

The Mechanism of Chrysoeriol in Improving Depression-like Behavior in Mice by Inhibiting Neuroinflammation: A Network Pharmacology Study

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ABSTRACT

This study aimed to investigate the effects and mechanisms of Chrysoeriol (CHR) in improving depression-like behavior in mice.

Network pharmacology analysis was conducted to identify CHR-related targets and signaling pathways associated with depression and neuroinflammation. The chronic unpredictable mild stress (CUMS) method was used to construct a depression mouse model. After CHR intervention, behavioral changes were assessed using the elevated plus maze and open field tests. Cortical proinflammatory cytokine levels were measured using enzyme-linked immunosorbent assay (ELISA) kits, and pathological changes were examined by hematoxylin and eosin (H&E) and Nissl staining.

Network pharmacology identified AKT1 among the core predicted targets of CHR. Elevated plus maze and open field test results indicated that CHR improved depression-like behavior in mice. CHR reduced neuroinflammatory cytokine levels and attenuated tissue and neuronal damage.

CHR alleviated CUMS-induced depression-like behaviors and reduced neuroinflammation and neuronal damage. Network pharmacology identified predicted core targets, including AKT1. Further molecular verification and safety evaluation are required to validate its antidepressant potential.

Keywords: Chrysoeriol; Depression; Network pharmacology; Neuroinflammation

INTRODUCTION

Depression is a complex mental disorder, and stress is an important inducer of depressive episodes.¹ Microglia are associated with stress-induced mental and behavioral disorders,² and targeting microglia-mediated neuroinflammation is a focus of pharmacological

treatment for depression.³ Neuroinflammation is one of the host defense mechanisms, resulting from the combined actions of various neuroglial and peripheral immune cells that protect the nervous system from pathogenic or infectious damage.⁴ In the acute phase, the neuroinflammatory response can produce a protective effect by maintaining neuronal homeostasis, but in the chronic phase, maladaptation related to neuroinflammation can lead to neuronal damage.⁵ Numerous studies have confirmed the association between neuroinflammation and depression, anxiety,

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and other mental disorders. Flavonoids are present in many traditional Chinese medicines and can exert antidepressant effects through various mechanisms, such as anti-inflammatory and antioxidant effects, including reducing the levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in the hippocampus of mice in a chronic unpredictable stress model, inhibiting the secretion of cyclooxygenase-2 (COX-2), reducing the expression of nitric oxide, and lowering the levels of TNF- α in the brain tissue of rats with lipopolysaccharide-induced depression.^{6,7}

Chrysoeriol (CHR) is a flavonoid compound. Studies have found that CHR has a variety of physiological activities, such as anti-inflammatory effects, relieving inflammatory responses, especially swelling and pain in arthritis, and its mechanism is related to the inhibition of inflammatory responses and the reduction of nuclear factor- κ B (NF- κ B) signal activation.⁸ CHR also has sedative and anti-anxiety effects, relieving anxiety and depressive symptoms, and its mechanism is related to the improvement of neurotransmitters in the brain. Additionally, CHR can improve sleep, reduce the number of night awakenings, and increase sleep depth and continuity, which is related to the regulation of melatonin signaling.^{9,10} Although CHR has certain applications in neurological diseases, the specific mechanisms underlying its effects remain unclear. Chrysoeriol (CHR) was identified as the major active component, and its mechanism of action was further investigated.

MATERIALS AND METHODS

Network Pharmacology Analysis

The structural information of CHR was obtained from the NCBI PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The SMILES string of CHR was imported into the Swiss Target Prediction database (<http://www.swisstargetprediction.ch>) and the SEA database (<https://sea.bkslab.org/>) to obtain its targets. The 3D structure SDF file of CHR was imported into the PharmMapper database (<http://www.lilab-ecust.cn/pharmmapper/>), with “Maximum Generated Conformations” set to 300 and “Targets Set” selected as “Human Protein Targets Only” to obtain its targets. The targets obtained from the 3 databases were combined, duplicates were removed, and the CHR targets were

unified into gene names using the UniProt database (<https://www.uniprot.org/>).

Acquisition of Targets for Depression and Neuroinflammation

Using the keywords “depression” and “neuroinflammation,” relevant genes were retrieved from 3 disease gene databases: GeneCards (<https://www.genecards.org/>), CTD database (<https://ctdbase.org/>), and OMIM database (<https://www.omim.org/>). The data obtained from the 3 databases were collated and entered as disease targets.

Prediction of Targets for Chrysoeriol in Treating Depression and Neuroinflammation

The online Venny tool (<https://bioinfogp.cnb.csic.es/tools/venny/>) was used to obtain the intersection of CHR-related targets and targets related to depression and neuroinflammation, yielding the effective therapeutic targets for CHR in treating depression and neuroinflammation.

Construction of Protein-Protein Interaction Network

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, <https://cn.string-db.org/>) is an online bioinformatics database that provides information on gene and protein interactions. The intersection targets were imported into the STRING database with a “minimum required interaction score ≥ 0.4 ” as the screening criterion. The protein-protein interaction (PPI) network diagram and TSV file were downloaded and saved. Finally, the PPI network was visualized, and a multidimensional network for “Chrysoeriol-depression + neuroinflammation” was constructed using Cytoscape software (version 3.9.0).

GO Functional Enrichment and KEGG Pathway Enrichment Analysis

R software was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on the core targets to explore the possible biological functions and main signaling pathways of CHR in treating depression and neuroinflammation. The enrichment results with $q < 0.05$ were selected, and the significant enrichment results were ranked in descending order according to the p . The GO terms and top 20 KEGG signaling pathways were reported.

CUMS Model

Male C57BL/6J mice (8 weeks old, 20–22 g; n=40) were obtained from a licensed SPF laboratory animal supplier and housed under specific-pathogen-free conditions (22±2°C, 50–60% relative humidity, 12-h light/dark cycle) with food and water available *ad libitum* except during scheduled CUMS stressors. After 7 days of acclimatization, animals were assigned by a computer-generated random-number sequence to Control, CUMS, CHR-L, and CHR-H groups (n=10/group). Drug administration was performed by a researcher not involved in behavioral scoring; behavioral testing and histological evaluation were conducted by investigators blinded to group allocation.

The experiment was double-blind. The chronic unpredictable mild stress (CUMS) method was used to construct the depression model: The model construction time for mice was 5 weeks, with 1 week for acclimatization and the remaining 4 weeks for model construction. Mice were subjected to 1 chronic unpredictable stressor daily, including: a. unpleasant odors; b. whole-body restraint; c. tail clipping; d. dampness; e. shaking; f. tilting; g. swimming in ice water; h. reversal of day and night; i. water deprivation; j. food deprivation. The timing and sequence of each stressor applied to the mice were random, with no repetition within 3 days, adhering to the principles of unpredictability and randomness. Finally, the open field test (OFT) and elevated plus maze were used to assess behavioral changes in mice to determine the success of the model.

Mice were divided into 4 groups: Control, CUMS, CHR-L, and CHR-H. The Control group consisted of unstressed control mice, while the other 3 groups were subjected to the CUMS method to construct the depression mouse model. In the CHR-L and CHR-H groups, CHR was administered by daily gavage at 10 mg/kg and 20 mg/kg, respectively, for 4 consecutive weeks, with the intervention period consistent with that of CUMS.

Sucrose Preference Test

A 2% sucrose solution was used for the sucrose preference test (SPT) experiment. On the first day, a bottle of 2% sucrose solution was placed in the mouse cage. On the second day, 2 bottles were placed, one containing 2% sucrose solution and the other containing distilled water. On the third day, no sucrose test was conducted, and the mice were deprived of food and

water. On the fourth day, 2% sucrose solution and distilled water were placed again for 3 hours, with the positions swapped after 1.5 hours. The consumption of 2% sucrose solution and distilled water by the mice was measured. Sucrose preference (%) = (Sucrose solution consumption) ÷ (Sucrose solution consumption + Distilled water consumption) × 100%.

Tail Suspension Test

Mice were suspended upside down from a hanging rod 20 cm above the ground, and the immobility time of the mice was recorded over a 4-minute period. Mice initially struggled when suspended but subsequently became motionless in the tail suspension test (TST).

Forced Swim Test

Male C57BL/6J mice (8 weeks old, 20–22 g; n=40) were obtained from a licensed SPF laboratory animal supplier and housed under specific-pathogen-free conditions (22±2 °C, 50–60% relative humidity, 12-h light/dark cycle) with food and water available *ad libitum* except during scheduled CUMS stressors. After 7 days of acclimatization, animals were assigned by a computer-generated random-number sequence to Control, CUMS, CHR-L, and CHR-H groups (n=10/group). Drug administration was performed by a researcher not involved in behavioral scoring; behavioral testing and histological evaluation were conducted by investigators blinded to group allocation.

Open Field Test

A test box measuring 40 × 40 × 40 cm was used. Mice were placed in the center of the open field, and their movement trajectories were recorded for 5 minutes using SMART behavioral testing software; total distance traveled and time spent in the center of the field were analyzed.

Elevated Plus Maze Test

The elevated plus maze consisted of 4 arms, with 2 open arms without walls and 2 closed arms. The maze was placed 40 cm above the ground, with each arm measuring 30 cm in length and 5 cm in width. Mice were placed on the platform facing the open arms, and their movement trajectories over 5 minutes were recorded using the SMART behavioral testing software, analyzing the time spent in the open arms and the number of entries into the open arms.

Enzyme-linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the levels of inflammatory cytokines IL-1 β , IL-6, and TNF- α in the cortex of mice. Cortical tissue was ground in liquid nitrogen, lysed with NP-40, and the supernatant was collected. The cytokine concentrations in cortical tissue supernatants were measured according to the ELISA kit instructions and expressed in pg/mL.

H&E Staining

Mouse brain tissue was fixed in 4% formalin solution for 24 hours, dehydrated in a gradient ethanol series, cleared with xylene, immersed in liquid paraffin for 90 minutes, and then sectioned. After treatment with xylene and anhydrous ethanol, the tissue was stained with hematoxylin for 8 minutes, washed with water, treated with hydrochloric acid ethanol, stained with eosin for 15 minutes, dehydrated in a gradient ethanol series, and finally treated with xylene. The hematoxylin and eosin (H&E) sections were mounted with neutral resin and observed under a microscope.

Nissl Staining

Mouse brain tissue sections were prepared as described for H&E staining and stained with Nissl stain for 2 to 5 minutes. The sections were washed with water until no color remained on the glass slide, differentiated with 1 mL/L glacial acetic acid, dried by shaking off excess moisture and baking in a 65 °C oven, and finally mounted with neutral resin.

Statistical Methods

All measurement data were expressed as mean $\pm$ SD. The statistical software SPSS 17.0 was used for analysis and processing. After homogeneity of variance tests, 2-sample t tests were used for comparisons between 2 groups, and 1-way ANOVA was used for comparisons among 3 or more groups, followed by LSD tests for pairwise comparisons between groups. All tests were 2-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Target Analysis of Chrysoeriol-depression-neuroinflammation

A total of 390 targets for CHR were obtained from the PharmMapper, Swiss Target Prediction, and SEA

databases. By querying the GeneCards, CTD, and OMIM disease databases, 4911 and 4329 targets related to depression and neuroinflammation were retrieved, respectively. The intersection of these targets with those of CHR yielded 173 potential targets related to the treatment of depression and neuroinflammation (Figure 1A). To further understand the potential associations between the core components of CHR and its targets with depression and neuroinflammation, the “Chrysoeriol-depression + neuroinflammation” network was constructed using Cytoscape software (version 3.9.0). The network contained 176 nodes (including 1 active ingredient node, 173 target nodes, and 2 disease nodes) and 519 edges. The average number of adjacent nodes was 5.898, the network heterogeneity was 3.371, the network density was 0.034, and the network centrality was 0.966. In the network, the nodes representing targets were circular, the node representing the active ingredient was quadrilateral, and the nodes representing diseases were inverted triangles. Each edge represented the interaction between CHR and its target, as well as the relationship between depression and neuroinflammation and the target (Figure 1B). To better understand the mechanism of action of CHR in treating depression and neuroinflammation, the PPI network was used to understand the interactions between the intersecting targets. The 173 intersecting targets related to depression and neuroinflammation were imported into the STRING database to clarify the relationships between the component targets, resulting in a network of 173 proteins and 2601 interactions. The color depth represented the Degree value, with higher Degree values indicating a greater role of the protein in the network and larger node areas. The drug-disease intersection and target PPI network are shown in Figure 1C. The screening criteria were based on Betweenness, Closeness, and Degree values, with targets above the average value defined as core targets. The top 10 targets by Degree value were *AKT1* (Degree=121), *ALB* (Degree=110), *CASP3* (Degree=93), *HSP90AA1* (Degree=92), *EGFR* (Degree=91), *ESR1* (Degree=89), *NFKB1* (Degree=87), *MMP9* (Degree=85), *PPARG* (Degree=85), and *PTGS2* (Degree=82) (Table 1). These 10 targets are considered core targets and play important roles in the occurrence and development of depression and neuroinflammation treated by CHR.

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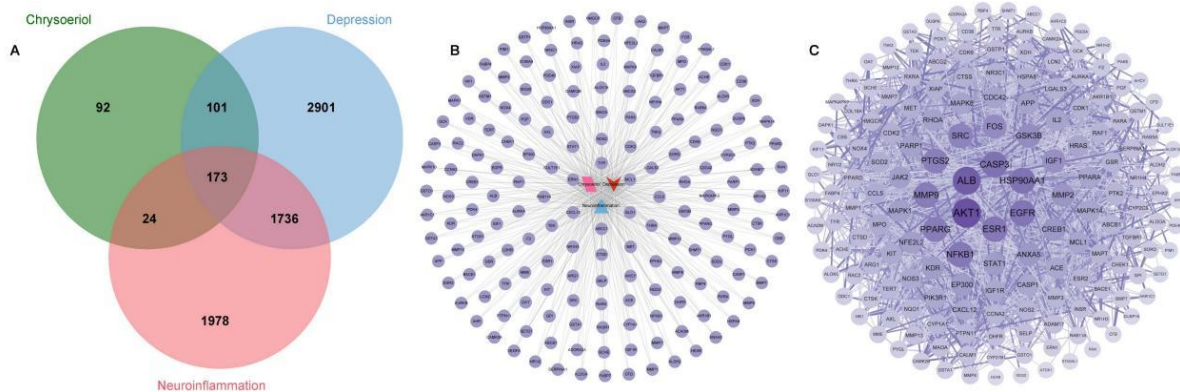


Figure 1. Target analysis of Chrysoeriol-depression-neuroinflammation. A, Target intersection diagram. B, “Chrysoeriol-depression + neuroinflammation” network diagram. C, Protein-protein interaction network diagram analyzed by Cytoscape.

Table 1. Core targets.

Gene	Degree	Betweenness centrality	Closeness centrality
<i>AKT1</i>	121	0.087247347	0.776255708
<i>ALB</i>	110	0.076877104	0.739130435
<i>CASP3</i>	93	0.024854388	0.688259109
<i>HSP90AA1</i>	92	0.052577366	0.68
<i>EGFR</i>	91	0.030853469	0.68
<i>ESR1</i>	89	0.040308373	0.669291339
<i>NFKB1</i>	87	0.018051544	0.671936759
<i>MMP9</i>	85	0.019317705	0.6640625
<i>PPARG</i>	85	0.037580143	0.6640625
<i>PTGS2</i>	82	0.02766063	0.658914729
<i>SRC</i>	80	0.020406651	0.648854962
<i>FOS</i>	73	0.022580093	0.62962963
<i>GSK3B</i>	72	0.015303534	0.63197026
<i>IGF1</i>	68	0.010710697	0.615942029
<i>MMP2</i>	64	0.008307054	0.607142857
<i>CREB1</i>	63	0.013826502	0.611510791
<i>ANXA5</i>	62	0.007729539	0.607142857
<i>EP300</i>	58	0.017659842	0.58419244
<i>STAT1</i>	58	0.013364024	0.592334495
<i>KDR</i>	57	0.006798322	0.590277778
<i>NFE2L2</i>	56	0.018511923	0.596491228
<i>MAPK1</i>	55	0.008417597	0.588235294
<i>PARP1</i>	53	0.006006396	0.586206897
<i>APP</i>	52	0.006701016	0.590277778
<i>CDC42</i>	52	0.007586524	0.580204778
<i>MAPK8</i>	52	0.005757704	0.582191781
<i>IL2</i>	51	0.010652289	0.58419244
<i>PPARA</i>	50	0.01096232	0.580204778
<i>MAPK14</i>	50	0.006886153	0.580204778

Table 1. Continued...

Gene	Degree	Betweenness centrality	Closeness centrality
<i>HRAS</i>	50	0.005608716	0.580204778
<i>ACE</i>	48	0.009130711	0.578231293
<i>CASP1</i>	48	0.006333031	0.578231293
<i>MPO</i>	45	0.007695387	0.570469799
<i>CCL5</i>	43	0.007958751	0.561056106
<i>SOD2</i>	42	0.012304931	0.564784053
<i>CTSS</i>	38	0.014830404	0.541401274
<i>LGALS3</i>	37	0.008603259	0.550161812
<i>HSPA8</i>	37	0.008406656	0.546623794
<i>GSR</i>	36	0.012640183	0.546623794
<i>CYP1A1</i>	33	0.009660545	0.544871795
<i>NQO1</i>	32	0.007087803	0.544871795

ACE: angiotensin I converting enzyme; AKT1: AKT serine/threonine kinase 1; ALB: albumin; ANXA5: annexin A5; APP: amyloid beta precursor protein; CASP1: caspase 1; CASP3: caspase 3; CCL5: C-C motif chemokine ligand 5; CDC42: cell division cycle 42; CREB1: cAMP responsive element binding protein 1; CTSS: cathepsin S; CYP1A1: cytochrome P450 family 1 subfamily A member 1; EGFR: epidermal growth factor receptor; EP300: E1A binding protein p300; ESR1: estrogen receptor 1; FOS: Fos proto-oncogene; GSK3B: glycogen synthase kinase 3 beta; GSR: glutathione-disulfide reductase; HRAS: HRas proto-oncogene; HSP90AA1: heat shock protein 90 alpha family class A member 1; HSPA8: heat shock protein family A member 8; IGF1: insulin like growth factor 1; IL2: interleukin 2; KDR: kinase insert domain receptor; LGALS3: galectin 3; MAPK1: mitogen-activated protein kinase 1; MAPK14: mitogen-activated protein kinase 14; MAPK8: mitogen-activated protein kinase 8; MMP2: matrix metalloproteinase 2; MMP9: matrix metalloproteinase 9; MPO: myeloperoxidase; NFE2L2: nuclear factor, erythroid 2 like 2; NFKB1: nuclear factor kappa B subunit 1; NQO1: NAD(P)H quinone dehydrogenase 1; PARP1: poly(ADP-ribose) polymerase 1; PPARA: peroxisome proliferator activated receptor alpha; PPARG: peroxisome proliferator activated receptor gamma; PTGS2: prostaglandin-endoperoxide synthase 2; SOD2: superoxide dismutase 2; SRC: SRC proto-oncogene, non-receptor tyrosine kinase.

Biological Function and Gene Pathway Enrichment Analysis of Intersecting Targets

The main biological processes involved in the target proteins were identified through KEGG and GO enrichment analyses. Using R software for GO functional enrichment and KEGG pathway enrichment analyses, the roles of the 173 intersecting targets related to depression and neuroinflammation in gene function and signaling pathways were investigated. The "Cluster Profiler" R package was applied to the 173 intersecting targets for GO enrichment analysis, yielding 2526 entries. Among these, 2253 entries were in biological processes (BP), mainly involving cellular response to chemical stress, response to oxidative stress, response to nutrient levels, response to xenobiotic stimulus, and cellular response to oxidative stress; 198 entries were in molecular functions (MF), primarily including nuclear receptor activity, ligand-activated transcription factor activity, carboxylic acid binding, monocarboxylic acid binding, and protein serine kinase activity; and 75 entries were in cellular components (CC), mainly

involving vesicle lumen, cytoplasmic vesicle lumen, secretory granule lumen, membrane raft, and membrane microdomain (Figure 2A–D). The analysis results indicated that the biological processes of inflammatory response and cell cycle have potential targets, all of which are closely related to the occurrence of depression and neuroinflammation, suggesting that the therapeutic effects of CHR on depression and neuroinflammation involve multiple biological processes.

KEGG pathway enrichment analysis was performed on the 173 intersecting targets related to depression and neuroinflammation using the "Cluster Profiler" package in R software. The results showed that there were 181 signaling pathways ($q < 0.05$) involved in the intervention of depression and neuroinflammation by CHR, mainly including Lipid and atherosclerosis, Fluid shear stress and atherosclerosis, Chemical carcinogenesis - reactive oxygen species, Efferocytosis, Prolactin signaling pathway, Chemical carcinogenesis - receptor activation, Relaxin signaling pathway, VEGF signaling pathway, Endocrine resistance, and AGE-

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RAGE signaling pathway in diabetic complications. The enrichment results were visualized (Figure 3A–C). In the “active ingredient-target-pathway” network, from the outside to the inside, signaling pathways were represented by diamond nodes, targets by circular nodes, and active ingredients by quadrilateral nodes. The larger the node area, the greater the degree value. The network contained 137 nodes (including 32 active ingredient nodes, 102 target nodes, 2 traditional Chinese medicine nodes, and 1 disease node) and 733 edges. The average number of adjacent nodes was 12.116, the network

heterogeneity was 1.269, the network density was 0.101, and the network centrality was 0.728. Through KEGG pathway enrichment analysis, it was understood that the targets of the active ingredients of CHR were closely related to multiple pathways, and the therapeutic effects on depression and neuroinflammation had characteristics of multi-component, multi-target, and multi-pathway interactions, producing multiple points of action at different levels, which were interconnected, jointly functioning, and multi-layer regulated (Figure 3D).

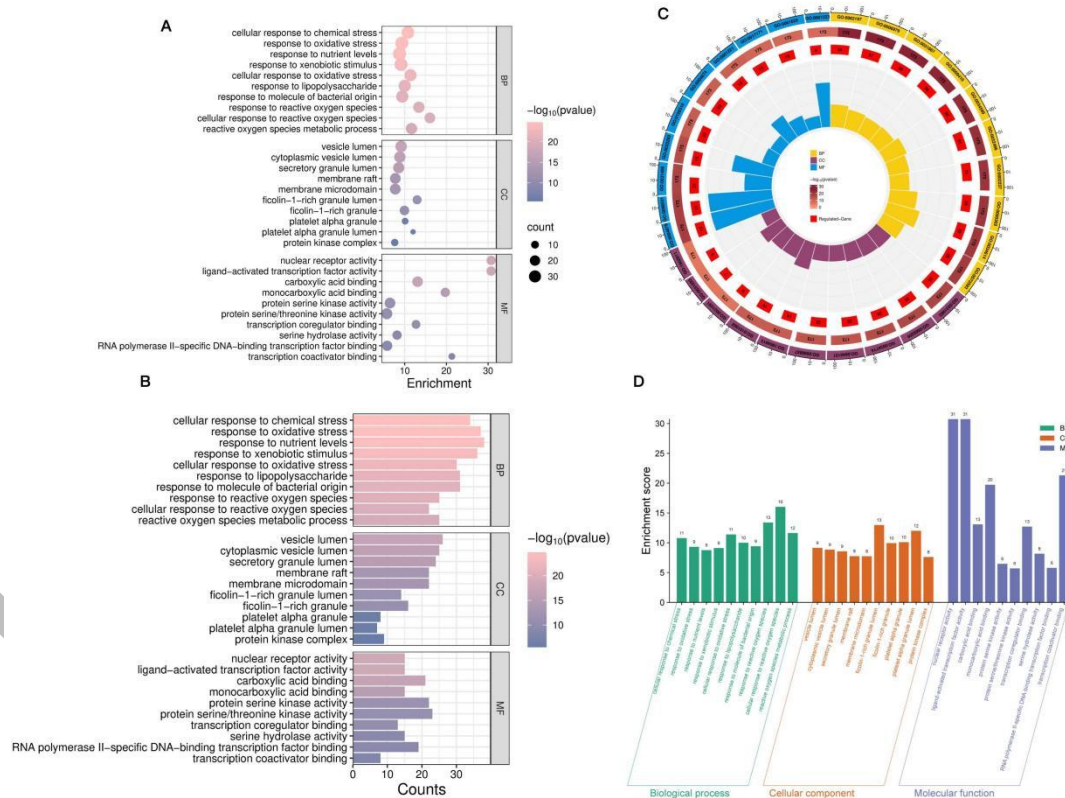


Figure 2. Gene Ontology (GO) analysis results. A–D, GO gene function enrichment analysis diagrams.

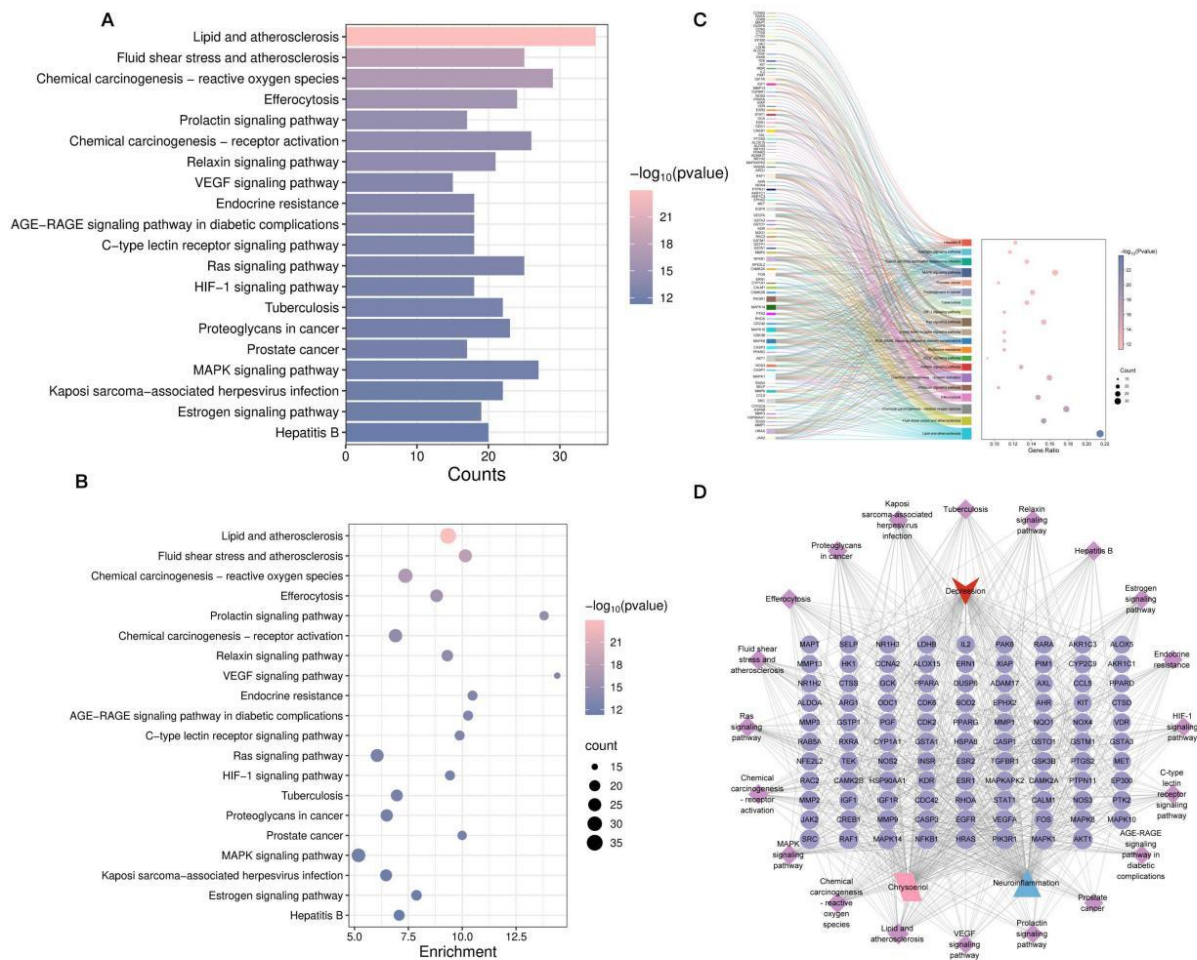


Figure 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis network diagram and target diagram. A–C, KEGG network analysis results. D, Active ingredient–target–pathway network diagram.

Chrysoeriol Improves Behavior in CUMS Mice

The SPT results showed that the sucrose preference of CUMS mice was lower than that of the Control group, and CHR could significantly improve the preference degree of mice, with a significant difference compared to the CUMS group (Figure 4A). The TST results indicated that the immobility time in the CUMS group was significantly higher than that in the Control group, while the immobility time in the CHR group was significantly shorter than that in the CUMS group (Figure 4B). The FST results showed that the immobility time of CUMS mice was higher than that of the Control group, while the swimming time was lower than that of the Control group. CHR could significantly increase the swimming time of mice (Figure 4C). The elevated plus maze test results showed that the time and number of entries into the open arms in the CUMS group

were significantly lower than those in the Control group, while CHR could increase the time and number of entries into the open arms of mice, with a significant difference compared to the CUMS group (Figure 4D–F). The OFT results showed that the total distance traveled and the time spent in the center of the field in the CUMS group were significantly lower than those in the Control group, while CHR could increase the time spent in the center of the field and the total distance traveled by mice (Figure 4G–I).

Chrysoeriol Inhibits Neuroinflammation and Neuronal Damage

The levels of inflammatory factors in the cortex were measured, and the results showed that the levels of IL-6, TNF- α , and IL-1 β in the CUMS group were higher than those in the Control group. CHR could reduce the

levels of inflammatory factors in a dose-dependent manner, with significant differences compared to the CUMS group (Figure 5A–C). Nissl and H&E staining showed that the neuronal cells in the Control group were normal without obvious damage, while neuronal cell

damage was evident in the CUMS group. CHR could inhibit neuronal cell damage, with a significantly higher number of surviving neuronal cells compared to the CUMS group (Figure 5D, E)

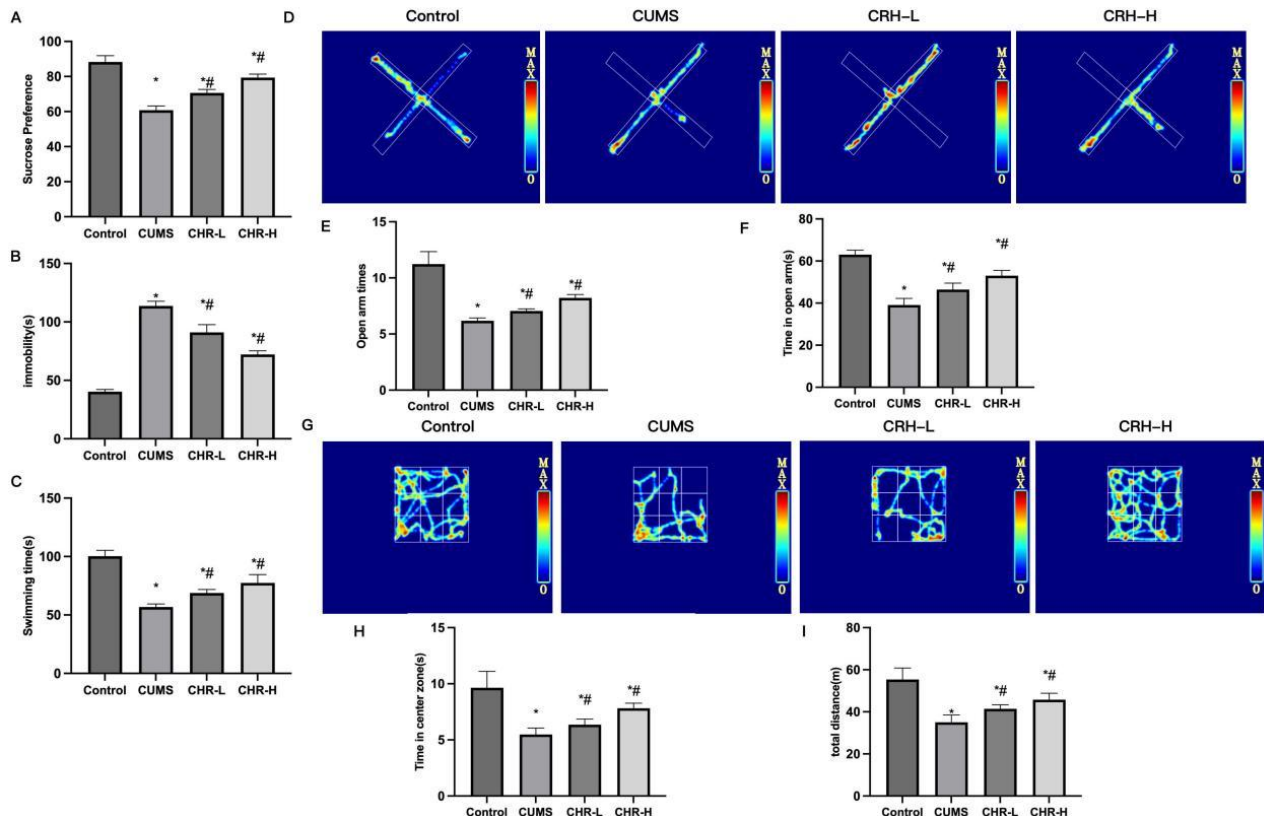


Figure 4. Chrysoeriol (CHR) improves behavior in chronic unpredictable mild stress (CUMS) mice. A, Sucrose preference test (SPT) (n=10); the sucrose preference of CUMS mice was lower than that of the Control group, and CHR could significantly improve the preference degree of mice, with a significant difference compared to the CUMS group. B, Tail suspension test (TST) (n=10); the immobility time in the CUMS group was significantly higher than that in the Control group, while the immobility time in the CHR group was significantly shorter than that in the CUMS group. C, Forced swim test (FST) (n=10); the immobility time of CUMS mice was higher than that of the Control group, while the swimming time was lower than that of the Control group. CHR could significantly increase the swimming time of mice. D–F, Elevated plus maze (n=10); the time and number of entries into the open arms in the CUMS group were significantly lower than those in the Control group, while CHR could increase the time and number of entries into the open arms of mice, with a significant difference compared to the CUMS group. G–I, Open field test (n=10); the total distance traveled and the time spent in the center of the field in the CUMS group were significantly lower than those in the Control group, while CHR could increase the time spent in the center of the field and the total distance traveled by mice. * $p < 0.05$ compared to Control; # $p < 0.05$ compared to CUMS.

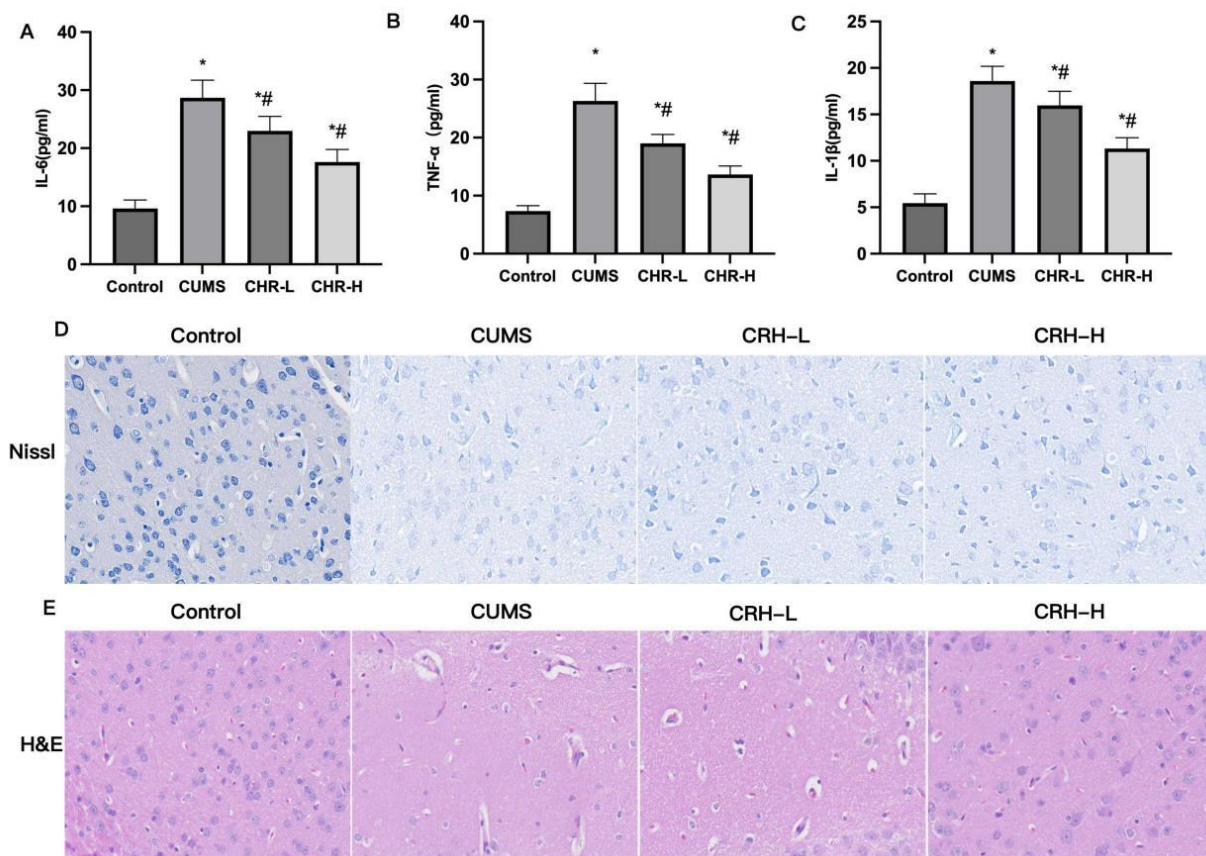


Figure 5. Chrysoeriol (CHR) inhibits neuroinflammation and neuronal damage. A–C, Enzyme-linked immunosorbent assay (ELISA) (n=10); the levels of interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β) in the chronic unpredictable mild stress (CUMS) group were higher than those in the Control group. CHR could reduce the levels of inflammatory factors in a dose-dependent manner, with significant differences compared to the CUMS group. D, E, Tissue staining (n=10); the neuronal cells in the Control group were normal without obvious damage, while neuronal cell damage was evident in the CUMS group. CHR could inhibit neuronal cell damage, with a significantly higher number of surviving neuronal cells compared to the CUMS group. * $p < 0.05$ compared to Control; # $p < 0.05$ compared to CUMS.

DISCUSSION

Depression is a complex mental disorder that has is a major public health concern because it can cause persistent low mood and functional impairment. Research into the etiology, pathophysiology, and mechanisms of depression is key to advancing methods for the prevention and treatment of depression.¹¹ In recent years, immune inflammation has been considered a potentially important factor in the development of major depressive disorder.¹² Various clinical and experimental results have shown that immune inflammation is involved in the occurrence of major depressive disorder and have revealed the pathological mechanisms by which immune inflammation leads to

depressive episodes. Immune inflammation is a nonspecific or specific recognition and elimination response of the immune system to antigens from external sources or within the body, and it is a natural defensive response of tissues to damaging factors.^{13,14} Experimental studies have shown that the role of immune inflammation in causing depression is mainly due to the release of inflammatory factors by immune cells, which cross the blood-brain barrier to activate or exacerbate immune cell responses in the brain,¹⁵ or cause structural and functional changes in brain regions related to the neuroendocrine axis, neurotransmitters, and emotion regulation, leading to the occurrence of depression.¹⁶

Depression and autoimmune diseases share common characteristics, such as genetic susceptibility and family history.¹⁷ It has been confirmed that immune inflammation is closely related to the pathophysiology of depression.¹⁸ The dysfunction of the innate and adaptive immune systems is a key cause of depression in patients. The immune system can regulate emotions, and the dysregulation of inflammatory responses in depressed patients is an important factor. Controlling immune inflammation secondary to early life trauma, acute stress responses, changes in the microbiota, genetic predisposition, etc, may be an effective method for the prevention and treatment of depression.^{19,20} Communication between the peripheral immune system and the brain immune system can lead to brain inflammatory processes, which in turn can impair neurogenesis and synaptic plasticity. Therefore, measuring brain inflammation and its possible correlation with peripheral inflammatory processes has become a focus of many recent studies. Current research has found that neuroinflammation plays an important role in depression, and inhibiting neuroinflammation has become an important method for treating depression.

Chrysoeriol is a natural flavonoid compound found in many traditional Chinese medicines and medicinal plants and has the potential to be a good candidate for new drug development. In the study of anti-inflammatory pharmacology, CHR has been shown to have therapeutic effects on acute inflammation in mouse ears induced by croton oil. It can block the activation of inflammatory signaling pathways by inhibiting the phosphorylation of nuclear transcription factor p-65 and STAT-3, inhibit the secretion of pro-inflammatory factors, such as iNOS, COX-2, IL-6, IL-1 β , and TNF- α , reduce tissue inflammatory cell infiltration, and inhibit ear swelling.^{22,23} In our study, CHR was investigated in the context of depression and neuroinflammation. Persistent neuroinflammation in the brain is a critical pathological driver of depression-like behavioral disorders. Overexpressed pro-inflammatory cytokines can activate the NF- κ B signaling cascade, further aggravating the release of inflammatory mediators and forming a positive feedback loop of neuroinflammation. Chronic neuroinflammation impairs hippocampal synaptic plasticity, suppresses the proliferation and differentiation of neural progenitor cells, downregulates the expression of brain-derived neurotrophic factor (BDNF), and disrupts the homeostasis of the hypothalamic-pituitary-adrenal (HPA) axis, ultimately

leading to anhedonia, despair, decreased locomotor activity, and other core depressive phenotypes observed in CUMS mice. In the present study, we observed that CHR simultaneously decreased cortical IL-1 β , IL-6, and TNF- α levels and alleviated multiple depression-like behaviors in CUMS mice, showing a correlative trend between anti-neuroinflammatory activity and antidepressant effects. Network pharmacology identified predicted CHR-related targets, such as *AKT1*, *ALB*, and *CASP3*. *AKT1* can exert its effects through downstream NF- κ B, activating inflammatory responses, while *CASP3* can mediate cell pyroptosis through GSDME, promoting the progression of inflammation.^{24,25} However, the expression and phosphorylation levels of core proteins, including AKT1, p-AKT, and NF- κ B p65, have not been verified by molecular experiments, such as western blot or quantitative polymerase chain reaction (qPCR), and the regulatory relationship between CHR and this pathway remains to be further confirmed in follow-up studies.

As a flavonoid compound, CHR has diverse targets. We verified the effects of CHR at the animal level. CUMS is a commonly used mouse model for depression. Mice exhibit significant cognitive impairment, with significantly reduced movement ability and reactivity. Behavioral tests, such as TST, FST, and OFT, showed that the movement ability of mice in the CUMS group was significantly reduced, and the mice appeared indifferent. CHR significantly improved the behavior of CUMS mice, with a significant increase in movement ability in a dose-dependent manner. Our detection of inflammatory factor levels showed that the levels of IL-6, TNF- α , and IL-1 β in the CUMS group were significantly higher than those in the Control group. Nevertheless, only cortical inflammatory cytokine levels were quantified in this study, and microglial activation markers (Iba1, CD68) were not detected. Reduced concentrations of pro-inflammatory cytokines cannot directly confirm that CHR inhibits the overactivation of microglia, which are the primary effector cells mediating neuroinflammation in the central nervous system. Future studies should further assess microglial morphological changes and activation status to fully confirm the anti-neuroinflammatory effect of CHR. High expression of neuroinflammatory factors can cause neuronal damage, and CHR can reduce the level of neuronal damage. Together, network pharmacology analysis and animal experiments supported the effects of CHR on depression-like behavior and

neuroinflammation, while the predicted mechanisms require molecular validation.

This study has several inherent limitations. First, the core signaling pathways and hub targets, such as the AKT1/NF- κ B axis screened by network pharmacology, lack direct molecular experimental verification via western blot, qPCR, or immunohistochemistry, and the regulatory interaction between CHR and these targets is based only on bioinformatic prediction without protein or gene expression evidence. Second, only 3 pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) were tested to evaluate neuroinflammation, while microglial activation markers Iba1 and CD68 were not detected, so changes in cytokine levels cannot fully reflect the activation status of central microglia. Third, all in vivo experiments used only the CUMS depression mouse model, and other depression models were not applied to verify the generalizability of CHR's antidepressant activity. Fourth, the range of inflammatory mediators assessed was limited, without quantification of IL-10, iNOS, COX-2, and other key inflammatory mediators to comprehensively assess CHR's anti-inflammatory capacity. Finally, only a 4-week CHR intervention was performed, and long-term efficacy, chronic toxicity, and organ safety assessments were absent, failing to provide evidence for the safety of long-term oral administration of CHR.

In summary, integrated network pharmacology prediction and CUMS mouse model experiments preliminarily demonstrated that CHR alleviates multiple depression-like behaviors and exerts neuroprotective effects by suppressing cortical neuroinflammatory responses in mice. Bioinformatics screening indicated that the multitarget regulatory effect of CHR is closely associated with hub genes, including *AKT1* and *CASP3*, though the underlying molecular signaling cascade remains unvalidated. The current preclinical animal data provide only a preliminary experimental basis for the antidepressant activity of CHR, rather than confirming definite therapeutic efficacy. Further comprehensive molecular validation, validation in multiple animal models, and long-term safety evaluation are required to support the development of CHR as a candidate antidepressant agent.

STATEMENT OF ETHICS

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of

Soochow University (approval no. ap-irb-sop-2024-242071) and were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Network-pharmacology source databases are publicly accessible as described in the Methods section.:

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Messaoud A, Mensi R, Douki W. Reduced peripheral availability of tryptophan and increased activation of the kynurenine pathway and cortisol correlate with major depression and suicide. *World J Biol Psychiatry*. 2019;20(9):703–11.
2. Liu Q, He H, Yang J, Feng X, Zhao F, Lyu J, et al. Changes in the global burden of depression from 1990 to 2017: findings from the Global Burden of Disease study. *J Psychiatr Res*. 2020;126:134–40.
3. Kalin NH. New insights into major depression and the treatment of bipolar depression. *Am J Psychiatry*. 2021;178(12):1071–4.
4. Pandey GN, Rizavi HS, Zhang H, Ren X, Bhaumik R, Dwivedi Y. Abnormal protein and mRNA expression of inflammatory cytokines in the prefrontal cortex of depressed individuals who died by suicide. *J Psychiatry Neurosci*. 2018;43(6):376–85.
5. Bouças AP, Rheinheimer J, Lagopoulos J. Why severe

- COVID-19 patients are at greater risk of developing depression: a molecular perspective. *Neuroscientist*. 2022;28(1):11–9.
6. Jabbi M, Arasappan D, Eickhoff SB, Cattaneo A, Höfle M, Daskalakis ZJ, et al. Neuro-transcriptomic signatures for mood disorder morbidity and suicide mortality. *J Psychiatr Res*. 2020;127:62–74.
 7. Barnes J, Mondelli V, Pariante CM. Genetic contributions of inflammation to depression. *Neuropsychopharmacology*. 2017;42(1):81–98.
 8. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand*. 2017;135(5):373–87.
 9. Syed SA, Beurel E, Loewenstein DA, Lowell JA, Craighead WE, Dunlop BW, et al. Defective inflammatory pathways in never-treated depressed patients are associated with poor treatment response. *Neuron*. 2018;99(5):914–24.
 10. Lamers F, Milaneschi Y, de Jonge P, Giltay EJ, Penninx BWJH. Metabolic and inflammatory markers: associations with individual depressive symptoms. *Psychol Med*. 2018;48(7):1102–10.
 11. Lushchak VI, Duszenko M, Gospodaryov DV, Garaschuk O. Oxidative stress and energy metabolism in the brain: midlife as a turning point. *Antioxidants (Basel)*. 2021;10(11):1715.
 12. Kowalczyk P, Sulejczak D, Kleczkowska P, Bukowska-Ośko I, Kucia M, Popiel M, et al. Mitochondrial oxidative stress—a causative factor and therapeutic target in many diseases. *Int J Mol Sci*. 2021;22(24):13384.
 13. Szebeni A, Szebeni K, DiPeri TP, Johnson LA, Stockmeier CA, Crawford JD, et al. Elevated DNA oxidation and DNA repair enzyme expression in brain white matter in major depressive disorder. *Int J Neuropsychopharmacol*. 2017;20(5):363–73.
 14. Jiménez-Fernández S, Gurpegui M, Garrote-Rojas D, Gutiérrez-Rojas L, Carretero MD, Correll CU, et al. Oxidative stress parameters and antioxidants in adults with unipolar or bipolar depression versus healthy controls: systematic review and meta-analysis. *J Affect Disord*. 2022;314:211–21.
 15. Kong Y, Liu C, Zhang C, Wang Y, Li X, Yang X. Association between serum uric acid levels and suicide attempts in adolescents and young adults with major depressive disorder: a retrospective study. *Neuropsychiatr Dis Treat*. 2022;18:1469–77.
 16. Zhang K, Bai Y, Song T, Liu H, Li Y, Chen Y. In vivo and in vitro evidence of protective effects of a natural flavone on rat myocardial ischemia-reperfusion and hypoxia-reoxygenation injuries. *J Cardiovasc Pharmacol Ther*. 2013;18(1):31–6.
 17. Wu JY, Chen YJ, Bai L, Liu Y, Fu X, Zhu PL, et al. Chrysoeriol ameliorates TPA-induced acute skin inflammation in mice and inhibits NF- κ B and STAT3 pathways. *Phytomedicine*. 2020;68(12):153173.
 18. Choi DY, Lee JY, Kim MR, Woo ER, Kim YG, Kang KW, et al. Chrysoeriol potently inhibits the induction of nitric oxide synthase by blocking AP-1 activation. *J Biomed Sci*. 2005;12(6):949–59.
 19. Limboonreung T, Tuchinda P, Chongthammakun S. Chrysoeriol mediates mitochondrial protection via PI3K/Akt pathway in MPP⁺-treated SH-SY5Y cells. *Neurosci Lett*. 2020;714(14):134545.
 20. Wigner P, Synowiec E, Czarny P, Bijak M, Talarowska M, Galecki P, et al. Effects of venlafaxine on the expression level and methylation status of genes involved in oxidative stress in rats exposed to a chronic mild stress. *J Cell Mol Med*. 2020;24(10):5675–94.
 21. Celikbilek A, Gocmen AY, Tanik N, Borekci E, Zararsiz G, Benli S. Serum lipid peroxidation markers are correlated with those in brain samples in different stress models. *Acta Neuropsychiatr*. 2014;26(1):51–7.
 22. Belvederi Murri M, Pariante C, Mondelli V, Masotti M, Atti AR, Mellacqua Z, et al. HPA axis and aging in depression: systematic review and meta-analysis. *Psychoneuroendocrinology*. 2014;41(5):46–62.
 23. Du X, Pang TY. Is dysregulation of the HPA-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Front Psychiatry*. 2015;6:32.
 24. Alcocer-Gómez E, de Miguel M, Casas-Barquero N, Núñez-Vasco J, Sánchez-Alcázar JA, Fernández-Rodríguez A, et al. NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. *Brain Behav Immun*. 2014;36:111–7.
 25. Zoubovsky SP, Hoseus S, Tumukuntala S, Rupprecht R, Wotjak CT, Murgatroyd C. Chronic psychosocial stress during pregnancy affects maternal behavior and neuroendocrine function and modulates hypothalamic CRH and nuclear steroid receptor expression. *Transl Psychiatry*. 2020;10:6.