Our results revealed that AC showed great effects on anti-depression through immune inflammatory modulation. These findings provide an insight into anti-depression therapy, which will be useful for the development of clinical application of AC.

Introduction

Depression, also known as major depressive disorder, is a serious mental illness characterized by constant feeling of low self-esteem and loss of interest or pleasure. The pathogenesis of depression is complex and involves multiple genetic and environmental factors, leading to poor treatment. Accumulating evidence supports the role of a dysregulated immune system in the pathogenesis of depression [1-3]. Unrestrained immune response results in immunopathology and inflammatory response. The induced immune inflammatory response in brain is manifested as release of pro-inflammatory cytokines from immune cells across the blood brain barrier and activation of neuroinflammation, and consequently affecting neurotransmission, neuronal growth and synaptic plasticity [4]. Amongst all the pro-inflammatory cytokines, interleukin-17 (IL-17) plays an important role in immune and inflammation [5]. Sustained increased level of IL-17 contributes to the impairment of brain blood barrier and activation of astrocytes and microglia, thereby inducing neuroinflammation and depression [6]. IL-17A, the prototypical and founding member of IL-17 family, is induced in the blood of depressed patients and patients who have elevated level of IL-17A have increased risk for depression, while reduced level of IL-17A predicts great anti-depressant effect [7, 8]. Thus, it is likely to develop antidepressant effect through

immune inflammatory modulation; this knowledge will deepen the understandings of preventing and counteracting depression.

Acanthopanacis Cortex (AC, the dried cortex of *Acanthopanar gracilistμlus* W. W. Smith) is a valuable Chinese herbal medicine, which has the effects of immune inflammatory modulation, anti-stress, anti-fatigue, sedation and analgesia [9]. AC belongs to the species of *Eleutherococcus* Maxim., and the chemical composition of AC mainly includes diterpenoids, triterpenoids, phenylpropanoids and lignans, which is similar to other herbs in *Eleutherococcus* Maxim. [10]. The functions of herbs from *Eleutherococcus* Maxim. involve neuroactivity, anti-fatigue, anti-stress and immune inflammatory modulation, and the peripheral functions of which are consistent with AC [11-13]. However, the central nervous system function of AC has not been clearly illustrated. Since herbs from *Eleutherococcus* Maxim. possess similar chemical composition and the majority of which are widely used in brain disease, it is reasonable to suggest that AC might be able to mediate resistance to brain disease, especially depression, through immune inflammatory modulation.

In the study, we aimed to investigate the effect of AC against depression as well as the underlying mechanism. Chemical candidates and their candidate targets in AC were obtained from traditional Chinese medicine systems pharmacology database (TCMSP) and Swiss Target Prediction. Disease enrichment and network pharmacology analysis was carried out to explain the antidepressant effect of AC. Chronic mild stress (CMS)-induced depressive mouse was selected for its similarity with the true state of depressive patients [14]. Thus, the CMS-induced depressive mouse model promises a stable and credible approach in evaluating the effect of AC, and the study on tissue samples from depressive mice indicates the underlying mechanism against depression. We evaluated whether AC had a beneficial effect on CMS-induced depressive mice, including improvement in depressive behavior and modulation of the levels of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines. We hypothesized that IL-17 signaling cascade, which was suppressed by AC, was one of the molecular mechanism for relieving neuroinflammation and depression. Therefore, we investigated whether AC exerted antidepressant-like effects and modulated IL-17 signaling in CMS-induced depressive mice. Our results could accelerate the development of anti-depression therapy, which will be useful for the clinical application of AC.

Methods

Chemicals and reagents

HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Ultra-pure water was processed by a Milli-Q purification system (Millipore, Molsheim, France). The chemical standards of syringin, chlorogenic acid, isochlorogenic acid A, isochlorogenic acid B and kaurenoic acid were purchased from Weikeqi-Biotech Co., Ltd. (Chengdu, China). All the standards have a purity of at least 98% based on HPLC profile. Imipramine was from Yuanye Bio-Technology Co., Ltd. (Shanghai, China) and other reagents were from Sigma-Aldrich (St. Louis, MO).

Chemical candidates in Acanthopanacis Cortex

The chemical candidates in AC were gathered from TCMSP (https://tcmspw.com/tcmsp.php) and relevant literature. The collected chemicals were screened according to SWISS ADME to illustrate the proposed model.

Candidate targets of the chemicals in Acanthopanacis Cortex

The candidate targets of the chemicals in AC were obtained from Swiss Target Prediction (http://www.swisstargetprediction.ch/). After removing duplicate values, a total of 340 candidate targets of AC were collected.

Disease enrichment analysis

Based on candidate targets of the chemicals collected above, disease enrichment analysis based on DisGeNET (https://www.disgenet.org/) was carried out using Enrichr (https://maayanlab.cloud/Enrichr/) [15]. The analysis was tested by *p* value which was computed using Fisher's exact test to assess the deviation from the expected rank.

Known therapeutic targets in the treatment of depression

The known therapeutic targets in depression were acquired from GeneCards database (http://www.genecards.org/). After filtering out low correlative targets (relevance score£5), a total of 1589 targets related to depression were collected (as shown in Additional file 1).

Network construction and analysis

Network construction was made by Cytoscape software (http://www.cytoscape.org/). Compound-target-disease (C-T-D) network was established by connecting the targets with the compounds and depression [16]. GO biological process and KEGG pathway analysis with FDR-adjusted *p*-values < 0.05 were employed and the data were collected by RStudio 1.1.463 for R statistical computing (http://www.rstudio.com/).

Preparation of Acanthopanacis Cortex extract

AC was obtained from Bozhou Market in Anhui China, and was morphologically authenticated by Dr. Min Wei, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences. The corresponding voucher specimens were deposited in Research Center of Medicinal Plants of Institute of Botany, Jiangsu Province and Chinese Academy of Science. The plant materials were tested for quality according to the requirements of Chinese Pharmacopeia (2020 Edition). In preparing the extract of AC, 100 g of the plant materials were minced and soaked in 800 mL of water for 2 h and extracted twice. The extract was combined and spray-dried to obtain the extract of AC. For chemical analysis, the extract was weighed accurately and sonicated in 5 mL of 80% methanol for 45 min. After centrifugation (12,000 rpm at 4 ^{o}C, 5 min), the supernatant was collected before HPLC analysis.

HPLC analysis

Agilent rapid analysis LC 1200 series system (equipped with a degasser, a binary pump, an auto-sampler, a DAD and a thermostated column compartment) was applied. A Waters XBridge C18 column (3.5 μm, 4.6 mm×150 mm) was used for separation. For the quantification of syringin, chlorogenic acid, isochlorogenic acid A and isochlorogenic acid B (277 nm), the mobile phase condition was acetonitrile (A) and 0.1% phosphoric acid in water (B), flow rate of 0.8 mL/min, injection volume of 10 μL, and column temperature was 30 ^{o}C. 0-10 min, linear gradient 86.0-70.0% (B); 10-15 min, linear gradient 70.0-60.0% (B); 15-20 min, linear gradient 60.0-5.0% (B). For the quantification of kaurenoic acid (202 nm), the mobile phase condition was acetonitrile (A) and 0.1% phosphoric acid in water (B), flow rate of 1.0 mL/min, injection volume of 10 μL, and column temperature was 30 ^{o}C. 0-15 min, isocratic gradient 15.0% (B) [17, 18].

Animal experiments

Male C57BL/6J mice (8- to 10-week-old at the start of experiments) were obtained from Changzhou Cavens Laboratory Animal Co. Ltd. (Changzhou, China). Animals were hosted on a 12 h light/dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.) under controlled temperature (22±2 ^{o}C) and humidity (50±10%), with standard diet and water ad libitum. Animals were acclimatized for 7 days. The experimental procedures had been approved by the Animal Experimentation Ethics Committee of China Pharmaceutical University and under the guidelines of “Principles of Laboratory Animal Care” (NIH publication No. 80-23, revised in 1996). All efforts were made to minimize suffering.

The procedures of CMS were conducted with some adjustments. Briefly, a series of stressors were applied onto the animals: (1) water deprivation for 24 h, (2) stroboscopic illumination for 2 h, (3) cage tilt (45°) for 15 h, (4) noise for 2 h, (5) soiled cage (200 mL water in 100 g sawdust bedding) for 15 h, (6) body restraint for 1 h, (7) forced swimming at 8 °C for 6 min, (8) tail-clipping restraint for 6 min, (9) food deprivation for 24 h and (10) day and night reverse. These stressors were randomly arranged in 1 week and repeated for 6 weeks. At the end of CMS procedures, sucrose preference test was carried out to evaluate the CMS model [19].

The mice were randomly divided into five groups (n = 12). The control and CMS model were given with saline. For the other three groups, AC at low dose (50.0 mg/kg/day), high dose (150 mg/kg/day) and imipramine (30 mg/kg/day) were intra-gastrically given 30 min before stress exposure for 6 weeks. The body weight of all mice was recorded every week.

Sucrose preference test was carried out at the end of CMS procedures. In brief, mice in each group were learned to adapt to 2 bottles of 1% sucrose solution (w/v) 72 h before the test, and 24 h later, one bottle of 1% sucrose solution (w/v) was replaced with tap water for 24 h. Then, mice were deprived of water and food for 24 h. Sucrose preference test was conducted at 17:00 p.m., where mice were kept in individual cages with two bottles, one with 100 mL of 1% sucrose solution (w/v) and the other with 100 mL of water. After 3 h, the volumes of consumed sucrose solution and water were recorded and the sucrose

preference was calculated by the following formula: sucrose preference = sucrose consumption/ (water consumption + sucrose consumption) x 100% [20].

Forced swimming test was carried out at the end of CMS procedures. Mice in each group were placed in large glass cylinders (50 cm height and 20 cm diameter) with 30 cm height water at 22±2 °C, so that mice were not able to support themselves by hind limbs. The test consisted of two parts: the first 15 min was for pre-swimming and then 24 h later, the swimming behavior was observed in 5 min, and the latency to float was measured and analyzed [21].

Real-time quantitative PCR

Total RNA was isolated from mouse hippocampus by RNAprep pure Tissue Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The concentrations of RNAs were detected by UV absorbance at 260 nm. cDNA was reverse transcribed from 1 μg samples of total RNA using RT SuperMix for qPCR (Vazyme, Nanjing, China), according to the protocol provided by the manufacturer. Real-time PCR was performed using SYBR Green Master Mix (Vazyme). The SYBR green signal was detected by qTOWER 2.0 (Analytic Jena AG, Germany). Primers used were: 18S-S: TGT GAT GCC CTT AGA TGT CC; 18S-AS: GAT AGT CAA GTT CGA CCG TC; IL-6-S: TAG TCC TTC CTA CCC CAA TTT CC; IL-6-AS: TTG GTC CTT AGC CAC TCC TTC; TNF-α-S: CAG GCG GTG CCT ATG TCT C; TNF-α-AS: CGA TCA CCC CGA AGT TCA GTA G; COX2-S: CGC ATC CTT TAC ATA ACA GAC G; COX2-AS: TAG GAG TTG AAG ATT AGT CCG C; MMP9-S: CAA AGA CCT GAA AAC CTC CAA C; MMP9-AS: GAC TGC TTC TCT CCC ATC ATC.

SDS-PAGE and immunoblotting

The mouse hippocampus was collected, and protein content was determined by Bradford method. Proteins (~20 μg) were separated on 8% SDS-polyacrylamide gels and transferred to a PVDF membrane. The PVDF membrane was blocked with 5% fat-free milk in tris-buffer saline/0.1% tween 20 (TBS-T), and then incubated in the primary antibodies diluted in 2.5% fat-free milk in TBS-T over night at 4 °C. The primary antibodies were: anti-phospho-JNK (Cell Signaling, Banvers, MA), anti-JNK (Cell Signaling), anti-phospho-Erk1/2 (Cell Signaling), anti-Erk1/2 (Cell Signaling), anti-phospho-GSK-3β (Cell Signaling) and anti-GSK-3β (Cell Signaling). After that, the PVDF membrane was rinsed with TBS-T and incubated for 2 h at room temperature in peroxidase (HRP)-conjugated anti-rabbit secondary antibody (Sangon, Shanghai, China), diluted in 2.5% fat-free milk in TBS-T. After intensive washing with TBS-T, the immune complexes were visualized using the enhanced chemiluminescence (ECL) method (Vazyme). The intensities of bands in control and samples, run on the same gel and under strictly standardized ECL conditions, were compared on an image analyzer, using a calibration plot constructed from a parallel gel with serial dilutions of one of the sample.

Measurement of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines

The levels of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines were determined by commercial ELISA kits (Lanpaibio, Shanghai, China; AbFrontier, Korea) according to the manufacturer's

instructions. In brief, the samples were added onto a 96-well plate with coating of anti-mouse serotonin (5-HT)/norepinephrine (NE)/dopamine (DA)/glutamate/nerve growth factor (NGF)/brain-derived neurotrophic factor (BDNF)/glial-cell derived neurotrophic factor (GDNF)/interleukin-1β (IL-1β)/interleukin-6 (IL-6)/IL-17/tumor necrosis factor-α (TNF-α) antibody, individually, and then incubated with HRP-labeled detection antibody at 37 °C for 90 min. After washing five times with PBS, substrate solution was added at 37 °C for another 15 min. At last, stop solution was added to stop the reaction and absorbance of 450 nm was measured immediately. The absorbance of non-specific blinding was taken consideration for sample analysis and each sample in duplicate was employed to minimize inter-assay variation.

Statistical analysis

All data were analyzed using one-way ANOVA or Student's t-test method. Differences with values of $p < 0.05$ were considered significant.

Results

Chemical candidates and targets

As 91 compounds were identified belonging to AC by TCMSP and relevant literature (as shown in Additional file 2), 16 compounds were selected according to oral bioavailability≥15% and drug-likeness≥0.1. Removing 4 compounds (gracilistone A, B, C and linalool) with no target information, 12 compounds with known target information were chosen for the following analysis. Another 13 compounds including caffeoylquinic acids, steroids and eleutherosides reported to have anti-inflammatory, anti-oxidative, anti-fatigue and learning-enhancing effects were added additionally, and finally 25 compounds were analyzed (as shown in Table 1) [22-24]. These 25 identified active compounds interacted with 340 target proteins (as shown in Additional file 3) based on a target fishing technique [25]; that is, on average, each of the compounds interacted with 13.6 target genes, which did fully explain the multiple-target effects of pharmacology by AC.

Disease enrichment analysis

With 340 targets of identified compounds in AC, we conducted disease enrichment analysis, which was sorted by combined score (Fig. 1). We found that 73 genes related to depression, e.g. MAPK8, MAPK1, GSK-3β, IL-6, TNF, were involved in disease enrichment. Under this scenario, it was reasonable that AC might be able to exert anti-depressant effect.

Network construction and analysis

Among the 340 obtained targets and 1589 disease targets, 120 potential targets (as shown in Additional file 4) were associated with depression, and they were reserved for further analysis. Network pharmacology provides a visual approach to understanding the complex relationship between disease and therapeutic spots [26]. In the present study, 120 potential targets and 25 involved compounds were

used to construct the C-T-D network for further cluster analysis (Fig. 2a). All the compounds connected with more than two targets and all of the 120 targets interacted with more than one compound, indicating that the effect of AC on anti-depression was the result of the interaction of multi-components, multi-targets, and multi-pathways.

Through the C-T-D network, a macroscopic visual of the relationship between AC, targets, and depression was obtained, but the underlying mechanism of AC against depression remained unclear. Therefore, 120 potential targets of depression underwent GO biological process and KEGG pathway analysis. GO biological process showed that these targets were enriched to 10 biological process terms, and regulation of inflammatory response and neurotransmitter levels as well as neuron death may indicate the possible mechanism of AC against depression (Fig. 2b). 12 protein targets were mapped to IL-17 signaling pathway (Fig. 2c). Depression is highly associated with immune and inflammation. IL-17 has a plethora of effects that could contribute to depression, including modulation of neuroinflammation, neurotransmission and neuron death, which may suggest the underlying mechanism of AC against depression.

Standardization of herbal extract

The extract of AC was prepared according to ancient preparation of Chinese herbs. The extraction efficiency was about 10.07±1.25% (mean ± SD, n = 3). Five chemicals were chosen to control the quality of the extract: syringin, chlorogenic acid, isochlorogenic acid A, isochlorogenic acid B and kaurenoic acid (Fig. 3). The amount was about 0.12±0.03 for syringin, 6.82±0.46 for chlorogenic acid, 1.52±0.14 for isochlorogenic acid A, 0.34±0.07 for isochlorogenic acid B and 21.53±2.07 for kaurenoic acid in mg/g of dried powder of extract (mean ± SD, n = 3). The established chemical parameters served as the control for repeatability of the below animal study.

Acanthopanacis Cortex relieves the depression-like behavior in CMS-induced depressive mice

Two animal behavior tests including sucrose preference and forced swimming were employed to evaluate the effect of AC against depression in mice. After the treatment of herbal extract for 6 weeks, AC (low dose: 50 mg/kg/day and high dose: 150 mg/kg/day) alleviated sucrose preference of CMS-induced depressive mice (Fig. 4a). In forced swimming test, the CMS-induced depressive mice doubled cumulative immobility time, while AC restored the cumulative immobility time (Fig. 4b). In body weight evaluation, CMS-induced depressive mice showed a decrease of body weight, while AC relieved the body loss (Fig. 4c). Imipramine (30 mg/kg/day) was set as a positive control.

Acanthopanacis Cortex restores the levels of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines in CMS-induced depressive mice

According to previous study, a systematic method was used to evaluate the anti-depressive efficiency of AC. The detected targets included: 5-HT, NE, DA, glutamate, NGF, BDNF, GDNF, IL-1β, IL-6, TNF-α and IL-17. In CMS-induced depressive mouse hippocampus, the amounts of 5-HT, NE and DA were decreased to

~50%, ~30% and ~50%, while the level of glutamate was increased to ~300%, respectively. The treatment of AC (low dose: 50 mg/kg/day and high dose: 150 mg/kg/day) and imipramine (30 mg/kg/day) restored the levels of these neurotransmitters (Fig. 5). The amounts of neurotrophic factors (NGF, BDNF and GDNF) were reduced to ~30%, which were up-regulated under AC and imipramine administration. The levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and IL-17) were increased to ~200%, which were restored by AC and imipramine treatment (Fig. 6). The induced-neuroinflammation could cause neuronal dysfunction, while the modulation of neurotrophic factors and neurotransmitters could prevent neuronal death, and improve neurotransmission and synaptic plasticity. These results indicated that AC might mediate resilience to stress-induced depression through modulating the levels of pro-inflammatory cytokines, neurotransmitters and neurotrophic factors.

Acanthopanacis Cortex regulates the phosphorylation of JNK, Erk1/2 and GSK-3β in CMS-induced depressive mice

For the underlying mechanism of AC against depression, IL-17 signaling molecules based on KEGG pathway analysis (Additional file 5), i.e. c-Jun N-terminal kinase (JNK), extracellular regulated protein kinases 1/2 (Erk1/2) and glycogen synthase kinase 3β (GSK-3β), were involved. The phosphorylation levels of JNK, Erk1/2 and GSK-3β were determined. In CMS-induced depressive mouse hippocampus, the phosphorylation of Erk1/2 and GSK-3β was reduced to ~60%, while the phosphorylation of JNK was increased to ~240%, respectively. The application of AC (low dose: 50 mg/kg/day and high dose: 150 mg/kg/day) and imipramine (30 mg/kg/day) restored the pathological change (Fig. 7). These results suggested that AC was able to suppress IL-17 signaling cascade, which may be one of the molecular mechanism for relieving neuroinflammation and depression.

Acanthopanacis Cortex regulates the expression of IL-6, TNF-α, COX2 and MMP9 in CMS-induced depressive mice

For the underlying mechanism of AC against depression, the downstream targets of IL-17 signaling based on KEGG pathway analysis (Additional file 5), i.e. IL-6, TNF-α, cyclooxygenase 2 (COX2) and matrix metallopeptidase 9 (MMP9), were involved. The mRNA levels of IL-6, TNF-α, COX2 and MMP9 were determined. In CMS-induced depressive mouse hippocampus, the mRNA levels of IL-6, TNF-α and COX2 were increased to ~240%, ~160% and ~350%, respectively. The application of AC (low dose: 50 mg/kg/day and high dose: 150 mg/kg/day) and imipramine (30 mg/kg/day) restored the pathological change (Fig. 8a-c). Furthermore, AC induced the expression of MMP9 (Fig. 8d), which could help tissue remodeling and repair following neuroinflammation. These results were consistent with our previous results that AC could modulate immune inflammatory response to mediate resilience to stress-induced depression through IL-17 signaling cascade.

Discussion

Depression is a serious mental illness which has been considered as one of the most disabling diseases worldwide. The etiology and pathogenesis of depression is complex and current evidence suggests that

immune and inflammation plays a critical role in occurrence and progression of depression [27]. IL-17 family is one kind of the key factors that modulate immune and inflammation [28]. In the brain, most evidence points to the pathogenic role for IL-17. IL-17 impairs the blood brain barrier integrity, activates CNS-resident cells causing hyperexcitability of neurons [29] and induces the production of pro-inflammatory cytokines and enzymes by astrocytes and microglial exacerbating neuroinflammation and neuronal dysfunction. Current studies indicate that elevated IL-17A level, the prototypical and founding member of IL-17 family, is observed in the blood of depressed patients and patients who have elevated levels of IL-17A have increased risk for depression and anxiety disorders [30]. Moreover, administration of IL-17A promotes depressive-like behaviors [31]. Thus, finding ways to modulate immune inflammatory response to mediate resilience to depression through IL-17 signaling cascade is of great importance.

AC, a valuable Chinese herbal medicine, has been used to treat autoimmune disease, fatigue and weakness for years. In China, Acanthopanax gracilistylus wine, which is made of AC in liquor, is considered as a health supplement product to treat rheumatic arthritis for its clinical application [32]. As a candidate therapy, AC particularly exerts anti-inflammation and hepatoprotection, showing potential for immune inflammatory modulation [33]. However, current study on pharmacological activity of AC mainly focuses on peripheral regulation, and the effect of AC on central nervous system remains to be unknown.

In the study, the effect of AC on central nervous system, especially depression, was investigated. AC had a plethora of effects against depression, including improvement in depressive behavior and modulation of the levels of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines. It is interesting that AC was able to suppress IL-17 signaling cascade and neuroinflammation in depressive mouse. The results were consistent with disease enrich and network pharmacology analysis that AC modulated immune inflammatory response to mediate resilience to depression through IL-17 signaling cascade. Furthermore, much more effort is required to elucidate the underlying mechanism of AC on IL-17 signaling as well as the chemical components responsible for immune inflammatory modulation.

Considering the major ingredients in AC should be crucial for anti-depression and immune inflammatory modulation, the majority of the identified 25 chemical components show various biological activities experimentally. For example, caffeoylquinic acids are experimentally identified which have anti-oxidative, anti-inflammatory and anti-depressant effects [34]. Syringin is reported to have immunomodulatory and sleep-potentiating effect in mice and human beings [35]. Chlorogenic acids possess various bioactivities, including anti-oxidation, immune inflammatory modulation, anti-depression, hepatoprotection and cardioprotection [36]. Kaurenoic acid is able to regulate inflammation and hepatic lipogenesis, and suppress SREBP-1c [37]. Anti-inflammatory effect of acanthoic acid is proved through regulation of LKB1/Sirt1/AMPK/ACC and LXRs pathway [38]. Therefore, it is reasonable that AC could be used as a new regimen for anti-depression for the chemical components could exert robust effect against depression through multi-targets, and further investigation may be carried out to determine whether the combination of these components could eliminate toxicity or side/adverse effects.

Conclusions

In this study, the anti-depressant effect of AC was investigated. AC had a beneficial effect on CMS-induced depressive mice, including improvement in depressive behavior and modulation of the levels of neurotransmitters, neurotrophic factors and pro-inflammatory cytokines. Moreover, AC was able to suppress IL-17 signaling cascade and thereby inhibiting neuroinflammation. These findings provide an insight into anti-depression therapy, which will be useful for the development of clinical application of AC; further research should focus on validating the active components of AC in cell and animal models.

Abbreviations

AC: Acanthopanacis Cortex; CMS: chronic mild stress; C-T-D: compound-target-disease; NGF: nerve growth factor; BDNF: brain-derived neurotrophic factor; GDNF: glial-cell derived neurotrophic factor; IL-1β: interleukin-1β; IL-6: interleukin-6; IL-17: interleukin-17; TNF: tumor necrosis factor; COX2: cyclooxygenase 2; MMP9: matrix metallopeptidase 9; GSK-3β: glycogen synthase kinase-3β; Erk1/2: extracellular signal-regulated kinase 1/2; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; TBS-T: tris-buffer saline/0.1% tween 20; HPR: peroxidase; ECL: enhanced chemiluminescence; TCMSP: traditional Chinese medicine systems pharmacology database; ANOVA: analysis of variance; FDR: false discovery rate.

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Experimentation Ethics Committee of China Pharmaceutical University and under the guidelines of “Principles of Laboratory Animal Care” (NIH publication No. 80-23, revised in 1996). The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

LY and MW designed research; LY and YQ conducted research; CL and PS collected data; ZL and TW analyzed data; LY wrote the paper; LY and MW revised the manuscript. All authors read and approved the final manuscript.

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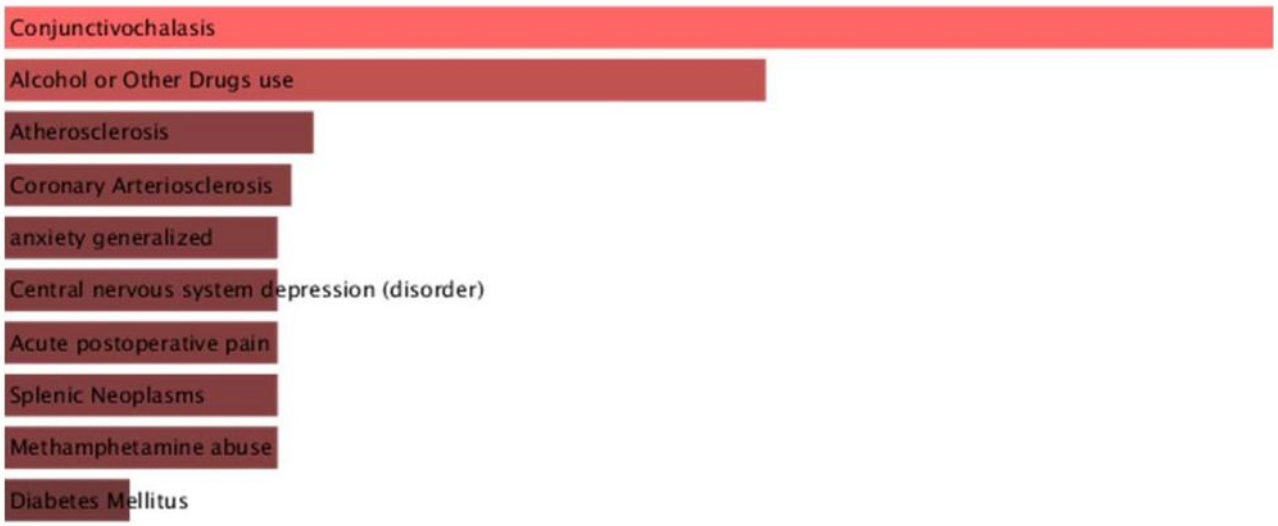
Table

Due to technical limitations Table 1 is available as a download in the Supplementary Files.

Figures



Index	Name	P-value	Adjusted P-value	Odds Ratio	Combined Score
1	Conjunctivochalasis	2.816e-8	2.019e-7	146.70	2550.48
2	Alcohol or other drugs use	1.081e-15	1.930e-14	59.90	2064.29
3	Atherosclerosis	1.677e-67	2.843e-64	10.60	1629.89
4	Coronary arteriosclerosis	6.713e-60	4.554e-57	11.81	1608.75
5	Methamphetamine abuse	0.000001198	0.000006745	117.01	1595.42
6	Splenic neoplasms	0.000001198	0.000006745	117.01	1595.42
7	Acute postoperative pain	0.000001198	0.000006745	117.01	1595.42
8	Central nervous system depression (disorder)	0.000001198	0.000006745	117.01	1595.42
9	Anxiety generalized	0.000001198	0.000006745	117.01	1595.42
10	Diabetes mellitus	4.046e-68	1.372e-64	9.37	1453.60

Figure 1

Disease enrichment analysis of AC. With 340 targets of the 25 identified chemicals in AC, disease enrichment analysis based on DisGeNET was carried out using Enrichr. The analysis was tested by p value which was computed using Fisher's exact test.

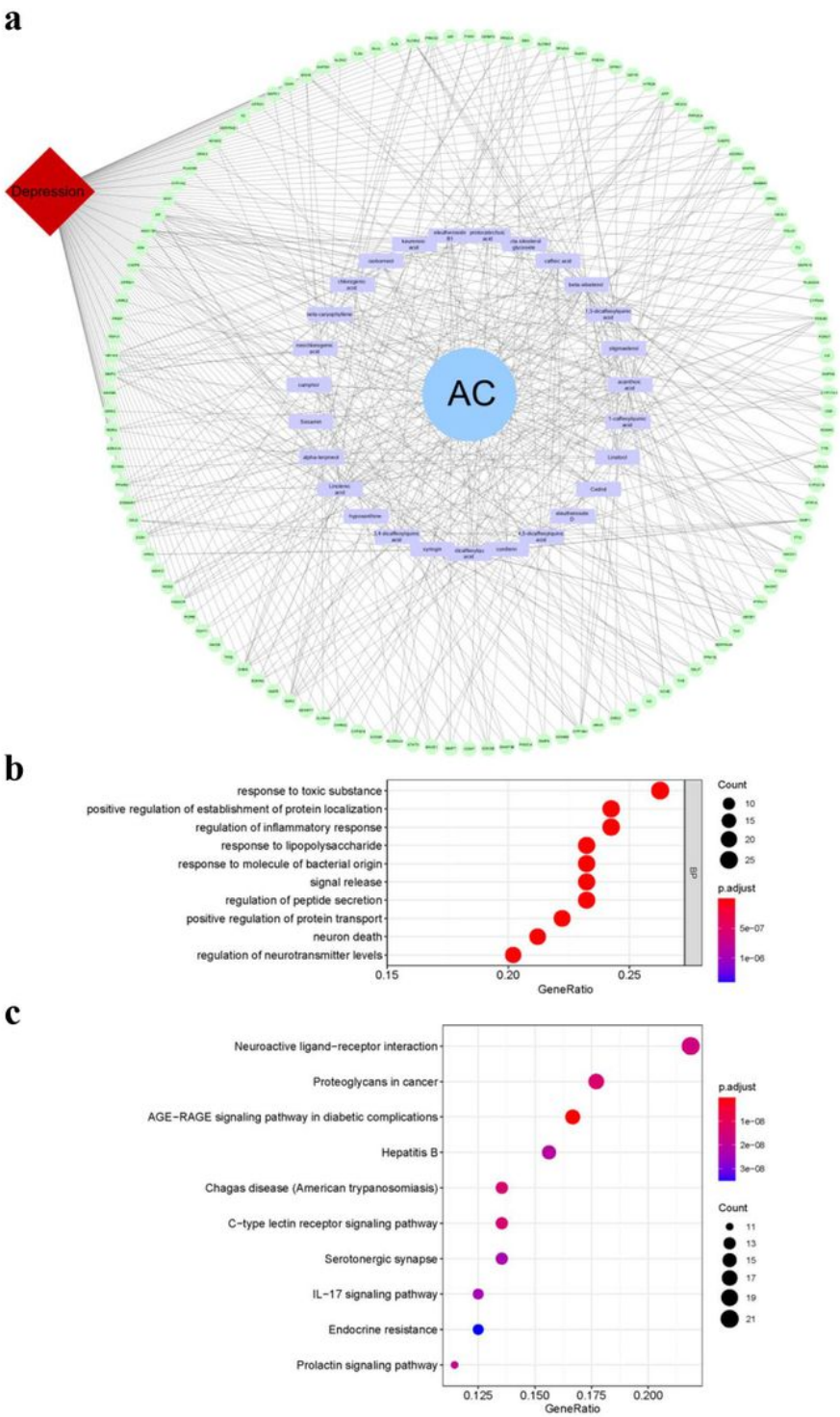


Figure 2

Network analysis of AC. C-T-D network a was established by connecting the targets with the compounds and depression. GO biological process b and KEGG pathway analysis c were performed by RStudio 1.1.463 for R statistical computing, FDR-adjusted p-values < 0.05.

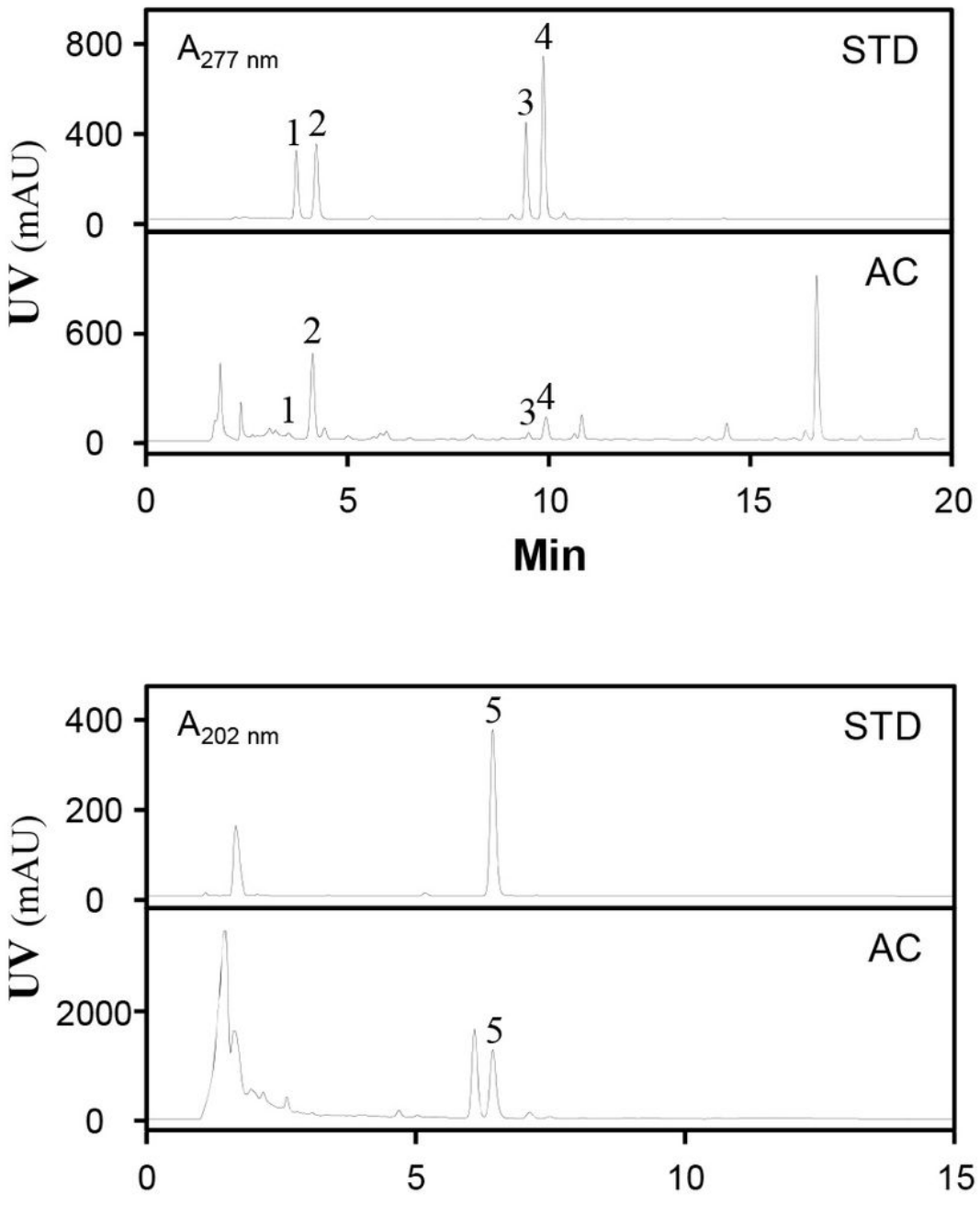


Figure 3

HPLC chromatogram of AC extract. The chromatographic method was described in method session. Syringing (1, 277 nm), chlorogenic acid (2, 277 nm), isochlorogenic acid A (4, 277 nm), isochlorogenic acid B (3, 277 nm) and kaurenoic acid (5, 202 nm) was made by a HPLC couple with a DAD detector. The detected wavelength was indicated. Representative chromatograms are shown, n = 3.

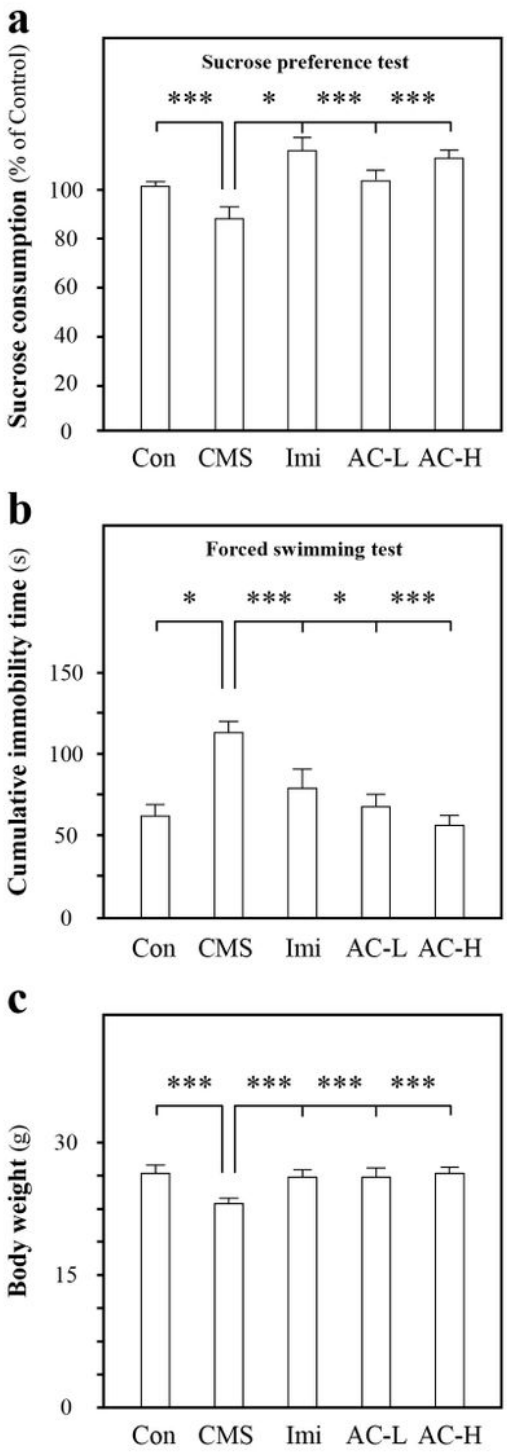


Figure 4

AC relieves the depression-like behavior in CMS-induced depressive mice. The CMS-induced depressive mice were randomly divided into five groups: control (Con), CMS, imipramine (Imi, 30 mg/kg/day), AC low dose (AC-L, 50 mg/kg/day) and AC high dose (AC-H, 150 mg/kg/day). After drug administration, sucrose preference a, forced swimming tests b, body weight c were carried out, as described in the method

session. Data are expressed as mean ± SEM, where n = 8, *p < 0.05, **p < 0.01, ***p < 0.001 compared with CMS.

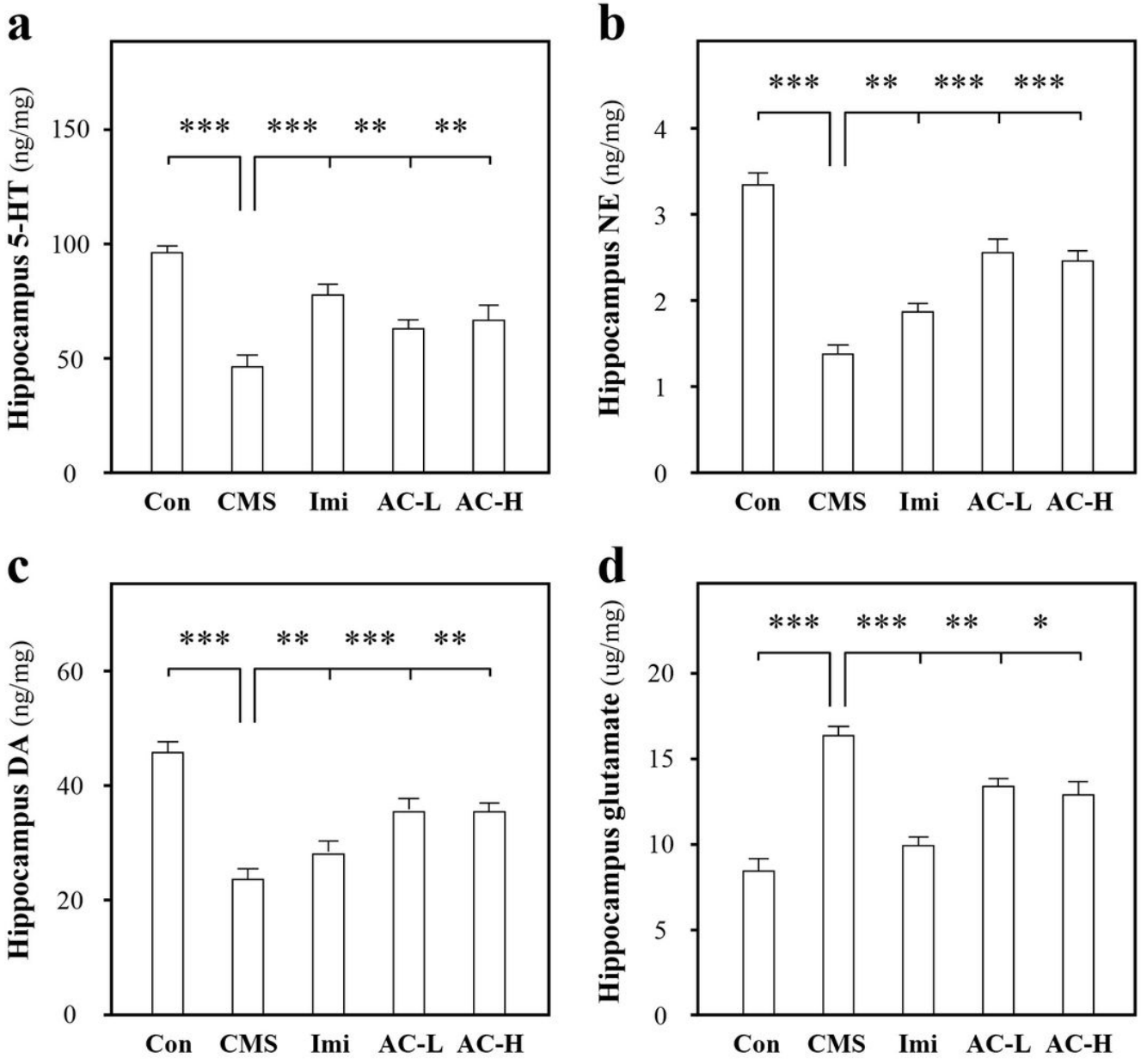


Figure 5

AC restores the levels of neurotransmitters in CMS-induced depressive mice. The CMS-induced depressive mice were randomly divided into five groups as described above. After drug administration, the hippocampus was collected. The amounts of 5-HT a, NE b, DA c and glutamate d in the extracts of hippocampus were detected using ELISA kits. Data are expressed in ng/mg or μg/mg, mean ± SEM, n = 7, *p < 0.05, **p < 0.01, ***p < 0.001 compared with CMS.

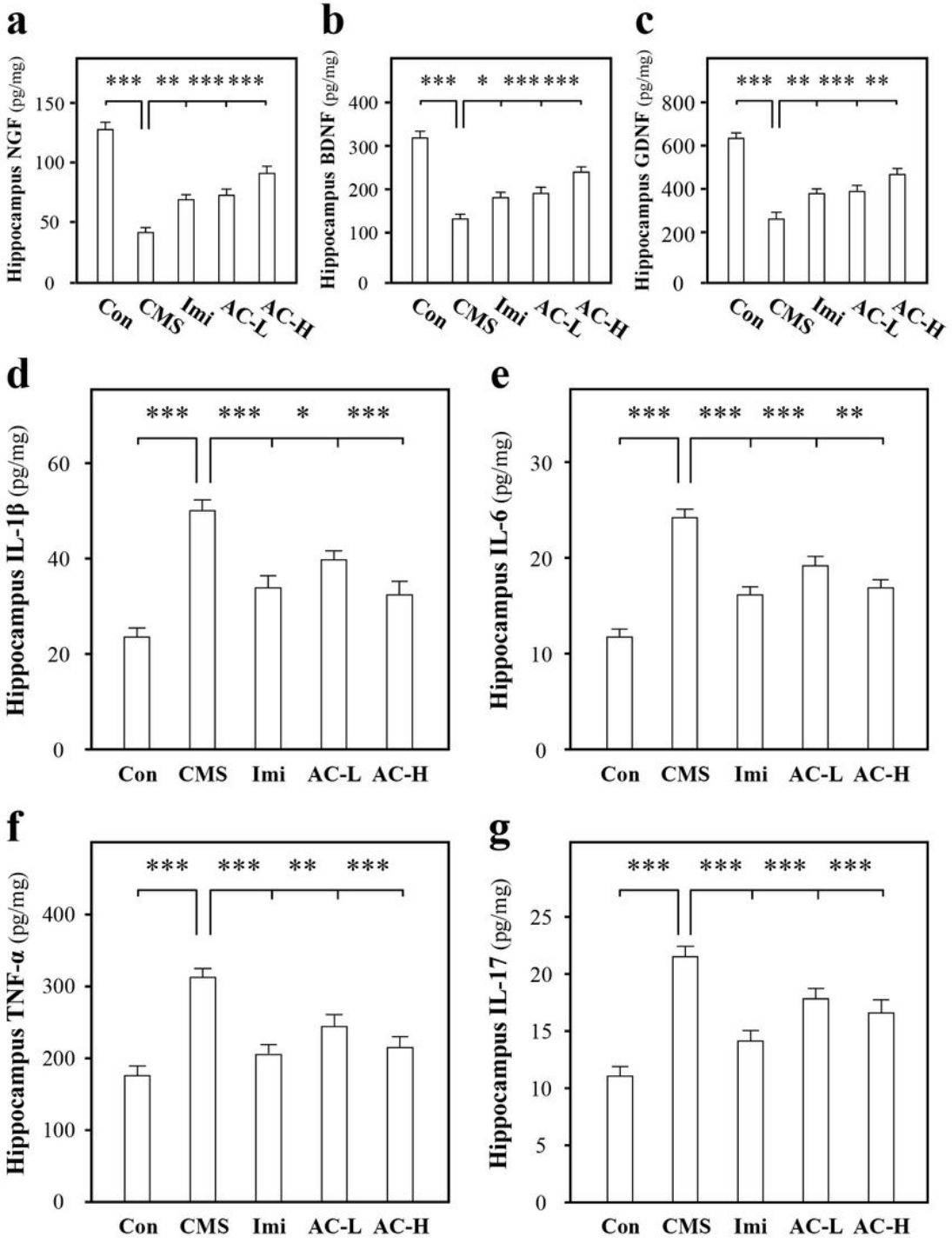


Figure 6

AC restores the levels of neurotrophic factors and pro-inflammatory cytokines in CMS-induced depressive mice. The CMS-induced depressive mice were randomly divided into five groups as described above. After drug administration, the hippocampus was collected. The amounts of NGF a, BDNF b, GDNF c, IL-1β d, IL-6 e, TNF-α f and IL-17 g in the extracts of hippocampus were detected using ELISA kits. Data are expressed in pg/mg, mean ± SEM, n = 7, *p < 0.05, **p < 0.01, ***p < 0.001 compared with CMS.

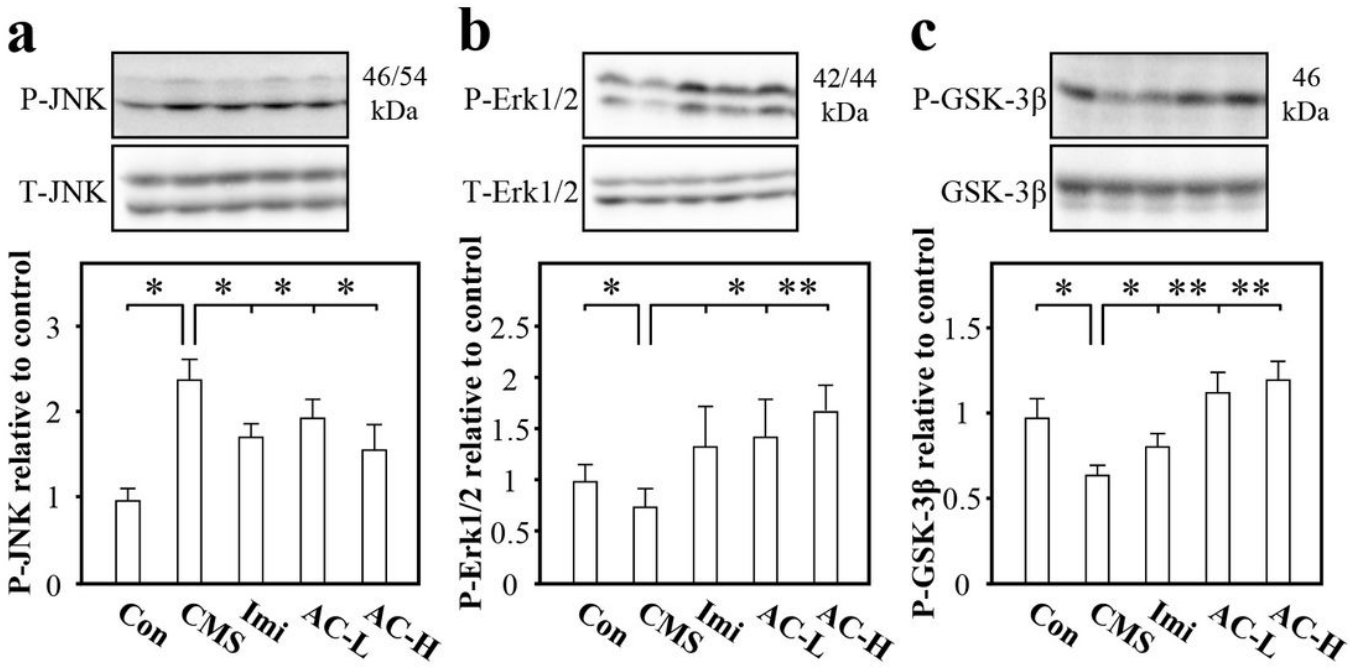


Figure 7

AC regulates the phosphorylation of JNK, Erk1/2 and GSK-3β in CMS-induced depressive mice. The CMS-induced depressive mice were randomly divided into five groups as described above. After drug administration, the hippocampus was collected. The phosphorylation of JNK a, Erk1/2 b and GSK-3β c were revealed by using specific antibodies. Data are expressed as fold of control, and in mean ± SEM, n = 4, *p < 0.05, **p < 0.01 compared with CMS.

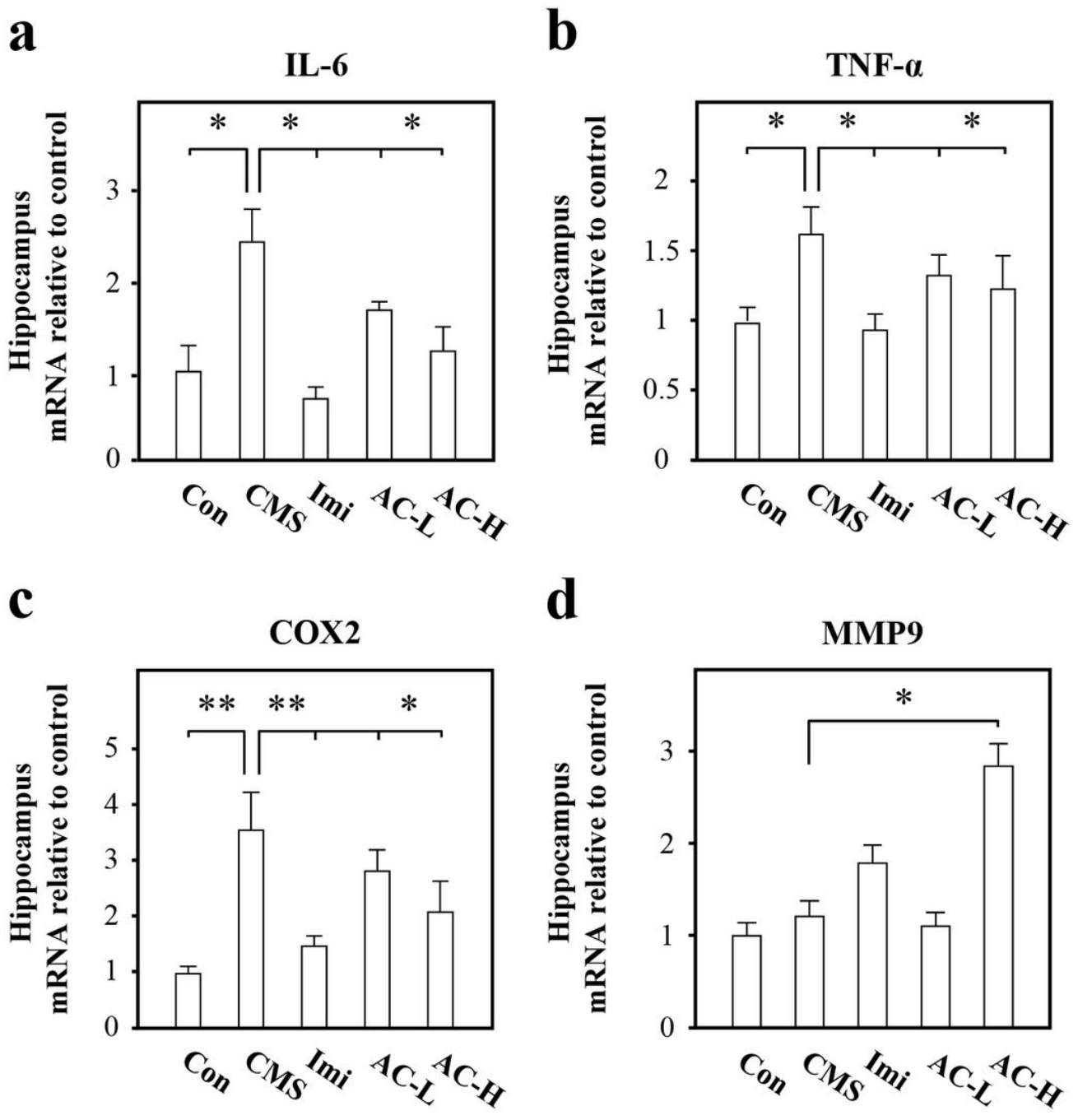


Figure 8

AC regulates the expression of IL-6, TNF-α, COX2 and MMP9 in CMS-induced depressive mice. The CMS-induced depressive mice were randomly divided into five groups as described above. After drug administration, the hippocampus was collected. The mRNA amount of IL-6 a, TNF-α b, COX2 c and MMP9 d were determined. Data are expressed as fold of control, and in mean ± SEM, n = 4, *p < 0.05, **p < 0.01 compared with CMS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Additionalfile1.docx
- Additionalfile2.docx
- Additionalfile3.docx
- Additonalfile4.docx
- Additonalfile5.docx