Mechanistic insights into the anti-depressant effect of curcumin based on network pharmacology and experimental validation

Jing Guo
Jianghan University
Meng Fang
Jianghan University
Zhe Xiong
Jianghan University
Ke Zhou
University of South China
Peng Zeng (zengp@hust.edu.cn)
University of South China

Research Article

Keywords: Depression, Curcumin, LPS, Network pharmacology, PI3K-Akt signaling pathway, Neuroinflammation

Posted Date: January 3rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2412991/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Naunyn-Schmiedeberg's Archives of Pharmacology on July 25th, 2023. See the published version at https://doi.org/10.1007/s00210-023-02628-w.

Abstract

Growing evidence supports the involvement of neuroinflammation in the pathophysiology of depression. Administrating curcumin could revert the depressive-like symptoms and weakened microglial activation and increased the level of pro-inflammatory cytokine. This study aimed to identify potential anti-depression targets and mechanisms of curcumin (CUR) by an approach of network pharmacology. GSEA and KEGG pathways showed the most significantly enriched pathway of CUR against depression was the PI3K-Akt pathway. Moreover, 52 targets were significantly correlated with PI3K-Akt signaling pathway and CUR-related targets. In addition, among these top 50 targets which were ranked by degree in the PPI network, there were 23 targets involved in the 52 intersection targets. Thus, our findings suggest that CUR exerts its anti-depression effects through PI3K-Akt signaling pathway. Furthermore, we investigated the anti-depression effects of CUR using a mouse model of depression induced by lipopolysaccharide (LPS). Administration of LPS alone (2 mg/kg/day, i.p.) extended the immobility time in the open filed test (OFT) and tail suspension test (TST), decreased sucrose consumption in the sucrose preference test (SPT). Pretreatment with CUR (50 mg/kg/day, i.p.) for 7 consecutive days relieved LPS-induced changes in the behavior tests, the activity of PI3K-Akt signaling pathway, neuronal damage in the PFC and inflammatory response. Moreover, inhibition of the PI3K-Akt signaling pathway by LY294002 (7.5 mg/kg/day, i.p.) blocks the therapeutic effects of CUR. In conclusion, our study indicate that CUR may be an effective antidepressant agent for LPS-induced mouse model, in part because of its anti-inflammatory actin through PI3K-Akt signaling pathway.

Introduction

Depression (also known as major depressive disorder, MDD) is the main cause of mental disorder that endangers the health of people worldwide, affect approximately 300 million persons globally as estimated by the World Health Organization (Charlson et al., 2019, Herrman et al., 2019). In China, depression is the second leading cause of long-term disability (Lu et al., 2021). Depression lacks effective treatments, with current antidepressants ineffective in 40% of patients (2018). The clinical treatment of depression is mainly based on chemical synthetic drugs, which have therapeutic effects but accompanied with adverse reactions, disadvantages of narrow antidepressant spectrum and a high recurrence rate (Du et al., 2019, Jiang et al., 2019). For example, the selective serotonin reuptake inhibitor fluoxetine has serious side effects, causing acute nausea, headache, sexual dysfunction and weight gain (Gerhard et al., 2016). In recent years, a series of active ingredients with antidepressant effect have been identified from traditional Chinese medicine or traditional Chinese medicine compound (Wang et al., 2019). Therefore, the development of high-efficiency, low-toxicity, and well-defined antidepressant traditional Chinese medicine products has become a major idea and direction for the treatment of depression.

Curcumin (CUR), as a yellow-orange bioactive ingredient extracted from the rhizome of turmeric. 6–10 months old turmeric rhizome contained > 5% w/w of CUR (Pantharos et al., 2022). As a highly lipophilic compound, CUR can cross the blood-brain barrier (BBB). Mice chronically fed CUR (2.5–10 mg/day for 4

months) had intracerebral CUR concentrations of 0.5 μg/g following oral administration(Begum et al., 2008). CUR exhibits a wide range of positive pharmacological activities, including anti-oxidant, anti-inflammation and anti-cancer (Wei et al., 2019, Ji et al., 2020). A growing number of studies suggest CUR has therapeutic effects on multiple brain disorders, especially in the treatment of MDD (Fusar-Poli et al., 2020), Alzheimer's disease (Zhang et al., 2010), Parkinson's disease (Siddique et al., 2013) and Huntington's disease (Sandhir et al., 2014). CUR ameliorates pain and depression through altering the serotonin and monoamine level, inhibiting monoamine oxidase, inhibiting glutamate release from the prefrontal cortex (PFC) (Kulkarni et al., 2008, Wang et al., 2008, Lin et al., 2011) and increasing brain-derived neurotrophic factor (BDNF) levels in the amygdala region (Zhang et al., 2012). Multiple clinical trials in adults with depression also found CUR (500 to 1500 mg/day) to have positive antidepressant effects (Lopresti, 2022). A meta-analysis also indicates that adding CUR to standard of care improved symptoms of depression and anxiety in people with depression (Fusar-Poli et al., 2020). Toxicity assessment is an important issue in drug development. CUR is generally thought to be a very safe drug with practically no toxicity. In preclinical systematic safety evaluation, no toxic effects were found in rats, dogs and monkeys administered at a dose of 3.5 g/kg CUR for 3 months (Sharma et al., 2007). In human trials, CUR was well tolerated and safe, and the frequency of adverse reactions was similar to placebo administration (Hewlings and Kalman, 2017).

Inflammation and depression are closely related, and interleukin-1 (IL-1), IL-6 and tumour necrosis factor-alpha (TNF-α) have been used as biomarkers in depression. As an effective activator of the inflammatory response (O'Connor et al., 2009), LPS-induced depression mouse model has been repeatedly shown to be an effective and predictive animal depression model (Henry et al., 2008, Adzic et al., 2015). The PI3K-Akt signaling pathway regulates and controls multiple cellular functions including proliferation, growth, and survival. Regarding inflammatory mediators, the PI3K-Akt pathway is directly associated with several inflammatory diseases (Chen et al., 2016). In depression, the PI3K-Akt signaling pathway plays a key role in glutamate uptake, glutamate receptor transport, and synaptic neurotransmission (Guillet et al., 2005, Budni et al., 2012, Shi et al., 2012). Animal studies have also indicated that the PI3K-Akt pathway is associated with depression (Ma et al., 2018, Zeng et al., 2021). Although CUR has multiple potential antidepressant mechanisms, it is still unclear whether the PI3K-Akt signaling pathway is the mechanism of its antidepressant effect. Therefore, the purpose of this study aimed to study the role of PI3K-Akt signaling pathway in the therapeutic effect of CUR against depression.

The PFC has become one of the most damaged region in depression (Pizzagalli and Roberts, 2022). In this study, we used the LPS-induced classical depression model to investigate antidepressant mechanism of CUR. The depression-like behaviors of mice were assessed by OFT, SPT and TST. Then, the molecular mechanism of CUR against depression was revealed through the systems pharmacology approach. We found that CUR exerts antidepressant effects through the PI3K-Akt signaling pathway and improves PFC neuronal damage and serum inflammatory response. These results revealed an important mechanism for the antidepressant effect of CUR, which may facilitate its clinical translation.

Materials And Methods

Collection of CUR-related target and pharmacological parameters evaluation

CUR-related targets were collected from the HERB database (http://herb.ac.cn/) (Fang et al., 2021) and PharmMapper server (http://lilab-ecust.cn/pharmmapper/index.html) (Liu et al., 2010). It should be noted that the related targets of CUR from HERB database were gathering from curated references, while targets from the PharmMapper server were drug target identification based on pharmacophore mapping method. AD. In addition, protein classification was performed using the Panther classification system (http://pantherdb.org/)(Mi et al., 2007).

Physicochemical and ADMET studies showed that the compounds that were most compliant with Lipinski's Rule of Five (RO5) could penetrate the BBB to high degrees and exhibited good oral bioavailability (Mohd Faudzi et al., 2020). Here, the SwissADME web service (http://www.swissadme.ch) (Huang et al., 2020) was used to evaluate the RO5 of the CUR. The toxicological parameters were calculated for CUR using the Protox II webserver (https://tox-new.charite.de/protox_II/) (Banerjee et al., 2018).

Gene Set Enrichment Analysis (Gsea)

GSE98793 dataset (Leday et al., 2018), the whole blood samples included 64 control healthy and 128 MDD samples, was collected from the Gene Expression Omnibus (GEO) database and annotated in the R software (R Foundation for Statistical Computing, Vienna, Austria) through annotation files. GSEA was used for enrichment analysis of MDD-related genes *via* the ClusterProfiler R package (version 3.12.0) (Yu et al., 2012), and a p value < 0.05 were considered significantly enriched. For GSEA data, the normalized enrichment score (NES) and *p* value were specified.

Kyoto Encyclopedia Of Genes And Genomes (Kegg) Pathway Enrichment Analysis

We performed gene ontology (GO) and KEGG pathway analyses using the ClusterProfiler R package (Yu et al., 2012) at Benjamini–Hochberg (BH) adjust the *p* value < 0.05. The ratio of the gene numbers to all gene numbers annotated in this term is the rich factor. The top 10 enriched terms of CUR targets were visualized using a web-based tool (www.bioinformatics.com.cn) (Zeng et al., 2022). We also downloaded the genes of PI3K-Akt signaling pathway from the PathCards database (http://pathcards.genecards.org/) (Belinky et al., 2015). The targets present in both the PI3K-Akt signaling pathway and CUR targets were screened out using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

Protein-protein Interaction (Ppi) Network Construction

STRING database, version 11.5 (https://string-db.org/) (Szklarczyk et al., 2019), was used to construct the PPI network. Only PPI interaction for *Homo sapiens* were considered and the combined score > 0.4 was requested. The edges' thickness represents the combined score. The Cytoscape plugin Network Analysis plugin was used to calculate degree values (the number of a node directly connected to other nodes), and constructed PPI network was visualized by Cytoscape software (version 3.7.1) (Shannon et al., 2003).

Molecular Docking And Molecular Dynamics Simulations

We used AutoDock Vina (The Scripps Research Institute, CA, USA) to perform molecular docking in this study. The protein-CUR interactions in the structures were analyzed by LigPlot. Molecular dynamics simulation was also performed employing GROMACS package (version 2019.6) combined with the Amber ff14SB force field. The conjugate gradient method is used to minimize the energy of the entire system and the steepest descent algorithm then processed 50,000 steps. When the temperature reaches 300 K, a density balance of 500 ps is performed with periodic boundary conditions, and in final, a 100 ns simulation of MD was carried out, which is on the basis of the initial structure of the docked complexes of substrate with protein.

Drugs And Antibodies

CUR (HPLC ≥ 97%, CAS# MB2147) was from Dalian Meilun Biological Technology Co., Ltd (Dalian, China). Carboxymethylcellulose sodium (CMC-Na, Cat# 30036365) was from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). LPS (CAS# MB5198) was from Dalian Meilun Biological Technology Co., Ltd (Dalian, China). LY294002 (PI3K inhibitor, CAS# 154447-36-6) was purchased from MCE (MedChemExpress). Total Akt (t-Akt, Cat# 9272) and phosphorylated Akt (p-Akt, Ser473; Cat# 4058), total PI3K (t-PI3K, Cat# 4695) and phosphorylated PI3K (p-PI3K, Tyr458; Cat# 4370), antibodies were purchased from Cell Signaling (Danvers, MA, USA). Anti-mouse IgG (Cat# 926-32211) and anti-rabbit (Cat# 926-32210) conjugated to IRDye® 800 CW were obtained from Li-Cor Bioscience (Lincoln, NE, USA). Sandwich enzyme-linked immunosorbent assay (ELISA) kits for IL-6 (Cat# RK00008), IL-1β (Cat# RK00006) and TNF-α (Cat# RK00027) were from Abclonal Technology (Wuhan, China).

Animal And Drug Treatment

Male adult C57BL/6J mice (12–14 weeks old) were purchased from Guangdong Medical Laboratory Animal Center (License No. SCXK-YUE 2022-0002, Guangdong, China). All mice were housed and maintained in the Medicine Animal Center of Jianghan University, and the experimental procedures were approved by the Medical Ethics Committee of Jianghan University with the approval YXLL2021-003.

CUR was dissolved in 0.5% CMC-Na. The experiment mice were divided into four groups (n = 8/group): saline-treated (control mice), LPS (2 mg/kg/day, intraperitoneally), LPS + CUR (50 mg/kg/day, intragastrically) and LPS + CUR + LY294002 (7.5 mg/kg/day, intraperitoneally). Animals received an intraperitoneal (i.p.) injection of saline or curcumin for 7 consecutive days. Mice received an i.p. injection of LPS or LY294002 once a day from the 5th day to the 7th day, LY294002 was administered 1 h prior to LPS treatment. Figure 4A showed the drug treatment schedule. The dose of LPS and CUR were based on the previous animal studies (da Silva Marques et al., 2021, Li et al., 2021). After 24 h of the last LPS injection, the behavior tests were performed and then body weight of mice was recorded. Finally, we sacrificed the animals and collected the brain tissues and serum.

Behavior Tests (Spt, Oft And Tst)

The OFT, SPT and TST were performed to detect exploratory activity and anxiety-like behavior as described previously in this study (Ning et al., 2018, Zeng et al., 2019, Qu et al., 2020). The number of crossing the center in OFT was recorded. Sucrose preference rate was calculated according to the formula: sucrose consumption/sucrose consumption plus water consumption (%). The amount time of immobility in TST was recorded.

Hematoxylin–eosin (He) Staining

Mice were sacrificed by isoflurane anesthesia and HE staining was performed according to our previous study (Guo et al., 2019). Images were obtained using an Olympus microscope (Tokyo, Japan).

Western Blotting And Elisa

Western blotting and ELISA
Western blotting was performed according to our previous methods (Guo et al., 2019, Zeng et al., 2019). The quantification of the western blotting was analyzed by Image J software (https://imagej.nih.gov/). The levels of IL-6, IL-1β and TNF-α in serum were quantified using ELISA kits (ABclonal, Wuhan, China) through the manufacturer's protocols. Finally, stop the reaction and measure the optical density was accordingly.

Statistical analysis

Data were presented as means ± SEM and plotted using GraphPad Prism. Statistical analysis was performed using SPSS 19.0 (SPSS, Chicago, IL). Multiple comparisons were performed using 1-way ANOVA and Tukey's multiple comparisons test. $p < 0.05$ was considered statistically significant.

Result

Pharmacological and toxicological properties of CUR

The 2D chemical structure of CUR is showed in Fig. 1A, it has three reactive functional groups containing one diketone moiety and two phenolic groups (Priyadarsini, 2013). The suitability of CUR for the use as a drug was based on RO5 (Lipinski et al., 2001) and drug likeness (Tao et al., 2013). CUR is fully compliant with the RO5 for orally bioavailable drugs. Furthermore, toxicological parameters including organ toxicity (hepatotoxicity) and toxicity end points (carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity) were predicted by Protox II webserver (Banerjee et al., 2018). The acute oral LD50 of CUR was 2000 mg/kg and only considered to be related to immunotoxicity (Fig. 1B). Combined with preclinical systematic safety evaluation (Sharma et al., 2007) and human trials (Hewlings and Kalman, 2017), CUR is considered to be a very safe drug. The above results indicated that CUR had satisfactory pharmacokinetic properties, meanwhile, it possessed no toxicity. Furthermore, these 358 target proteins of CUR were categorized into 9 different classes based on their cellular functions, of which metabolite interconversion enzyme (PC00262) and protein modifying enzyme (PC00260) were the most enriched class (Fig. 1C). To provide insight into the biological functions of CUR, we performed GO based biological process enrichment analysis using all 358 CUR targets as input list. Analysis of BP terms showed that most of the CUR targets were related to response to oxidative stress (GO:0006979), cellular response to chemical stress (GO:0062197), positive regulation of cytokine production (GO:0001819), response to nutrient levels (GO:0031667), neuron death (GO:0070997), response to lipopolysaccharide (GO:0032496) and regulation of inflammatory response (GO:0050727) (Fig. 1D). 47 CUR targets are involved in the neuron death (GO:0070997) and form a PPI network that includes 46 nodes and 462 edges. Notably, AKT1, HIF1A, TP53, CASP3, TNF, STAT3, MTOR, CTNNB1 (β-catenin), CASP8 and SIRT1 play a key role in this PPI network.

Pi3k-akt Signaling Pathway Is A Key Signaling Pathway For Cur Antidepressant Activity

By applying GSEA, we identified signaling pathways closely related to depression. The MDD dataset, GSE98793, contains the gene expression from the whole blood of 128 persons (64 MDD patients and 64 healthy controls). GSEA revealed that genes identified by GSE98793 were significantly enriched in the PI3K-Akt signaling pathway in MDD patients (NES = 1.44, $p = 0.002$) (Fig. 2A). We therefore identified the PI3K-Akt pathway as a candidate key signaling pathway for depression. We further identified the KEGG pathways regulated by CUR, and a total of 358 targets were collected from the HERB database (Fang et al., 2021) and PharmMapper server (Liu et al., 2010). Specifically, 176 and 193 CUR targets were obtained from HERB database and PharmMapper server, respectively. KEGG pathway enrichment analysis of 358 CUR targets was conducted using the ClusterProfiler R package (version 3.12.0) (Yu et al., 2012). A total of 159 KEGG pathways were significantly enriched (adjusted p values < 0.05) in our study. As shown, the m n n n most significantly enriched pathways were PI3K-Akt signaling pathway (hsa04151, adjust p value = 8.06E-14, 40 targets), MAPK signaling pathway (hsa04010), FoxO signaling pathway (hsa04068), apoptosis (hsa04210) and JAK-STAT signaling pathway (hsa04630) (Fig. 2B).

We obtained 525 genes involved in this signaling pathway from the PathCards database (Belinky et al., 2015). Ultimately, taking the intersection of the genes in PI3K-Akt signaling pathway and CUR, 52 targets were screened out (Fig. 2C). We further constructed a PPI network by the STRING database (version 11.5) (Szklarczyk et al., 2019), and obtained a PPI network containing 52 nodes and 712 edges (Fig. 2D). In the PPI network, AKT1, TP53, MYC, MTOR, CTNNB1, EGFR, VEGFA, MAPK3, CASP3 and CCND1 have the highest degree value. We also constructed a PPI network of 525 genes of the PI3K-Akt signaling pathway. Among the top 50 targets ranked by degree in the PPI network, there were 23 CUR targets that targeting PI3K Akt signaling pathway (Fig. 2E). The above results show that the PI3K-Akt signaling pathway, which is closely associated with depression, is a key signaling pathway targeted by CUR.

Molecular docking and molecular dynamics simulations of the interaction between CUR and it's targets involved in the PI3K-Akt signaling pathway

As shown in Fig. 2, PI3K-Akt signaling pathway is the main signaling pathway for CUR antidepressant activity. The molecular docking studies showed that the CUR targets involved in the PI3K-Akt signaling pathway were well combined with CUR (Fig. 3A). Among the targets, CUR showed the highest binding energy with RXRA (-9.8 kcal/mol), followed by ERBB4 (-8.6 kcal/mol), RAC1 (-8.6 kcal/mol), PTGS2 (-8.5 kcal/mol), JAK2 (-8.3 kcal/mol), and MTOR (-8.2 kcal/mol). AKT1 is the most important subtype of Akt isoforms and the activation of Akt1 was assessed by Ser473 phosphorylation of Akt relative to total Akt. Figure 3B, C demonstrates that CUR binds tightly in the AKT1 binding pockets and stabilized by hydrogen bonding. Specifically, CUR formed potential interactions with residues Glu228, Lys276 and Ser7 of AKT1 *via* hydrogen bonds. The distances between CUR and Glu228, Lys276, Ser7 were 2.91, 3.08 and 2.65, 3.02, 3.31 Å, respectively (Fig. 3C). Moreover, with the aim of investigating the dynamic interactions between CUR and AKT1, molecular dynamics simulation was also performed employing GROMACS package (version 2019.6) combined with the Amber ff14SB force field. The RMSD from the average structure of backbone atoms for each MD trajectory was calculated as well for exploring the "position stability" of the original conformation for each complex. Figure 3D is plotted the RMSD of backbone atoms of the complex system and the result shows that after 25 ns, the conformation of all systems has reached a steady-state because the RMSD for the original structure of complex reached at about 5 Å which indicates the stability of the structures.

Cur Ameliorates Lps-induced Depression-like Behaviors Through The Pi3k-akt Signaling Pathway

In this study, we employed the LPS-induced depression mice model (Sekio and Seki, 2014, Jeon et al., 2017, Li et al., 2021) to assess the antidepressant effect of CUR. The experimental paradigm was shown in Fig. 4A. In the OFT, zone crossing numbers in LPS group (16.75 ± 1.24) was decreased compared with that in CON mice (30.38 ± 1.63), whereas treatment with CUR led to a significant increase (24.13 ± 1.60). PI3K inhibitor, LY294002, can block PI3K-Akt signaling and block the antidepressant effect of CUR (Fig. 4B, F). The sucrose preference percentage of LPS mice (69.51 ± 4.38) was significantly reduced

compared with that of CON mice (86.99 ± 2.22). However, the sucrose preference percentage of LPS + CUR mice (86.13 ± 2.32) treated CUR significantly increased compared with LPS mice (Fig. 4C). The results of TST revealed that LPS exposure (165.8 ± 6.75 s) significantly increased immobility times compared with CON mice (75.63 ± 3.95 s), while CUR at dose of 50 mg/kg/day treatments (105.4 ± 6 s) significantly reduced immobility times versus LPS group (Fig. 4D). Inhibition of the PI3K-Akt signaling pathway by LY294002 blocks the antidepressant effects of CUR. The body weight between the 4 groups showed no difference (about 22 g) (Fig. 4E). These results suggest that CUR relieves LPS-induced depression-like behaviors, and inhibition of PI3K-Akt signaling pathway can block the antidepressant effect of CUR.

Cur Treatment Eliminated Lps-induced Neuronal Damage In The Pfc

As shown in Fig. 5, the neurons in the prefrontal cortex of CON mice have regular morphology, neat arrangement, uniform chromatin, and normal interneuron gap; the neurons in the prefrontal cortex of each group have different degrees of cell body swelling and nuclear pyknosis. The pathological phenomena such as cytoplasmic condensed and deep staining, disordered arrangement and widening of the gap were the most serious in the LPS group. This neuron morphology was reverse by CUR.

Cur Reduced Lps-induced Inflammatory Response Through Pi3k-akt Signaling Pathway

We investigated the anti-inflammatory effects of CUR. LPS administration could induce the expression of proinflammatory cytokines such as IL-6, IL-1β and TNF-α (Bhatia et al., 2022, Zhu et al., 2022). To further verify CUR has anti-inflammatory effects, we measured the level of IL-6, IL-1β and TNF-α in serum by ELISA. The levels of TNF-α, IL-1β and IL-6 in the LPS group were higher than the CON mice (Fig. 6A-C), while the levels of TNF-α, IL-1β and IL-6 in the LPS + CUR- group were decreased, respectively. (Fig. 6A-C). Whereas treatment with LY294002 resulted in a significant increase of TNF-α, IL-1β and IL-6 (Fig. 6A-C). The above results suggested that indicated that LPS-treatment significantly enhanced neuroinflammation which could be rescued by CUR treatment, however, blocking the PI3K pathway could reversed the anti-inflammatory effects of CUR.

Effects Of Cur On Pi3k-akt Signaling Pathway-related Proteins In Pfc

It is well known that prefrontal cortex is involved in the depression-like phenotype. Akt1 is widely expressed in the brain and highly expressed in the PFC (Fig. S1). Herein, these proteins expressed in PFC of mice were measured by Western blotting. In LPS mice, the levels of p-PI3K (Tyr458) (0.66 ± 0.07) and p-Akt (Ser473) (0.30 ± 0.01) were significantly decreased than the CON mice. After CUR treatment, the levels of p-PI3K and p-Akt in LPS + CUR mice were significantly increased ($p < 0.05$). However, the levels of p-

PI3K and p-Akt were significantly decreased in LPS + CUR + LY294002 mice. There was no difference in t-PI3K and t-Akt among the four groups (Fig. 7A-C).

Discussion

Neuroinflammation in the brain has been implicated in the pathogenesis of depression (Brites and Fernandes, 2015, Yirmiya et al., 2015) and excessive neuroinflammation has been demonstrated to induce neuronal damage in the PFC of depression rats model induced by chronic unpredictable mild stress (CUMS) (Fan et al., 2018a, Fan et al., 2018b). The PFC plays an important role in translating emotional information (Pan et al., 2014). This study took PFC as the research object to explore the antidepressant mechanism of CUR. CUR belongs to the new generation antidepressants and the most commonly prescribed drug for the treatment of depression. In addition, previous findings on the molecular and cellular mechanisms of CUR suggest that CUR exerts anti-inflammatory effect by inhibiting cyclooxygenase 2 (COX-2), nuclear factor kappa beta (NF-κβ) pathway and activating NOD-like receptor protein 3 (NLRP3) inflammasome. Here, we demonstrated that CUR could reverse the alterations of inflammatory mediators (i.e., TNF-α, IL-1β and IL-6) induced by LPS. Another finding was that CUR treatment was able to restore PI3K-Akt activity (phosphorylation), which ultimately alleviated depression-like phenotypes. These results not only provide a new antidepressant mechanism of CUR but also provide new options for further exploration of therapeutic targets for depression.

In this study, we examined LPS-treated animals to study the antidepressant role of CUR. Previous experimental data support a strong association between depression and neuroinflammation (Singhal et al., 2014, Tohidpour et al., 2017, Ali et al., 2020). Elevated cytokines are first identified in MDD patients (Nanni et al., 2012, Kreisel et al., 2014). A postmortem study showed the pro-inflammatory cytokines genes are elevated in the frontal cortex of depressed people compared with the healthy controls (Shelton et al., 2011). LPS, the bacterial endotoxin, is widely used to establish a depression animal model related to inflammation (Dantzer, 2001, Ohgi et al., 2013, Ma et al., 2014, Zhang et al., 2014, Yao et al., 2015). Administration of LPS induces the expression of pro-inflammatory cytokines such as TNF-α and IL-1β in brain. Depression-like behaviors induced by peripheral administration of LPS peaked 2 to 6 hours after dosing (Zhang et al., 2016). In this study, 3 days of LPS injection created depressive tendency mice measured by OFT, SPT and TST. Furthermore, pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α were activated in the serum in response to peripheral administration of LPS. Multiple lines of evidence for the therapeutic effect of anti-inflammatory therapy on infection-induced depression-like symptoms continue to support a causal relationship between depression and neuroinflammation.

IL-1β, IL-6 and TNF-α are thought to be important factors in the neuroinflammation, and have been implicated in the treatment of depression and stress-related mood disorders (Tuglu et al., 2003, Raison et al., 2006). For example, preclinical studies have shown that administration of IL-1β produces multiple stress-like effects, downregulation of BDNF, and impairment of memory function (Koo and Duman, 2009). Furthermore, knockout the IL-1β receptor could attenuated the social defeat of mice (Wohleb et al., 2011).

Chronic alterations in peripheral and cerebral IL-6 levels may contribute to depressive symptoms in a number of ways (Hodes et al., 2016). IL-6 and IL-1β could also work together and synergistically induce psycho-neuro-immunological changes in depressive patients (Maes et al., 1993). IL-6 also increase the level of indoleamine 2, 3-dioxygenase (IDO), leading a decrease the production of tryptophan and tryptophan catabolites (TRYCATs), which is associated with the symptoms of depression (Maes et al., 2014). In addition, previous experimental data support that TNF-α can disrupt the BBB integrity (Cheng et al., 2018) and induce the depression-like behavior of mice (Zhe et al., 2017). With regards to studies of human patients, a previous report showed that CUR reduced the pro-inflammatory cytokines in in patients with depression compared with the placebo group (Yu et al., 2015). Moreover, studies in other clinical trials indicate that CUR can reduce the plasma levels of TNF-α, IL-6, and CRP in patients (Zhou et al., 2011, Aggarwal et al., 2013, Sahebkar, 2014). The results of our present study showed CUR could reversed LPS-induced behavioral and cytokines (TNF-α, IL-1β and IL-6) changes. Thus, one of the antidepressant mechanisms of CUR could be through its anti-inflammatory effect.

Depression is strongly associated with neuroinflammation and involves multiple targets and pathways. The regulation and release of pro-inflammatory cytokines are related with the PI3K-Akt signaling pathway. Previous studies have demonstrated a role for PI3K-Akt pathway in cancer (Fakhri et al., 2020, Fakhri et al., 2022).Furthermore, a recent review suggested this pathway plays the critical role in depression (Fakhri et al., 2021). PI3K/Akt/mTOR plays an important role in cell apoptosis, proliferation, migration and other processes. Also, this pathway can organize a major intracellular network for neoplastic synaptic function through the surviving cell transmission(Matsuda et al., 2019). In depression, there is evidence that PI3K-Akt pathway is associated with ERK1/2, GSK-3β and BDNF towards potential neuroprotective role (Lv et al., 2014). Natural products are major modulators of PI3K-Akt signaling pathway that inhibits the neuroinflammation (IL-6, IL-1β) (Jiang et al., 2018), nauroapoptosis (Bax/Bcl-2) (Cao and Qiao, 2019) and neuronal oxidative stress (Krogh et al., 2014) in depression. PI3K-Akt signaling pathway appears to play important roles in several functions as mentioned before. From the PPI network, 23 core targets were screened out ranked by degree (AKT1, EGFR, CTNINB1, MAPK3, MYC, TP53, VEGFA, TNF, IL6, MAPK1, MTOR, CCND1, CASP3, CDC42, JAK2, STAT1, PTK2, KIT, IGF1R, PIK3CB, HGF, INSR and MET). Evidence implicated that AKT is a good candidate molecule for MDD susceptibility. AKT1, a subtype of AKT, play an important role in depression. It is associated with anxiety symptoms and suicidal tendency of patients with depressive disorder(Yang et al., 2012). In this study, CUR treatment augmented the ratios of p-Akt (Ser473)/t-Akt and p-PI3K (Tyr458)/t-PI3K in the PFC of LPS + CUR mice. These results suggested that the effect of CUR on depression is directly related to the PI3K-Akt signaling pathway.

In addition to targeting proteins, CUR can interact with microRNAs and alter their function. MiR-155 was negatively correlated with the level of BDNF in the hippocampus, and the increase of miR-55 inhibits the expression of BDNF (Huan et al., 2021). Previous report has indicated that CUR inhibits LPS-induced inflammatory responses by inhibiting miR-155 (Ma et al., 2017). Therefore, the epigenetic mechanism by which CUR exerts antidepressant effect needs to be further clarified. further studies are needed to elucidate the epigenetic mechanisms by which CUR exerts antidepressant effects. In addition to depression, dysregulation of the PI3K-Akt signaling pathway is found in a variety of human diseases,

including cancers, diabetes, cardiovascular diseases. Further research is needed to treat the above diseases with CUR through PI3K-Akt signaling pathway.

Conclusion

Our results demonstrated that LPS-induced neuroinflammation plays a role in depression by inhibiting PI3K-Akt activity and associated neuronal damage. CUR could reverse these effects through increasing PI3K-Akt activity, which ultimately eliminating depressive-like symptoms and the neuroinflammation.

Abbrevistions

BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BH	Benjamini–Hochberg
COX-2	Cyclooxygenase 2
CUMS	Chronic unpredictable mild stress
CUR	Curcumin
ELISA	Sandwich enzyme-linked immunosorbent assay
GEO	Gene Expression Omnibus
GSEA	Gene set enrichment analysis
HE	Hematoxylin-eosin staining
i.p.	Intraperitoneal
IDO	Indoleamine 2, 3-dioxygenase
IL-1	Interleukin-1
KEGG	Kyoto Encyclopedia of Genes and Genomes
LPS	Lipopolysaccharide
MDD	Major depressive disorder
NES	Normalized enrichment score
NF-κβ	Nuclear factor kappa beta
NLRP3	NOD-like receptor protein 3
OFT	Open field test
PFC	prefrontal cortex
PPI	Protein-Protein Interaction
RO5	Lipinski's Rule of Five
SPT	Sucrose preference test
TNF-α	Tumour necrosis factor-alpha
TRYCATs	Tryptophan catabolites
TST	Tail suspension test

Declarations

Ethics approval Not applicable.

Competing interests The authors declare no competing interests.

Author contributions Conceptualization, P.Z. and M.F.; investigation, P.Z.; methodology, P.Z., J.G., and Z.X.; supervision, P.Z. and J.G.; visualization, P.Z., Z.X., M.F., and K.Z.; writing—original draft, J.G.; writing—review and editing, P.Z. All authors have read and agreed to the published version of the manuscript.

Funding This work was supported by grants from the Research Foundation of Scientific Research Program of Jianghan University (Grant No. 2021yb 131), the Research Foundation of Scientific Research Program of University of South China (Grant No. 220XQD090) and the Research Foundation of Education Bureau of Hunan Province (Grant No. 22B0416).

Availability of data and materials All data of this study are included in this published article.

References

1. (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England) 392: 1789-1858.http://dx.doi.org/10.1016/s0140-6736(18)32279-7
2. Adzic M, Djordjevic J, Mitic M, Brkic Z, Lukic I, Radojcic M (2015) The contribution of hypothalamic neuroendocrine, neuroplastic and neuroinflammatory processes to lipopolysaccharide-induced depressive-like behaviour in female and male rats: Involvement of glucocorticoid receptor and C/EBP-β. Behavioural brain research 291: 130-139.http://dx.doi.org/10.1016/j.bbr.2015.05.029
3. Aggarwal BB, Gupta SC, Sung B (2013) Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. Br J Pharmacol 169: 1672-1692.http://dx.doi.org/10.1111/bph.12131
4. Ali T, Hao Q, Ullah N, Rahman SU, Shah FA, He K, Zheng C, Li W, Murtaza I, Li Y, Jiang Y, Tan Z, Li S (2020) Melatonin Act as an Antidepressant via Attenuation of Neuroinflammation by Targeting Sirt1/Nrf2/HO-1 Signaling. Frontiers in molecular neuroscience 13: 96.http://dx.doi.org/10.3389/fnmol.2020.00096
5. Banerjee P, Eckert AO, Schrey AK, Preissner R (2018) ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic acids research 46: W257-w263.http://dx.doi.org/10.1093/nar/gky318
6. Begum AN, Jones MR, Lim GP, Morihara T, Kim P, Heath DD, Rock CL, Pruitt MA, Yang F, Hudspeth B, Hu S, Faull KF, Teter B, Cole GM, Frautschy SA (2008) Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. The Journal of pharmacology and experimental therapeutics 326: 196-208.http://dx.doi.org/10.1124/jpet.108.137455
7. Belinky F, Nativ N, Stelzer G, Zimmerman S, Iny Stein T, Safran M, Lancet D (2015) PathCards: multi-source consolidation of human biological pathways. Database (Oxford) 201510.1093/database/bav006. http://dx.doi.org/10.1093/database/bav006

8. Bhatia R, Sharma S, Bhadada SK, Bishnoi M, Kondepudi KK (2022) Lactic Acid Bacterial Supplementation Ameliorated the Lipopolysaccharide-Induced Gut Inflammation and Dysbiosis in Mice. Frontiers in microbiology 13: 930928.http://dx.doi.org/10.3389/fmicb.2022.930928
9. Brites D, Fernandes A (2015) Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. Frontiers in cellular neuroscience 9: 476.http://dx.doi.org/10.3389/fncel.2015.00476
10. Budni J, Lobato KR, Binfaré RW, Freitas AE, Costa AP, Martín-de-Saavedra MD, Leal RB, Lopez MG, Rodrigues AL (2012) Involvement of PI3K, GSK-3β and PPARγ in the antidepressant-like effect of folic acid in the forced swimming test in mice. Journal of psychopharmacology (Oxford, England) 26: 714-723.http://dx.doi.org/10.1177/0269881111424456
11. Cao LH, Qiao JY (2019) PI3K-AKT Signaling Activation and Icariin: The Potential Effects on the Perimenopausal Depression-Like Rat Model. 2410.3390/molecules24203700. http://dx.doi.org/10.3390/molecules24203700
12. Charlson F, van Ommeren M, Flaxman A, Cornett J, Whiteford H, Saxena S (2019) New WHO prevalence estimates of mental disorders in conflict settings: a systematic review and meta-analysis. Lancet (London, England) 394: 240-248.http://dx.doi.org/10.1016/s0140-6736(19)30934-1
13. Chen CW, Chen CC, Jian CY, Lin PH, Chou JC, Teng HS, Hu S, Lieu FK, Wang PS, Wang SW (2016) Attenuation of exercise effect on inflammatory responses via novel role of TLR4/PI3K/Akt signaling in rat splenocytes. Journal of applied physiology (Bethesda, Md : 1985) 121: 870-877.http://dx.doi.org/10.1152/japplphysiol.00393.2016
14. Cheng Y, Desse S, Martinez A, Worthen RJ, Jope RS, Beurel E (2018) TNFα disrupts blood brain barrier integrity to maintain prolonged depressive-like behavior in mice. Brain, behavior, and immunity 69: 556-567.http://dx.doi.org/10.1016/j.bbi.2018.02.003
15. da Silva Marques JG, Antunes FTT, da Silva Brum LF, Pedron C, de Oliveira IB, de Barros Falcão Ferraz A, Martins MIM, Dallegrave E, de Souza AH (2021) Adaptogenic effects of curcumin on depression induced by moderate and unpredictable chronic stress in mice. Behavioural brain research 399: 113002.http://dx.doi.org/10.1016/j.bbr.2020.113002
16. Dantzer R (2001) Cytokine-induced sickness behavior: where do we stand? Brain, behavior, and immunity 15: 7-24.http://dx.doi.org/10.1006/brbi.2000.0613
17. Du Y, Li W, Li Y, Yang J, Wang X, Yin S, Wang X, Velez de-la-Paz OI, Gao Y, Chen H, Yin X, Shi H (2019) Repeated arctigenin treatment produces antidepressant- and anxiolytic-like effects in mice. Brain Res Bull 146: 79-86.http://dx.doi.org/10.1016/j.brainresbull.2018.12.005
18. Fakhri S, Iranpanah A, Gravandi MM, Moradi SZ, Ranjbari M, Majnooni MB, Echeverría J, Qi Y, Wang M, Liao P, Farzaei MH, Xiao J (2021) Natural products attenuate PI3K/Akt/mTOR signaling pathway: A promising strategy in regulating neurodegeneration. Phytomedicine : international journal of phytotherapy and phytopharmacology 91: 153664.http://dx.doi.org/10.1016/j.phymed.2021.153664
19. Fakhri S, Khodamorady M, Naseri M, Farzaei MH, Khan H (2020) The ameliorating effects of anthocyanins on the cross-linked signaling pathways of cancer dysregulated metabolism.

Pharmacol Res 159: 104895.http://dx.doi.org/10.1016/j.phrs.2020.104895

20. Fakhri S, Moradi SZ, Farzaei MH, Bishayee A (2022) Modulation of dysregulated cancer metabolism by plant secondary metabolites: A mechanistic review. Semin Cancer Biol 80: 276-305.http://dx.doi.org/10.1016/j.semcancer.2020.02.007
21. Fan C, Song Q, Wang P, Li Y, Yang M, Liu B, Yu SY (2018a) Curcumin Protects Against Chronic Stress-induced Dysregulation of Neuroplasticity and Depression-like Behaviors via Suppressing IL-1β Pathway in Rats. Neuroscience 392: 92-106.http://dx.doi.org/10.1016/j.neuroscience.2018.09.028
22. Fan C, Song Q, Wang P, Li Y, Yang M, Yu SY (2018b) Neuroprotective Effects of Ginsenoside-Rg1 Against Depression-Like Behaviors via Suppressing Glial Activation, Synaptic Deficits, and Neuronal Apoptosis in Rats. Frontiers in immunology 9: 2889.http://dx.doi.org/10.3389/fimmu.2018.02889
23. Fang S, Dong L, Liu L, Guo J, Zhao L, Zhang J, Bu D, Liu X, Huo P, Cao W, Dong Q, Wu J, Zeng X, Wu Y, Zhao Y (2021) HERB: a high-throughput experiment- and reference-guided database of traditional Chinese medicine. Nucleic acids research 49: D1197-d1206.http://dx.doi.org/10.1093/nar/gkaa1063
24. Fusar-Poli L, Vozza L, Gabbiadini A, Vanella A, Concas I, Tinacci S, Petralia A, Signorelli MS, Aguglia E (2020) Curcumin for depression: a meta-analysis. Crit Rev Food Sci Nutr 60: 2643-2653.http://dx.doi.org/10.1080/10408398.2019.1653260
25. Gerhard DM, Wohleb ES, Duman RS (2016) Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity. Drug discovery today 21: 454-464.http://dx.doi.org/10.1016/j.drudis.2016.01.016
26. Guillet BA, Velly LJ, Canolle B, Masmejean FM, Nieoullon AL, Pisano P (2005) Differential regulation by protein kinases of activity and cell surface expression of glutamate transporters in neuron-enriched cultures. Neurochemistry international 46: 337-346.http://dx.doi.org/10.1016/j.neuint.2004.10.006
27. Guo J, Xu C, Ni S, Zhang S, Li Q, Zeng P, Pi G, Liu E, Sun DS, Liu Y, Wang Z, Chen H, Yang Y, Wang JZ (2019) Elevation of pS262-Tau and Demethylated PP2A in Retina Occurs Earlier than in Hippocampus During Hyperhomocysteinemia. Journal of Alzheimer's disease : JAD 68: 367-381.http://dx.doi.org/10.3233/jad-180978
28. Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, Bailey MT, Sheridan JF, Godbout JP (2008) Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. J Neuroinflammation 5: 15.http://dx.doi.org/10.1186/1742-2094-5-15
29. Herrman H, Kieling C, McGorry P, Horton R, Sargent J, Patel V (2019) Reducing the global burden of depression: a Lancet-World Psychiatric Association Commission. Lancet (London, England) 393: e42-e43.http://dx.doi.org/10.1016/s0140-6736(18)32408-5
30. Hewlings SJ, Kalman DS (2017) Curcumin: A Review of Its Effects on Human Health. Foods (Basel, Switzerland) 610.3390/foods6100092. http://dx.doi.org/ 10.3390/foods6100092
31. Hodes GE, Ménard C, Russo SJ (2016) Integrating Interleukin-6 into depression diagnosis and treatment. Neurobiology of stress 4: 15-22.http://dx.doi.org/10.1016/j.ynstr.2016.03.003

32. Huan Z, Mei Z, Na H, Xinxin M, Yaping W, Ling L, Lei W, Kejin Z, Yanan L (2021) lncRNA MIR155HG Alleviates Depression-Like Behaviors in Mice by Regulating the miR-155/BDNF Axis. Neurochemical research 46: 935-944.http://dx.doi.org/10.1007/s11064-021-03234-z
33. Huang J, Liang Y, Tian W, Ma J, Huang L, Li B, Chen R, Li D (2020) Antitumor Activity and Mechanism of Robustic Acid from Dalbergia benthami Prain via Computational Target Fishing. Molecules (Basel, Switzerland) 2510.3390/molecules25173919. http://dx.doi.org/ 10.3390/molecules25173919
34. Jeon SA, Lee E, Hwang I, Han B, Park S, Son S, Yang J, Hong S, Kim CH, Son J, Yu JW (2017) NLRP3 Inflammasome Contributes to Lipopolysaccharide-induced Depressive-Like Behaviors via Indoleamine 2,3-dioxygenase Induction. The international journal of neuropsychopharmacology 20: 896-906.http://dx.doi.org/10.1093/ijnp/pyx065
35. Ji P, Wang L, Chen Y, Wang S, Wu Z (2020) Hyaluronic acid hydrophilic surface rehabilitating curcumin nanocrystals for targeted breast cancer treatment with prolonged biodistribution. 8: 462-472.http://dx.doi.org/10.1039/c9bm01605h
36. Jiang CY, Qin XY, Yuan MM, Lu GJ, Cheng Y (2018) 2,3,5,4'-Tetrahydroxystilbene-2-O-beta-D-glucoside Reverses Stress-Induced Depression via Inflammatory and Oxidative Stress Pathways. 2018: 9501427.http://dx.doi.org/10.1155/2018/9501427
37. Jiang N, Lv JW, Wang HX, Lu C, Wang Q, Xia TJ, Bao Y, Li SS, Liu XM (2019) Dammarane sapogenins alleviates depression-like behaviours induced by chronic social defeat stress in mice through the promotion of the BDNF signalling pathway and neurogenesis in the hippocampus. Brain Res Bull 153: 239-249.http://dx.doi.org/10.1016/j.brainresbull.2019.09.007
38. Koo JW, Duman RS (2009) Evidence for IL-1 receptor blockade as a therapeutic strategy for the treatment of depression. Current opinion in investigational drugs (London, England : 2000) 10: 664-671.http://dx.doi.org/
39. Kreisel T, Frank MG, Licht T, Reshef R, Ben-Menachem-Zidon O, Baratta MV, Maier SF, Yirmiya R (2014) Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. Molecular psychiatry 19: 699-709.http://dx.doi.org/10.1038/mp.2013.155
40. Krogh J, Benros ME, Jørgensen MB, Vesterager L, Elfving B, Nordentoft M (2014) The association between depressive symptoms, cognitive function, and inflammation in major depression. Brain, behavior, and immunity 35: 70-76.http://dx.doi.org/10.1016/j.bbi.2013.08.014
41. Kulkarni SK, Bhutani MK, Bishnoi M (2008) Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology (Berl) 201: 435-442.http://dx.doi.org/10.1007/s00213-008-1300-y
42. Leday GGR, Vértes PE, Richardson S, Greene JR, Regan T, Khan S, Henderson R, Freeman TC, Pariante CM, Harrison NA, Perry VH, Drevets WC, Wittenberg GM, Bullmore ET (2018) Replicable and Coupled Changes in Innate and Adaptive Immune Gene Expression in Two Case-Control Studies of Blood Microarrays in Major Depressive Disorder. Biol Psychiatry 83: 70-80.http://dx.doi.org/10.1016/j.biopsych.2017.01.021

43. Li W, Ali T, Zheng C, Liu Z, He K, Shah FA, Ren Q, Rahman SU, Li N, Yu ZJ, Li S (2021) Fluoxetine regulates eEF2 activity (phosphorylation) via HDAC1 inhibitory mechanism in an LPS-induced mouse model of depression. 18: 38.http://dx.doi.org/10.1186/s12974-021-02091-5
44. Lin TY, Lu CW, Wang CC, Wang YC, Wang SJ (2011) Curcumin inhibits glutamate release in nerve terminals from rat prefrontal cortex: possible relevance to its antidepressant mechanism. Prog Neuropsychopharmacol Biol Psychiatry 35: 1785-1793.http://dx.doi.org/10.1016/j.pnpbp.2011.06.012
45. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced drug delivery reviews 46: 3-26.http://dx.doi.org/10.1016/s0169-409x(00)00129-0
46. Liu X, Ouyang S, Yu B, Liu Y, Huang K, Gong J, Zheng S, Li Z, Li H, Jiang H (2010) PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. Nucleic acids research 38: W609-614.http://dx.doi.org/10.1093/nar/gkq300
47. Lopresti AL (2022) Potential Role of Curcumin for the Treatment of Major Depressive Disorder. CNS drugs 36: 123-141.http://dx.doi.org/10.1007/s40263-022-00901-9
48. Lu J, Xu X, Huang Y, Li T, Ma C, Xu G, Yin H, Xu X, Ma Y, Wang L, Huang Z, Yan Y, Wang B, Xiao S, Zhou L, Li L, Zhang Y, Chen H, Zhang T, Yan J, Ding H, Yu Y, Kou C, Shen Z, Jiang L, Wang Z, Sun X, Xu Y, He Y, Guo W, Jiang L, Li S, Pan W, Wu Y, Li G, Jia F, Shi J, Shen Z, Zhang N (2021) Prevalence of depressive disorders and treatment in China: a cross-sectional epidemiological study. The lancet Psychiatry 8: 981-990.http://dx.doi.org/10.1016/s2215-0366(21)00251-0
49. Lv QQ, Wu WJ, Guo XL, Liu RL, Yang YP, Zhou DS, Zhang JX, Liu JY (2014) Antidepressant activity of astilbin: involvement of monoaminergic neurotransmitters and BDNF signal pathway. Biological & pharmaceutical bulletin 37: 987-995.http://dx.doi.org/10.1248/bpb.b13-00968
50. Ma F, Liu F, Ding L, You M, Yue H, Zhou Y, Hou Y (2017) Anti-inflammatory effects of curcumin are associated with down regulating microRNA-155 in LPS-treated macrophages and mice. Pharm Biol 55: 1263-1273.http://dx.doi.org/10.1080/13880209.2017.1297838
51. Ma L, Li XP, Ji HS, Liu YF, Li EZ (2018) Baicalein Protects Rats with Diabetic Cardiomyopathy Against Oxidative Stress and Inflammation Injury via Phosphatidylinositol 3-Kinase (PI3K)/AKT Pathway. Med Sci Monit 24: 5368-5375.http://dx.doi.org/10.12659/msm.911455
52. Ma M, Ren Q, Zhang JC, Hashimoto K (2014) Effects of Brilliant Blue G on Serum Tumor Necrosis Factor-α Levels and Depression-like Behavior in Mice after Lipopolysaccharide Administration. Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology 12: 31-36.http://dx.doi.org/10.9758/cpn.2014.12.1.31
53. Maes M, Anderson G, Kubera M, Berk M (2014) Targeting classical IL-6 signalling or IL-6 trans-signalling in depression? Expert opinion on therapeutic targets 18: 495-512.http://dx.doi.org/10.1517/14728222.2014.888417
54. Maes M, Scharpé S, Meltzer HY, Bosmans E, Suy E, Calabrese J, Cosyns P (1993) Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pituitary-

adrenal axis in severe depression. Psychiatry Res 49: 11-27.http://dx.doi.org/10.1016/0165-1781(93)90027-e

55. Matsuda S, Ikeda Y, Murakami M, Nakagawa Y, Tsuji A, Kitagishi Y (2019) Roles of PI3K/AKT/GSK3 Pathway Involved in Psychiatric Illnesses. Diseases (Basel, Switzerland) 710.3390/diseases7010022. http://dx.doi.org/ 10.3390/diseases7010022
56. Mi H, Guo N, Kejariwal A, Thomas PD (2007) PANTHER version 6: protein sequence and function evolution data with expanded representation of biological pathways. Nucleic acids research 35: D247-252.http://dx.doi.org/10.1093/nar/gkl869
57. Mohd Faudzi SM, Abdullah MA, Abdull Manap MR, Ismail AZ, Rullah K, Mohd Aluwi MFF, Mazila Ramli AN, Abas F, Lajis NH (2020) Inhibition of nitric oxide and prostaglandin E(2) production by pyrrolylated-chalcones: Synthesis, biological activity, crystal structure analysis, and molecular docking studies. Bioorg Chem 94: 103376.http://dx.doi.org/10.1016/j.bioorg.2019.103376
58. Nanni V, Uher R, Danese A (2012) Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. Am J Psychiatry 169: 141-151.http://dx.doi.org/10.1176/appi.ajp.2011.11020335
59. Ning LN, Zhang T, Chu J, Qu N, Lin L, Fang YY, Shi Y, Zeng P, Cai EL, Wang XM, Wang Q, Lu YM, Zhou XW, Zhang Q, Tian Q (2018) Gender-Related Hippocampal Proteomics Study from Young Rats After Chronic Unpredicted Mild Stress Exposure. Molecular neurobiology 55: 835-850.http://dx.doi.org/10.1007/s12035-016-0352-y
60. O'Connor JC, Lawson MA, André C, Moreau M, Lestage J, Castanon N, Kelley KW, Dantzer R (2009) Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Molecular psychiatry 14: 511-522.http://dx.doi.org/10.1038/sj.mp.4002148
61. Ohgi Y, Futamura T, Kikuchi T, Hashimoto K (2013) Effects of antidepressants on alternations in serum cytokines and depressive-like behavior in mice after lipopolysaccharide administration. Pharmacol Biochem Behav 103: 853-859.http://dx.doi.org/10.1016/j.pbb.2012.12.003
62. Pan Y, Chen XY, Zhang QY, Kong LD (2014) Microglial NLRP3 inflammasome activation mediates IL-1β-related inflammation in prefrontal cortex of depressive rats. Brain, behavior, and immunity 41: 90-100.http://dx.doi.org/10.1016/j.bbi.2014.04.007
63. Pantharos P, Sukcharoen P, Phadungrakwittaya R, Akarasereenont P, Booranasubkajorn S, Lumlerdkij N (2022) Utilization of UPLC-PDA and GC-MS/MS coupled with metabolomics analysis to identify bioactive metabolites in medicinal turmeric at different ages for the quality assurance. Phytomedicine : international journal of phytotherapy and phytopharmacology 102: 154157.http://dx.doi.org/10.1016/j.phymed.2022.154157
64. Pizzagalli DA, Roberts AC (2022) Prefrontal cortex and depression. 47: 225-246.http://dx.doi.org/10.1038/s41386-021-01101-7
65. Priyadarsini KI (2013) Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Des 19: 2093-2100.http://dx.doi.org/10.2174/138161213805289228

66. Qu N, Wang XM, Zhang T, Zhang SF, Li Y, Cao FY, Wang Q, Ning LN, Tian Q (2020) Estrogen Receptor α Agonist is Beneficial for Young Female Rats Against Chronic Unpredicted Mild Stress-Induced Depressive Behavior and Cognitive Deficits. Journal of Alzheimer's disease : JAD 77: 1077-1093.http://dx.doi.org/10.3233/jad-200486
67. Raison CL, Capuron L, Miller AH (2006) Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends in immunology 27: 24-31.http://dx.doi.org/10.1016/j.it.2005.11.006
68. Sahebkar A (2014) Are curcuminoids effective C-reactive protein-lowering agents in clinical practice? Evidence from a meta-analysis. Phytotherapy research : PTR 28: 633-642.http://dx.doi.org/10.1002/ptr.5045
69. Sandhir R, Yadav A, Mehrotra A, Sunkaria A, Singh A, Sharma S (2014) Curcumin nanoparticles attenuate neurochemical and neurobehavioral deficits in experimental model of Huntington's disease. Neuromolecular Med 16: 106-118.http://dx.doi.org/10.1007/s12017-013-8261-y
70. Sekio M, Seki K (2014) Lipopolysaccharide-induced depressive-like behavior is associated with α -adrenoceptor dependent downregulation of the membrane GluR1 subunit in the mouse medial prefrontal cortex and ventral tegmental area. The international journal of neuropsychopharmacology 1810.1093/ijnp/pyu005. http://dx.doi.org/ 10.1093/ijnp/pyu005
71. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research 13: 2498-2504.http://dx.doi.org/10.1101/gr.1239303
72. Sharma RA, Steward WP, Gescher AJ (2007) Pharmacokinetics and pharmacodynamics of curcumin. Adv Exp Med Biol 595: 453-470.http://dx.doi.org/10.1007/978-0-387-46401-5_20
73. Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirnics K (2011) Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. Molecular psychiatry 16: 751-762.http://dx.doi.org/10.1038/mp.2010.52
74. Shi HS, Zhu WL, Liu JF, Luo YX, Si JJ, Wang SJ, Xue YX, Ding ZB, Shi J, Lu L (2012) PI3K/Akt signaling pathway in the basolateral amygdala mediates the rapid antidepressant-like effects of trefoil factor 3. Neuropsychopharmacology 37: 2671-2683.http://dx.doi.org/10.1038/npp.2012.131
75. Siddique YH, Khan W, Singh BR, Naqvi AH (2013) Synthesis of alginate-curcumin nanocomposite and its protective role in transgenic Drosophila model of Parkinson's disease. ISRN Pharmacol 2013: 794582.http://dx.doi.org/10.1155/2013/794582
76. Singhal G, Jaehne EJ, Corrigan F, Toben C, Baune BT (2014) Inflammasomes in neuroinflammation and changes in brain function: a focused review. Frontiers in neuroscience 8: 315.http://dx.doi.org/10.3389/fnins.2014.00315
77. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV (2019) STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic acids research 47: D607-d613.http://dx.doi.org/10.1093/nar/gky1131

78. Tao W, Xu X, Wang X, Li B, Wang Y, Li Y, Yang L (2013) Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease. Journal of ethnopharmacology 145: 1-10.http://dx.doi.org/10.1016/j.jep.2012.09.051
79. Tohidpour A, Morgun AV, Boitsova EB, Malinovskaya NA, Martynova GP, Khilazheva ED, Kopylevich NV, Gertsog GE, Salmina AB (2017) Neuroinflammation and Infection: Molecular Mechanisms Associated with Dysfunction of Neurovascular Unit. Frontiers in cellular and infection microbiology 7: 276.http://dx.doi.org/10.3389/fcimb.2017.00276
80. Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E (2003) Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. Psychopharmacology (Berl) 170: 429-433.http://dx.doi.org/10.1007/s00213-003-1566-z
81. Wang R, Xu Y, Wu HL, Li YB, Li YH, Guo JB, Li XJ (2008) The antidepressant effects of curcumin in the forced swimming test involve 5-HT1 and 5-HT2 receptors. European journal of pharmacology 578: 43-50.http://dx.doi.org/10.1016/j.ejphar.2007.08.045
82. Wang YS, Shen CY, Jiang JG (2019) Antidepressant active ingredients from herbs and nutraceuticals used in TCM: pharmacological mechanisms and prospects for drug discovery. Pharmacol Res 150: 104520.http://dx.doi.org/10.1016/j.phrs.2019.104520
83. Wei Z, Zhu J, Cheng Y, Huang Q (2019) Ovotransferrin fibril-stabilized Pickering emulsions improve protection and bioaccessibility of curcumin. Food research international (Ottawa, Ont) 125: 108602.http://dx.doi.org/10.1016/j.foodres.2019.108602
84. Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF (2011) β-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. The Journal of neuroscience : the official journal of the Society for Neuroscience 31: 6277-6288.http://dx.doi.org/10.1523/jneurosci.0450-11.2011
85. Yang C, Sun N, Ren Y, Sun Y, Xu Y, Li A, Wu K, Zhang K (2012) Association between AKT1 gene polymorphisms and depressive symptoms in the Chinese Han population with major depressive disorder. Neural regeneration research 7: 235-239.http://dx.doi.org/10.3969/j.issn.1673-5374.2012.03.014
86. Yao W, Zhang JC, Dong C, Zhuang C, Hirota S, Inanaga K, Hashimoto K (2015) Effects of amycenone on serum levels of tumor necrosis factor-α, interleukin-10, and depression-like behavior in mice after lipopolysaccharide administration. Pharmacol Biochem Behav 136: 7-12.http://dx.doi.org/10.1016/j.pbb.2015.06.012
87. Yirmiya R, Rimmerman N, Reshef R (2015) Depression as a microglial disease. Trends Neurosci 38: 637-658.http://dx.doi.org/10.1016/j.tins.2015.08.001
88. Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 16: 284-287.http://dx.doi.org/10.1089/omi.2011.0118
89. Yu JJ, Pei LB, Zhang Y, Wen ZY, Yang JL (2015) Chronic Supplementation of Curcumin Enhances the Efficacy of Antidepressants in Major Depressive Disorder: A Randomized, Double-Blind, Placebo-

Controlled Pilot Study. J Clin Psychopharmacol 35: 406-410.http://dx.doi.org/10.1097/jcp.0000000000000352

90. Zeng P, Shi Y, Wang XM, Lin L, Du YJ, Tang N, Wang Q, Fang YY, Wang JZ, Zhou XW, Lu Y, Tian Q (2019) Emodin Rescued Hyperhomocysteinemia-Induced Dementia and Alzheimer's Disease-Like Features in Rats. The international journal of neuropsychopharmacology 22: 57-70.http://dx.doi.org/10.1093/ijnp/pyy090
91. Zeng P, Su HF, Ye CY, Qiu SW, Shi A, Wang JZ, Zhou XW, Tian Q (2022) A Tau Pathogenesis-Based Network Pharmacology Approach for Exploring the Protections of Chuanxiong Rhizoma in Alzheimer's Disease. Frontiers in pharmacology 13: 877806.http://dx.doi.org/10.3389/fphar.2022.877806
92. Zeng P, Wang XM, Ye CY, Su HF, Fang YY, Zhang T, Tian Q (2021) Mechanistic insights into the anti-depressant effect of emodin: an integrated systems pharmacology study and experimental validation. Aging 13: 15078-15099.http://dx.doi.org/10.18632/aging.203072
93. Zhang C, Browne A, Child D, Tanzi RE (2010) Curcumin decreases amyloid-beta peptide levels by attenuating the maturation of amyloid-beta precursor protein. The Journal of biological chemistry 285: 28472-28480.http://dx.doi.org/10.1074/jbc.M110.133520
94. Zhang JC, Wu J, Fujita Y, Yao W, Ren Q, Yang C, Li SX, Shirayama Y, Hashimoto K (2014) Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. The international journal of neuropsychopharmacology 1810.1093/ijnp/pyu077. http://dx.doi.org/10.1093/ijnp/pyu077
95. Zhang JC, Yao W, Hashimoto K (2016) Brain-derived Neurotrophic Factor (BDNF)-TrkB Signaling in Inflammation-related Depression and Potential Therapeutic Targets. Curr Neuropharmacol 14: 721-731.http://dx.doi.org/10.2174/1570159x14666160119094646
96. Zhang L, Xu T, Wang S, Yu L, Liu D, Zhan R, Yu SY (2012) Curcumin produces antidepressant effects via activating MAPK/ERK-dependent brain-derived neurotrophic factor expression in the amygdala of mice. Behavioural brain research 235: 67-72.http://dx.doi.org/10.1016/j.bbr.2012.07.019
97. Zhe Q, Sulei W, Weiwei T, Hongyan L, Jianwei W (2017) Effects of Jiaotaiwan on depressive-like behavior in mice after lipopolysaccharide administration. Metabolic brain disease 32: 415-426.http://dx.doi.org/10.1007/s11011-016-9925-8
98. Zhou H, Beevers CS, Huang S (2011) The targets of curcumin. Current drug targets 12: 332-347.http://dx.doi.org/10.2174/138945011794815356
99. Zhu H, Tong S, Yan C, Zhou A, Wang M, Li C (2022) Triptolide attenuates LPS-induced activation of RAW 264.7 macrophages by inducing M1-to-M2 repolarization via the mTOR/STAT3 signaling. Immunopharmacol Immunotoxicol: 1-8.http://dx.doi.org/10.1080/08923973.2022.2093738

Figures

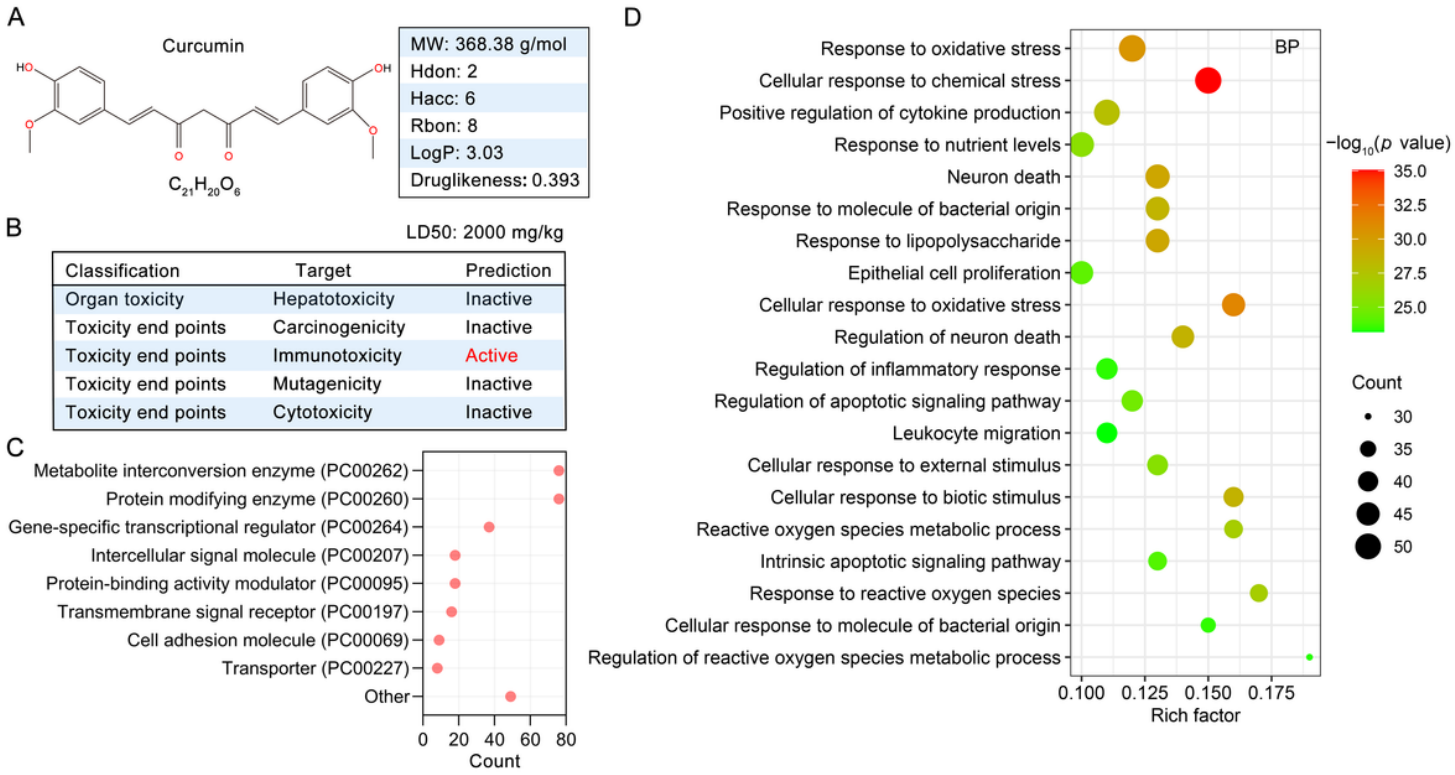

Figure 1

Pharmacological and toxicological parameters of Curcumin (CUR). **A** Chemical structure of CUR and its pharmacological properties. **B**Toxicological parameters of CUR. **C** Panther classification was used categorize common targets of CUR targets. D: Top 10 significantly enriched biological process terms for 358 CUR targets. The x-axis, rich factor (the ratio of targets in the background terms). Bubble size, the number of genes enriched. Bubble color, *p* value.

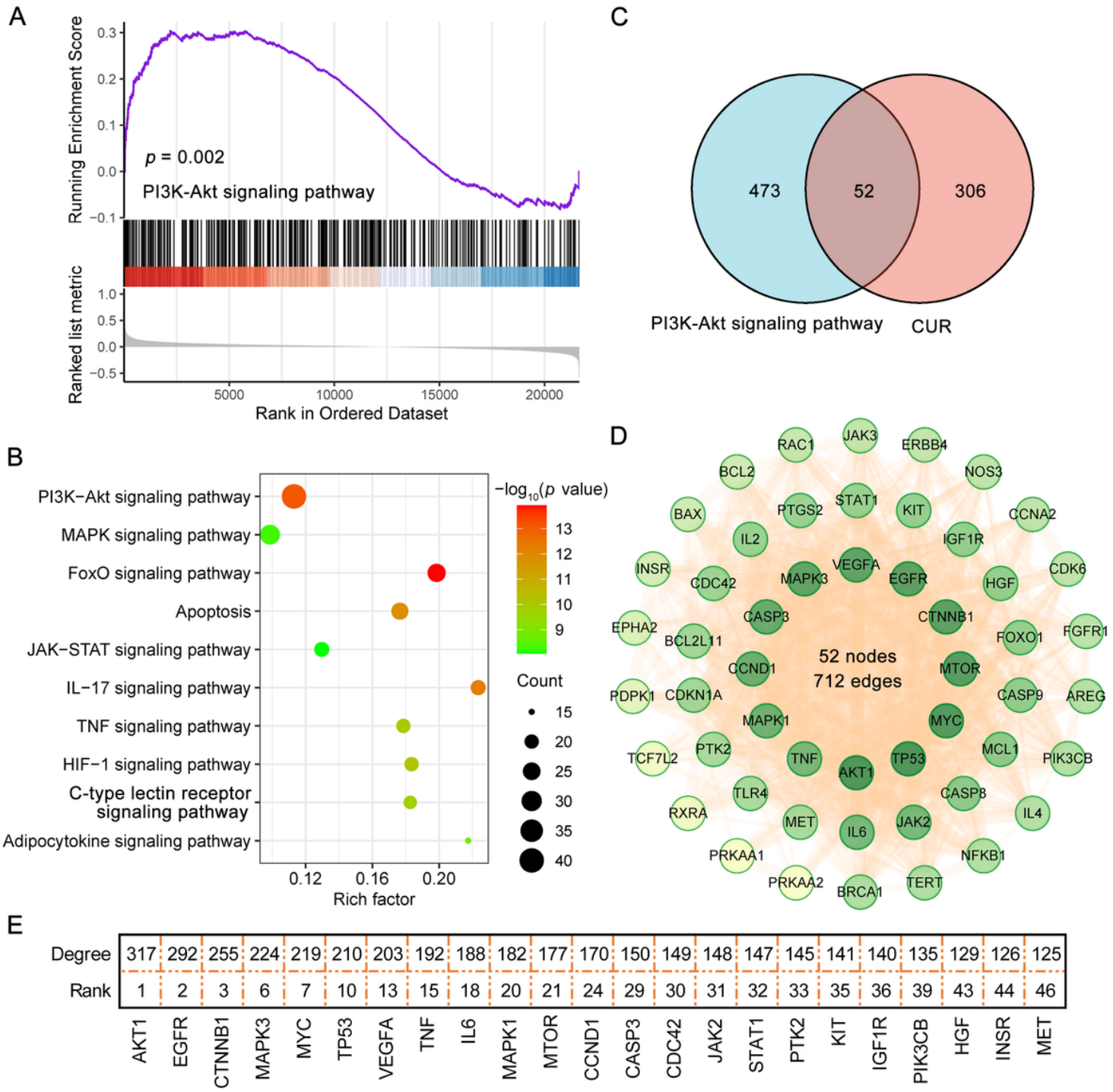

Figure 2

PI3K-Akt signaling pathway is a key pathway for CUR antidepressant activity. **A**GSEA demonstrated significant enrichment for members of the PI3K-Akt signaling pathway in major depressive disorder (MDD) patients. Data was obtained from GSE98793. **B** Top 10 significantly enriched KEGG pathways for 358 CUR targets. **C** Venn diagram showing overlapping of PI3K-Akt signaling pathway involved targets and CUR targets. **D**Establishment of PPI network of the CUR antidepressant targets using STRING database (version 11.5). The degree of each node was reflected by the color: the higher degree was

represented with deeper color. **E** Among the 525 targets involved in the PI3K-Akt signaling pathway, 23 of the top 50 targets in the PPI network ranked by degree were CUR targets.

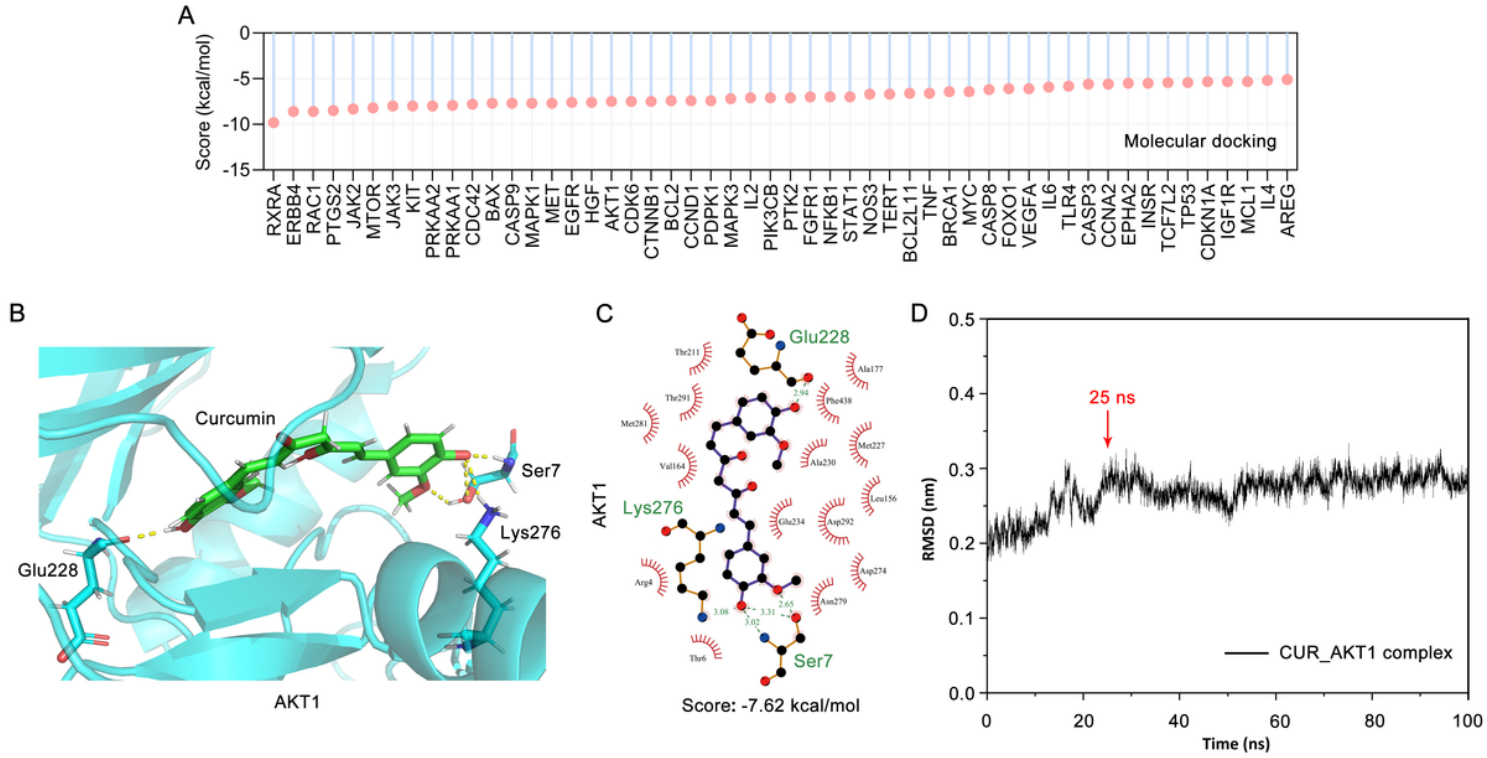

Figure 3

Amino Acid Residues and Molecular Dynamics Simulation of CUR Binding to AKT1. **A** Docking score (kcal/mol) of CUR with its targets involved in the PI3K-Akt signaling pathway. **B** Pattern diagram of molecular docking of CUR with Akt1 (PDB ID: 3MV5). **C** LigPlus schematic 2D representation the interactions between CUR and Akt1. **D** The molecular dynamics simulation between CUR and AKT1.

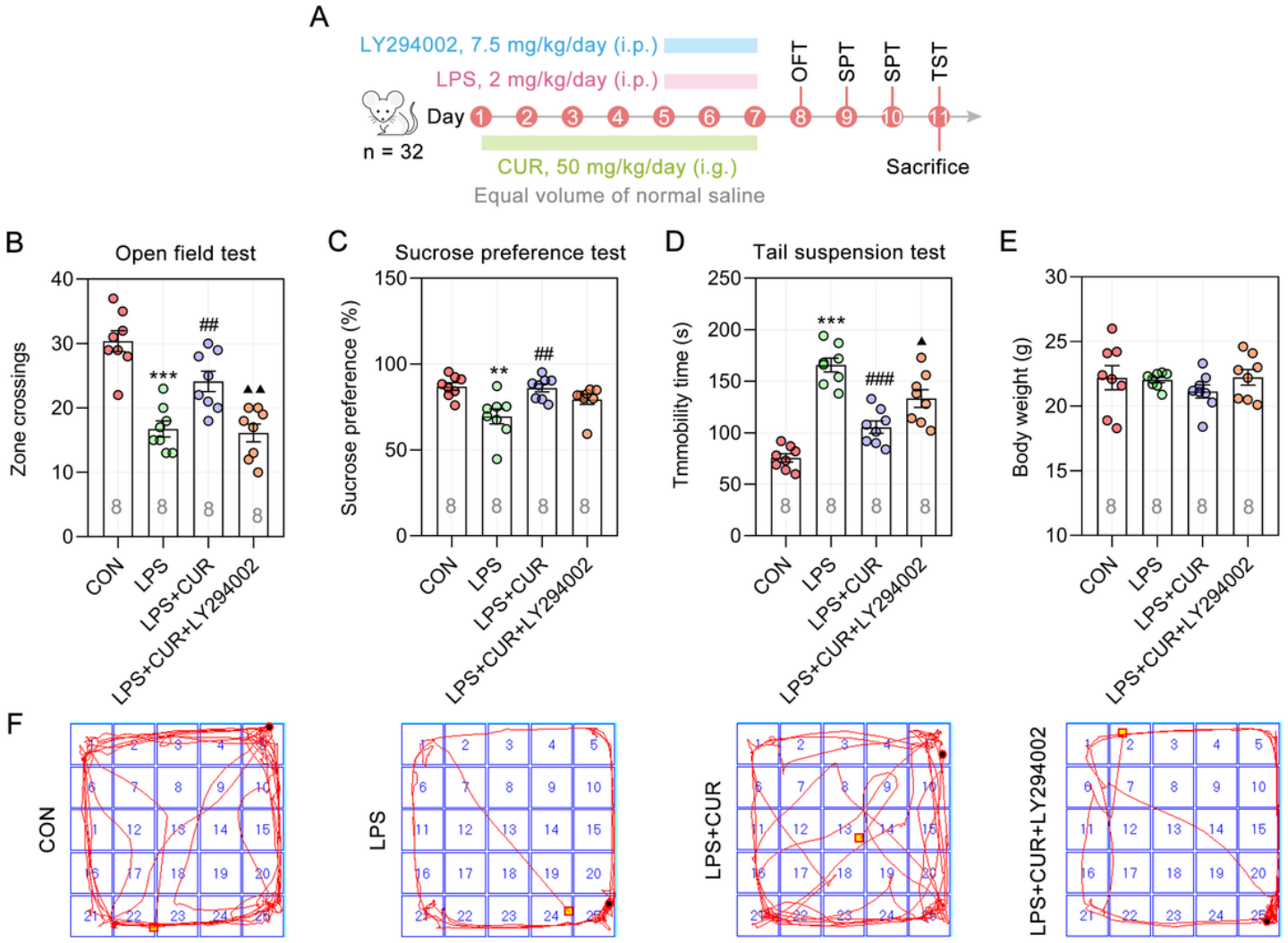

Figure 4

CUR ameliorates LPS-induced depression-like behaviors *via* the PI3K-Akt signaling pathway. **A** A flowchart demonstrates the experimental timeline. Thirty-two C57BL/6J male mice were divided into four groups, control group (saline-treated mice), LPS group (2 mg/kg/day, i.p.), LPS+CUR (50 mg/kg/day, i.p.) and LPS+CUR+LY294002 (7.5 mg/kg/day, i.p.). **B** The zone crossing numbers in the OFT (n = 8 per group). **C** Percentage of sucrose water consumption in the SPT on day 10. **D** The immobility times in the FST. **E** Body weight was measured on day 11. **F** Representative activity tracking in the OFT in CON, LPS, LPS+CUR and LPS+CUR+LY294002 mice. ** $p < 0.01$, *** $p < 0.001$ LPS vs CON. ## $p < 0.01$, ### $p < 0.001$ LPS+CUR vs LPS. ▲ $p < 0.05$, ▲▲ $p < 0.01$ LPS+CUR+LY294002 vs LPS+CUR.

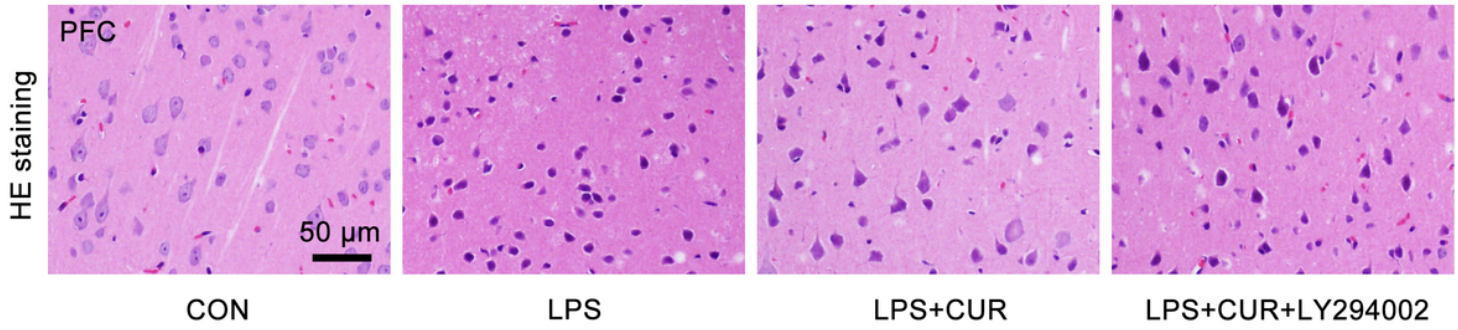

Figure 5

Representative HE staining images in the PFC of each group. Scale bar = 50 μm.

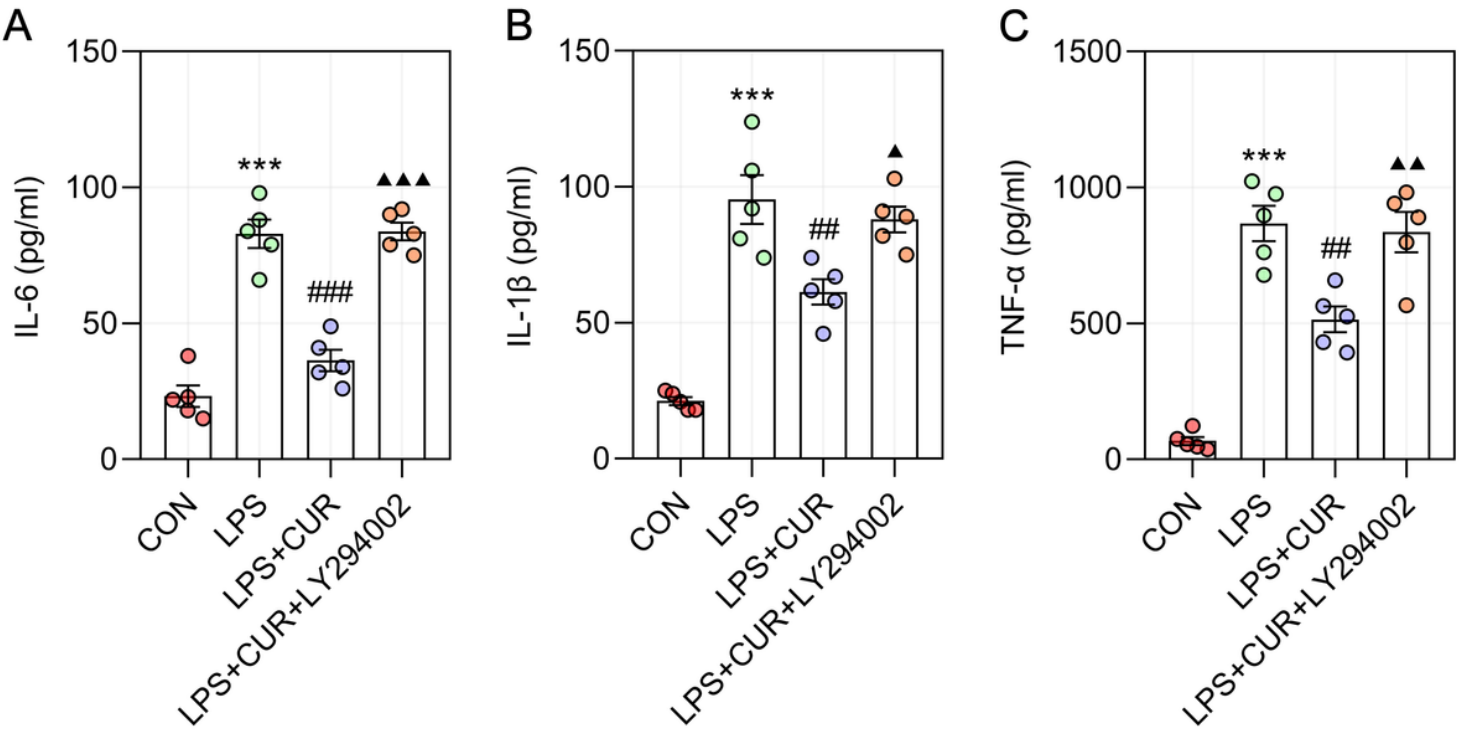

Figure 6

CUR treatment significantly reduced LPS-induced inflammatory response through PI3K-Akt signaling pathway. Serum protein levels of proinflammatory cytokine IL-6 (**A**), IL-1β (**B**), and TNF-α (**C**) were measured by ELISA (n = 5). Data were expressed as the means ± SEM. *** $p < 0.001$ LPS vs CON. ## $p < 0.01$, ### $p < 0.001$ LPS+CUR vs LPS. ▲ $p < 0.05$, ▲▲ $p < 0.01$, ▲▲▲ $p < 0.001$ LPS+CUR+LY294002 vs LPS+CUR.

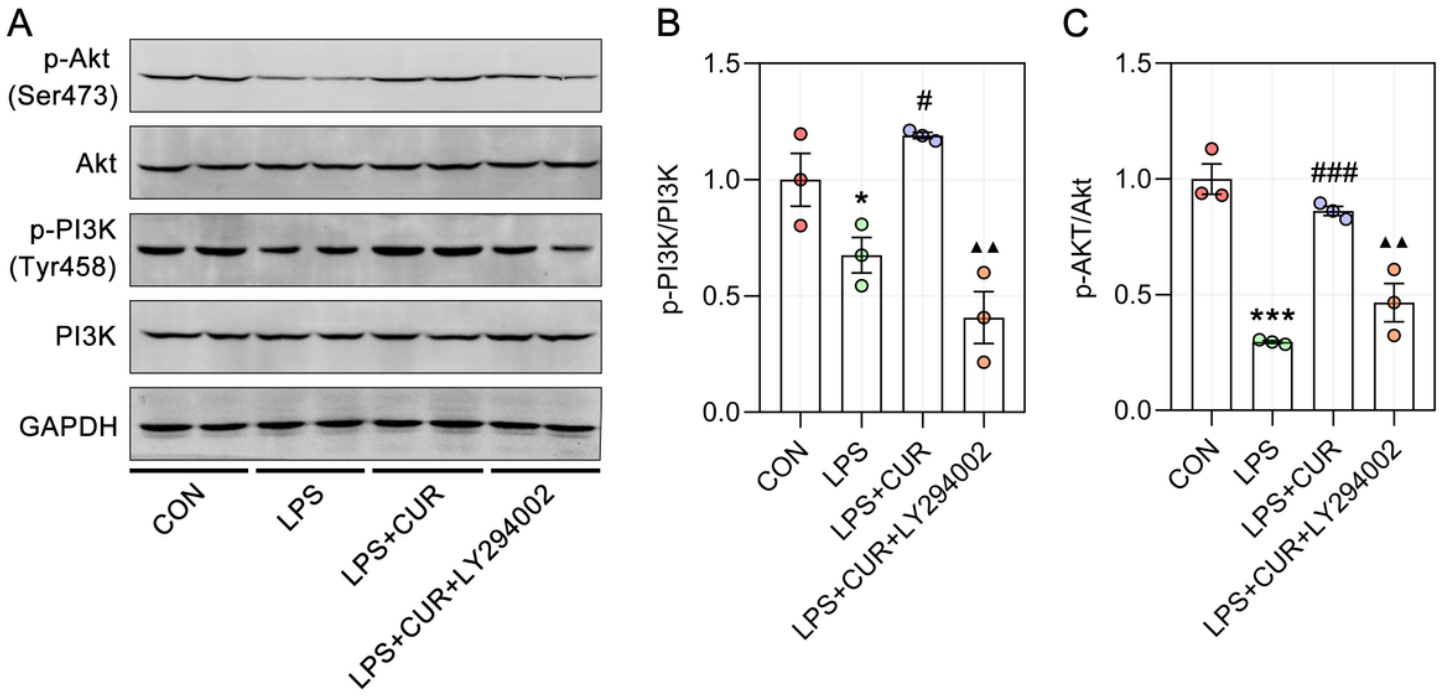

Figure 7

Effects of CUR on proteins of the PFC involved in PI3K-Akt signaling pathway associated. **A** Representative Western blotting bands of PI3K-Akt signaling pathway associated proteins. (**B, C**) The Levels of total PI3K and phosphorylated PI3K (p-PI3K, Tyr458), total Akt and phosphorylated Akt (p-Akt, Ser473) in PFC were detected by Western blotting and quantitatively analyzed (n = 3 per group). * $p < 0.05$, *** $p < 0.001$ LPS vs CON. # $p < 0.05$, ### $p < 0.001$ LPS+CUR vs LPS. ▲▲ $p < 0.01$ LPS+CUR+LY294002 vs LPS+CUR.

Supplementary Files

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

- FigureS1.tif