Ind. J. Pharm. Edu. Res., 2026; 60(4):1596-1605.
https://www.ijper.org

Original Article

Tanshinone IIA Ameliorates Chronic Unpredictable Mild Stress Induced Depression-Like Behaviors: Integrating Network Pharmacology and Molecular Mechanism Evaluation

Qun Li[1,#], Yuancheng Jin[1,#], Yuhua Wu[1], Menglu Zhu[1], Saiwei Wu[1], Guoqing Zhang[3,*], Zhujin Song[1,2,*]

[1]Department of Pharmacy, the Fourth Affiliated Hospital of School of Medicine and International School of Medicine, International Institutes of Medicine, Zhejiang University, Yiwu, CHINA.
[2]Department of Basic Theory of Traditional Chinese Medicine, School of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, CHINA.
[3]Department of Clinical Nutrition, West China Hospital, Sichuan University, Chengdu, CHINA.
[#]Qun Li and Yuancheng Jin are the first authors of the present study.

ABSTRACT

Background: Depression is a commonly occurring mental disease and many active ingredients of herbs have been proven to effectively alleviate depression. **Objectives:** To investigate the effects of Tanshinone IIA (Tan IIA) on depression and determine potential mechanisms. **Materials and Methods:** The antidepressant mechanism of Tan IIA was predicted by network pharmacology. Then, we established Chronic Unpredictable Mild Stress (CUMS)-induced depression in a mouse model for validation and the mice were administered with Tan IIA during the 15th day-the 28th day (20 and 40 mg/kg). And related proteins were determined by western blotting or immunofluorescence. The levels of cytokines and neurotransmitters were detected by ELISA. Synaptic changes were visualized by Golgi staining and western blotting. **Results:** Network pharmacology predicted PI3K/Akt pathway and TNF-α/TNFR1 pathway played significant roles in the treatment of depression by Tan IIA. And CUMS mice was used to verify. Tan IIA reduced the immobility time of CUMS mice in Forced Swim Test (FST) and Tail Suspension Test (TST). Meanwhile, CUMS mice consumed more sucrose after Tan IIA treatment and it improved the body weight. Importantly, Tan IIA alleviated CUMS-stimulated release of IL-1β and TNF-α, activation of astrocytes and microglia, reduction of synaptic proteins (PSD95, Arc, BDNF). Moreover, Tan IIA promotes the phosphorylation of PI3K/Akt and inhibits the expression of TNFR1 in the hippocampal. **Conclusion:** Tan IIA improves depression-like behaviors possibly through inhibiting neuroinflammation, increasing synaptic plasticity and improving neurotransmitter levels in the hippocampus.

Keywords: Tanshinone IIA, Depression, CUMS, Network pharmacology.

Correspondence:
Dr. Zhujin Song[1,2]
[1]Department of Pharmacy, the Fourth Affiliated Hospital of School of Medicine and International School of Medicine, International Institutes of Medicine, Zhejiang University, Yiwu, CHINA.
[2]Department of Basic Theory of Traditional Chinese Medicine, School of Basic Medical Science, Zhejiang Chinese Medical University, Hangzhou, CHINA.
Email: 8023034@zju.edu.cn

Dr. Guoqing Zhang
Department of Clinical Nutrition, West China Hospital, Sichuan University, Chengdu, CHINA.
Email: gqzhang@wchscu.edu.cn

Received: 24-04-2025;
Revised: 09-06-2025;
Accepted: 19-09-2025.

INTRODUCTION

Depression is a common neuropsychiatric disease and affects 15%-20% of the global population.[1] According to the WHO survey, the number of people with depression in the world exceeds 300 million.[2] Alarmingly, depression has developed into the leading cause of disability and is also an important factor in economic burden.[3] Although depression is associated with neurotransmitter,[4-6] hyperactivity of HPA axis,[7] neuronal apoptosis,[6] neuroinflammation,[8] oxidative stress,[9] and gut microbiota,[10] the specific pathogenesis of depression is still unclear and effective drugs for depression with few side effects is lacking.

Salvia miltiorrhiza is mainly used to treat various cardiovascular and cerebrovascular diseases.[11-13] According to the structural characteristics of *Salvia miltiorrhiza*, it is divided into water-soluble polyphenol compounds and lipophilic chemical substances, including Tan I and Tan IIA.[14-16] Tan IIA has definite anti-inflammatory, antioxidant and neuroprotective effects.[17,18] Moreover, previous studies have also confirmed Tan IIA can improve Aβ-induced cognitive impairment and behavioral changes by inhibiting neuroinflammation.[19,20] Currently, Tan IIA may alleviate depression and this effect is achieved through the

DOI: 10.5530/ijper.20260395

ERK/CREB/BDNF pathway.[21] However, the specific mechanism of Tan IIA's antidepressant effects remains unclear.

The hippocampus is one of the key brain regions in the brain involved in regulating learning, memory and emotion,[22] and neuroinflammation and synaptic plasticity in this brain region are considered to be closely related to depression.[23] Meanwhile, neuroinflammation can cause synaptic degeneration and neuronal cell death.[24,25] And PI3K-Akt pathway can induce inflammation and also lead to synaptic damage, so this pathway is significant for depression.[26,27] Although Tan IIA can alleviate many neurological diseases, the exact molecular mechanism of its antidepressant effects is still not clear. Therefore, we aimed to investigate the antidepressant effects of Tan IIA and explore the role of PI3K-Akt pathway in the treatment of depression with Tan IIA.

MATERIALS AND METHODS

Potential targets of depression and predicted targets of Tan IIA

We collected potential targets from databases, including OMIM database (http://www.omim.org); GeneCards(https://www.genecards.org/); DisGeNET (https://www.disgenet.org/); Therapeutic Target Database (http://bidd.nus.edu.sg/group/cjttd/); and DrugBank (https://www.drugbank.ca/). The search terms were depression, depressive disorder and antidepressant.

SwissTarget Prediction database (http://www.swisstargetprediction.ch/), SEA (https://sea.bkslab.org/), STITCH (http://stitch.embl.de/) and PharmMapper database (http://lilab.ecust.edu.cn/pharmmapper/) were used to predict the targets of Tan IIA. The UniProt database (http://www.uniprot.org/) was applied to standardize these targets.

PPI network construction and hub targets analysis

The common targets of Tan IIA and depression were constructed in the String Database to construct a Protein-Protein Interaction (PPI) network and Homo sapiens were selected as the target organism. Topological analysis was performed on the four dimensions of MNC, MCC, Degree and Betweenness centrality using Cytoscape plug-in CytoHubba. The common goal is identified as the pivot goal by intersecting the first fifteen goals of the four dimensions.

Enrichment analysis and functional prediction

Gene Ontology (GO) annotation and Genomes (KEGG) pathway enrichment analysis were performed to predict the potential functions and pathways using the Comparative Toxicogenomics Database (CTD; http://ctdbase.org/). In order to explore the functional interactions of biological processes, the Cytoscape plug-in ClueGO and Cluepedia was applied.

Animals and drugs

Male C57BL/6 mice (22-26 g, 8 weeks old) were purchased from the Animal Center of Zhejiang Chinese Medical University. And then housed in a room with a temperature of 20-24°C and a relative humidity of 40-55%, a 12/12 hr light/dark cycle. Animal health was managed by caretakers. All experiments were conducted in accordance with the Guidelines for the Care and Use of Experimental Animals published. The experimental procedures were approved by the Ethics Committee of Zhejiang Chinese Medical University (Hangzhou, China; No approval: 20210322-03). The efforts were made to reduce the suffering of mice, as well as the number of mice.

The dose of Tan IIA was chosen according to previous research.[21,28] Tan IIA was purchased from Solarbio (HPLC≥98%, Beijing, China, Cat No: IT0530). Tan IIA was administered to mice at two doses (20 mg/kg, 40 mg/kg). Fluoxetine was purchased from Macklin (HPLC=99%, Shanghai, China, Cat No: 56296-78-7) and the dose of fluoxetine is 10 mg/kg.[29]

Chronic unpredictable mild stress (CUMS)-induced depression model

The mice were randomly divided into 6 groups: Control, CUMS, CUMS+Tan IIA (20 mg/kg, 40 mg/kg), CUMS+Fluoxetine (10 mg/kg), Control+Tan IIA (40 mg/kg). And they were adapted during the 1st week. CUMS was employed to model depression in mice and ten conditions were selected, including: (1) Fasting (24 hr); (2) Water deprivation (24 hr); (3) Exposure to a peculiar smell (2 hr); (4) Humid bedding (2 hr); (5) Thermal-stimulation (45°C, 5 min); (6) Ice-stimulation (4°C, 5 min); (7) Tail-clipping (3 min); (8) Tilted cage (2 hr); (9) Light (24 hr); (10) Restraint (2 hr). And two of them were randomly selected each day for three weeks.[8,30]

Tail Suspension Test (TST)

Mice were taped to the edge of a shelf about 30 cm from the bottom of the soundproof box. Mice were allowed to move freely for 6 min and recorded by Supermaze software (Shanghai Xinruan Information Technology Co., Ltd). The immobility time of mice during the last 4 min was recorded and quantified.

Forced Swimming Test (FST)

24 hr after TST, the mice were subjected to FST. They were successively placed in a glass container with 1200 mL water and the water temperature was maintained at 20-22°C. The mice swam freely for 6 min and recorded by Supermaze software. The immobility time of mice during the last 4 min was recorded and quantified.

Sucrose Preference Test (SPT)

The SPT was performed on the 7th, 14th, 21st day. During the 1st day-6th day, they were trained to acquaint sucrose. During the

CUMS, the SPT was performed after water deprivation. And the bottles containing 1% sucrose and the bottles containing water were placed randomly to avoid positional preference. The sucrose preference index=sucrose consumption/(sucrose consumption+water consumption).

Western blotting

The Hippocampus (HIP) was homogenized with protein lysate for 30 min. After homogenization, the EP tubes were placed in a centrifuge (4°C, 12000 rpm, 5 min). The concentration was detected by kits (Beyotime Institute of Biotechnology). Then, they were isolated by SDS-PAGE (12% gel, 115V, 70 min) and transferred onto the NC membranes (150 mA, 60-120 min). The membranes were blocked with 5% skimmed milk (dissolved in PBS containing 0.1% Tween-20) for 2-3 hr at room temperature and then incubated overnight with primary antibody at 4°C. Finally, the proteins and a peroxidase-conjugated goat anti-rabbit IgG (1:10000, Bioworld, BS13278) were incubated at room temperature for 1.5-2 hr. The band intensity was quantified by ImageJ v10 (National Institutes of Health). The quantification of target proteins was correlated with GAPDH.

The primary antibodies used were p-PI3K (1:1000, Abcam, ab278545), PI3K (1:1000, CST, 4255S), p-Akt (1:1000, CST, 4060T), Akt (1:1000, CST, 2920S), Iba1 (1:500, CST, 17198S), GFAP (1:1000, Proteintech Group, Inc, 16825-1), PSD95(1:1000, CST, 2507S), Arc(1:1000, Synaptic Systems, 156003), BDNF(1:500, Novus, NB100-98682), IL-1β (1:1000, CST, 31202S), TNF-α (1:1000, Proteintech Group, Inc, 17590-1), TNFR1 (1:1000, Proteintech Group, Inc, 21574-1-AP) and GAPDH (1:1000, Xianzhi Biological, AB-P-R001).

Immunofluorescence

The whole brain was fixed in 4% PFA for one week and then dehydrated with 30% sucrose (4°C, 24 hr). The frozen sections were blocked in 0.1 M PBS (10% goat serum+0.4% Triton X-100) for 1 hour and incubated in the primary antibody (4°C, overnight). Then, sections were washed in 0.1m PBS 5 times. Finally, they were incubated with either goat anti-mouse IgG H&L (Alexa Fluor®488) (1:100, ZSGB-BIO, ZF-0312) or goat anti-rabbit IgG H&L (Alexa Fluor®594) (1:100, ZSGB-BIO, ZF-0311). The images were obtained by Olympus FV 1000 confocal laser scanning microscope (Olympus, Tokyo, Japan). The primary antibodies used were GFAP (1:100, Proteintech Group, Inc, 16825-1-AP), Iba1 (1:100, CST, 17198S).

ELISA

The venous blood of the mice was collected to detect the levels of neurotransmitters and cytokines. The blood was clotted for 20 min, then centrifuged (15 min, 3500 rpm) and serum was finally collected. Levels of neurotransmitters and cytokines were detected in the HIP and Prefrontal Cortex (PFC). 10 mg HIP (PFC) was mixed with 100 uL of PBS (pH7.4), then centrifugation had been performed (15 min, 2500 rpm, 4°C) and collected supernatant. Thereafter, the levels of 5-HT, NE, IL-1β and TNF-α were determined according to the instructions provided by the 5-HT (JL12087), NE (JL13969), IL-1β (JL18442) and TNF-α (JL10484) ELISA kits. Finally, the absorbance (OD value) was quickly read on a microplate reader at a detection wavelength of 450 nm.

Statistical analysis

Data were presented as means±SEM. Statistical analyses were performed using GraphPad Prism 8.0.2. Kruskal Wallis test was used for statistical analysis. Kruskal Wallis test and Dunn test were used for multiple comparison tests.$p<0.05$ was considered significant.

RESULTS

The target of Tan IIA against depression

In this study, 2492 depression-related targets and 331 Tan IIA targets were found through the database and the Venn diagram showed that depression and Tan IIA shared 94 targets (Figure 1A). At the same time, the results of MNC, MCC, Degree and Betweenness indicate a total of 12 intersecting targets (AKT, Caspase3, ESR1, PTGS2, EGFR, NOS3, IL-2, CCND1, MMP9, EZH2, MAPK14, EDN1) (Figures 1B-F) and they were identified as the hub targets.

GO and KEGG enrichment analysis

GO enrichment showed metabolic process, response to stress and biological regulations were the main part in the biological processes. And intracellular space was obvious. Molecular functional results highlighted protein binding and signaling receptor binding (Figure 1G). KEGG indicated that Ras pathway, PI3K/Akt pathway, calcium pathway, Rap1 pathway and TNF pathway are very valuable (Figure 1H). ClueGO analysis showed enriched terms were mainly regulation of synaptic transmission, positive regulation of smooth muscle cell proliferation, regulation of monooxygenase activity (Figure 1I). Meanwhile, pathway-target network showed PI3K/Akt pathway was enriched for most key targets including Akt, EGFR, IL-6, MAPK1, TP53 and VEGFA (Figure 1J).

Tan IIA reduces CUMS-induced depression-like behaviors

We investigated antidepressant effect of Tan IIA by CUMS mice. During the CUMS, body weight was recorded weekly. Then, SPT, TST and FST were performed (Figure 2A). During the adaptive period, there was no significant difference in the body weight between the groups (p=0.9879) (Figure 2B). However, after the stress, the body weight of CUMS mice decreased significantly, but either 20 mg/kg or 40 mg/kg Tan IIA could inhibit the weight loss of mice (p=0.0006) (Figure 2C). In the FST, the immobility time of CUMS mice increased (p=0.0025) (Figure 2D). The results of

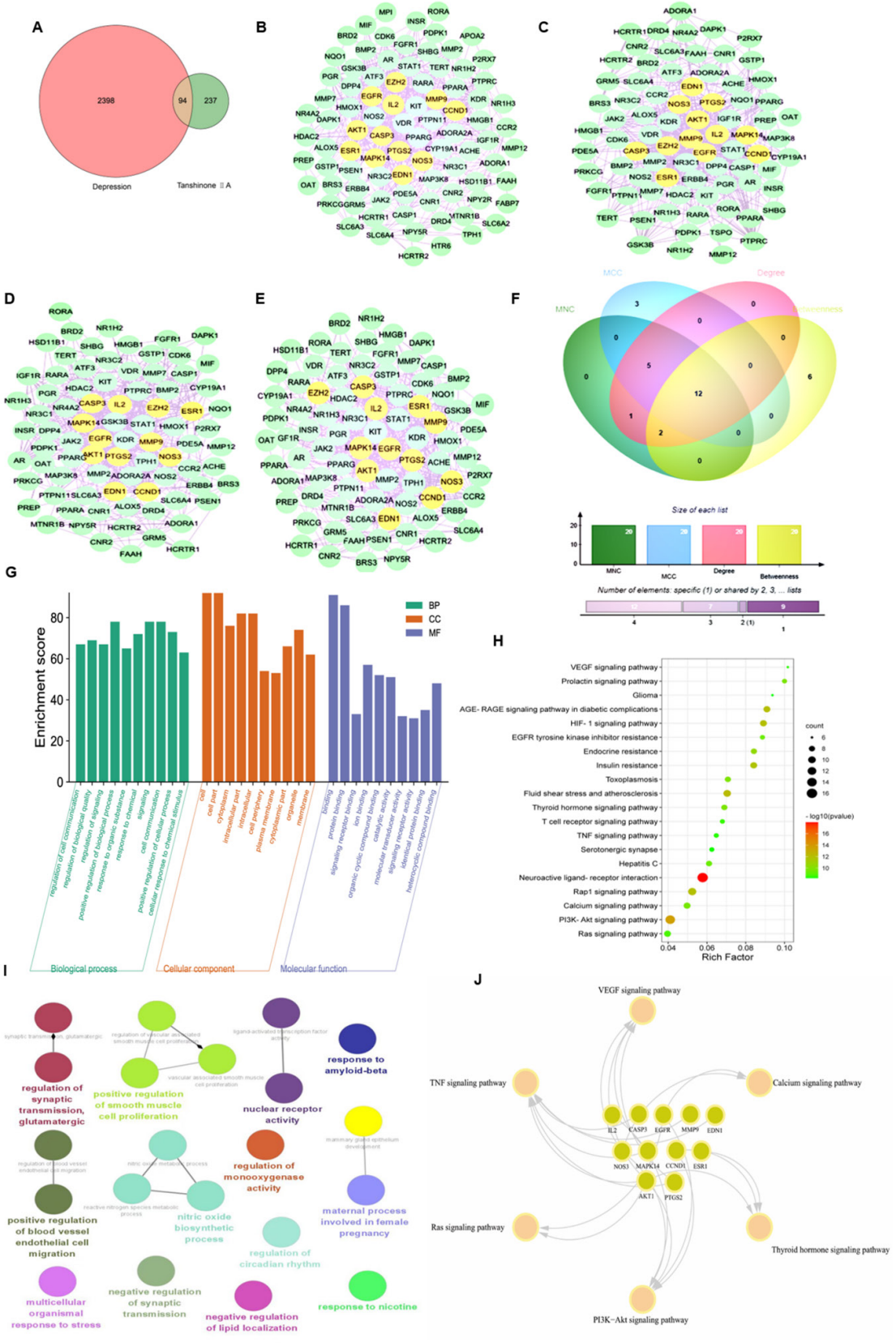

Figure 1: Potential targets of Tan IIA in depression and enrichment analysis the shared targets of Tan IIA in depression. (A) 94 shared targets were identified of Tan IIA against depression. (B-F) 12 intersected targets based on the MNC, MCC, Degree and Betweenness, respectively. (G) Go enrichment analysis. (H) KEGG pathway analysis. (I) ClueGO enrichment analysis. (J) A corresponding network of hub targets and valuable pathways.

TST are the same as well (p=0.0008) (Figure 2E). But Tan IIA could improve CUMS mice. Similar to body weight, there was no difference in sucrose between groups during the adaptive period (p=0.9522) (Figure 2F). However, mice were stimulated for three weeks, the intake of sugar water in the CUMS mice decreased. After Tan IIA treatment, the intake of sucrose in CUMS mice tended to be a normal level (p=0.0022) (Figure 2G).

Tan IIA improves the levels of neurotransmitters and cytokines in CUMS mice

Imbalance of 5-HT and NE is realized as a key mechanism of depression.[31] In our results, the CUMS mice showed lower levels of 5-HT in HIP (p=0.0029), PFC (p=0.0029) and serum (p=0.0024) (Figure 3A) than control group. Similarly, NE in HIP (p=0.0018), PFC (p=0.0018) and serum (p=0.0021) (Figure 3A) of CUMS mice decreased. In contrast, administration of Tan IIA to CUMS mice improved the levels of 5-HT and NE.

The connection between inflammation and depression is well-known to researchers.[32] And we found that TNF-α in HIP (p=0.0017), PFC (p=0.0018) and serum (p=0.0026) of CUMS mice all increased (Figure 3B). And IL-1β in the HIP (p=0.0024), PFC (p=0.0018) and serum (p=0.0024) of CUMS mice also increased (Figure 3B). But after administration of Tan IIA, the levels of IL-1β and TNF-α were decreased to a certain extent. And western blotting showed Tan IIA inhibited TNF-α (p=0.0035) and IL-1β (p=0.0031) in the HIP of CUMS mice (Figures 3C, 3D), which were consistent with ELISA. Moreover, network pharmacology suggested that TNF pathway may play a major role on the treatment of depression by Tan IIA. And we found the expression of TNFR1 in the HIP of CUMS mice were significantly higher than control group and Tan IIA prohibited the upregulation of TNFR1 (p=0.0024) (Figure 3D).

Tan IIA inhibits the activation of astrocytes and microglia in the hippocampus

Inflammation always induces over-activation of glial cells.[33,34] The over-activation of microglia and astrocytes in the DG regions was limited by Tan IIA (Figures 3G-3H). In addition, we found the expression of GFAP (p=0.0043) and Iba1 (p=0.0038) (Figures 3C, 3F) in the HIP of CUMS mice increased compared with the control group.

Tan IIA ameliorates CUMS mice through PI3K/Akt pathway in the hippocampus

Through network pharmacology, it is understood PI3K/Akt pathway may be a critical pathway for Tan IIA to improve

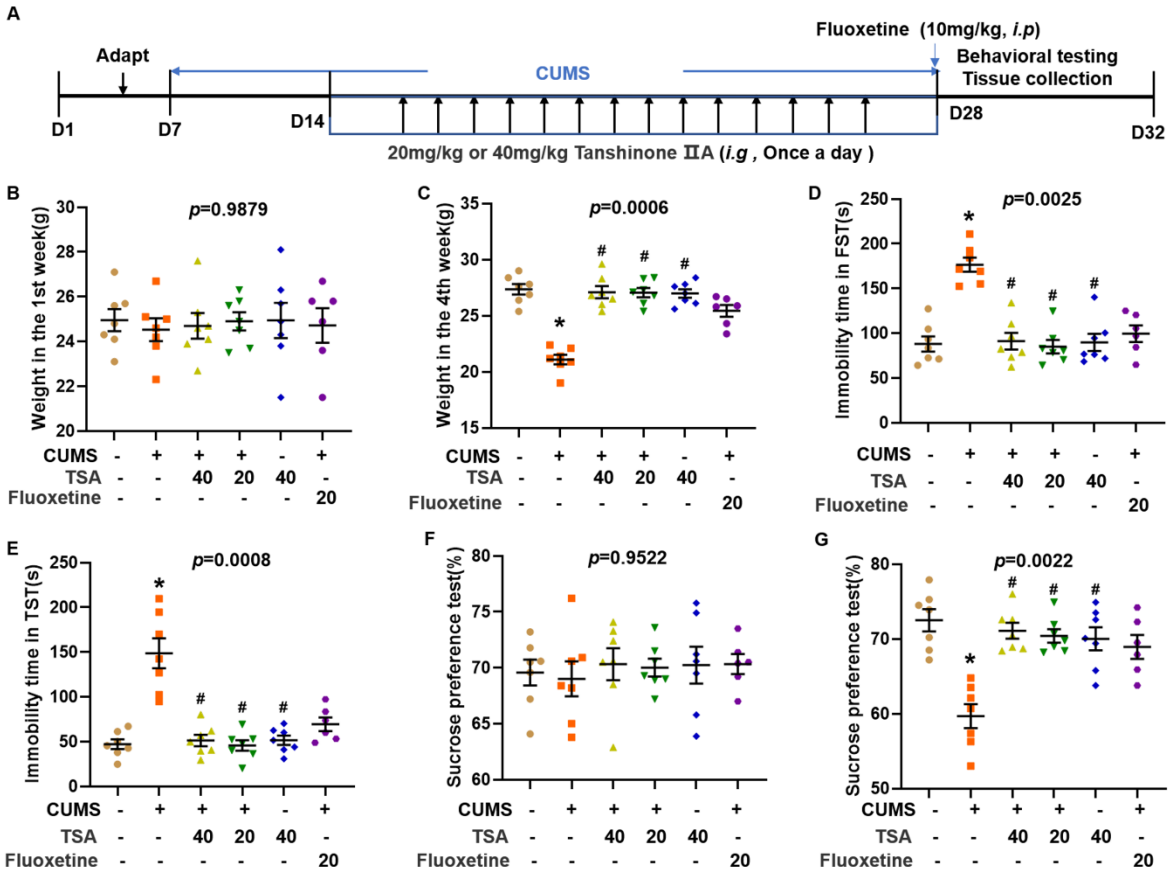

Figure 2: Tan IIA reduces CUMS-induced depression-like behaviors. (A) Schematic diagram of experimental designs; (B-C) Body weight in the 1st week and 4th week (n=6 or 7); (D) Immobility time in the FST (n=6 or 7); (E) Immobility time in the TST (n=6 or 7); (F-G) SPT in the 1st week and 4th week. Data are presented as the mean±SEM. $^{*}p$<0.05 vs Control, $^{\#}p$<0.05 vs CUMS.

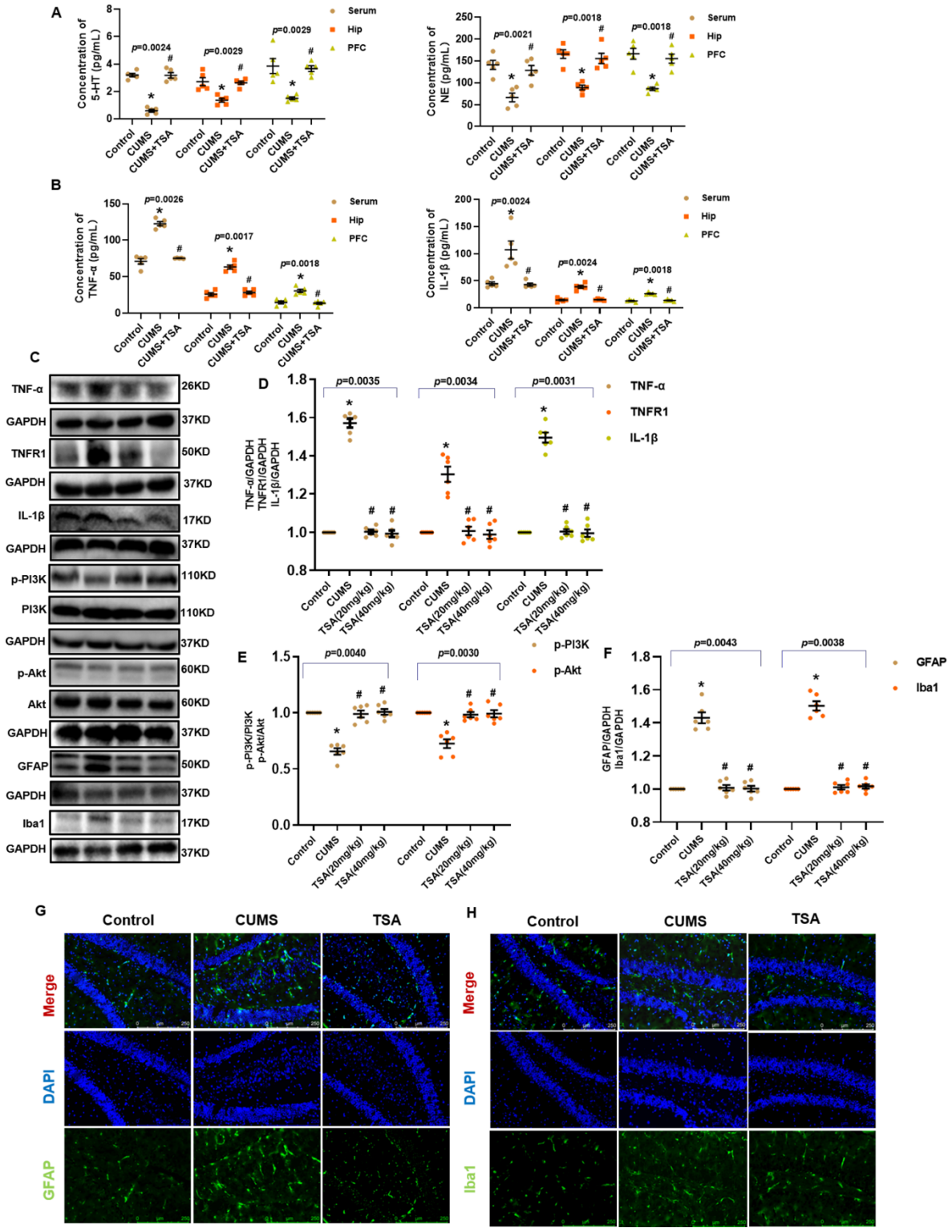

Figure 3: Tanshinone IIA ameliorated neurotransmitter and cytokines levels in CUMS mice, suppressed the overactivation of hippocampal astrocytes and microglia, and promoted the phosphorylation of PI3K and Akt. (A-B) The levels of 5-HT, NE, TNF-α and IL-1β in the HIP, PFC and serum (n=5); (C) Representative blots of TNF-α, TNFR1, IL-1β, p-PI3K, p-Akt, GFAP, Iba1 and GAPDH; (D) The levels of TNF-α, TNFR1 and IL-1β were analyzed (n=6); (E) The level of p-PI3K and p-Akt were analyzed (n=6); (F) The levels of GFAP and Iba1 were analyzed (n=6); (G) Representative image of immunofluorescence for GFAP in DG (GFAP: Green; Nuclear: Blue); (H) Representative image of immunofluorescence for Iba1 in DG (Iba1: Green; Nuclear: Blue). $^{*}p<0.05$ vs Control, $^{\#}p<0.05$ vs CUMS.

depression, hence we have re-verified this point. The results indicated the phosphorylation of PI3K (p=0.0040) and Akt (p=0.0030) decreased in the hippocampus of CUMS mice (Figures 3C, 3E), whereas Tan IIA promoted the p-PI3K and p-Akt in the HIP of CUMS mice.

Tan IIA improved synaptic plasticity in the hippocampus of CUMS mice

Synaptic modulation is a potential and important pathway for treating depression.[35] In our results, the expressions of BDNF (p=0.0037), PSD95 (p=0.0043) and Arc (p=0.0031) decreased in the HIP of CUMS mice, while Tan IIA could improve the expression of BDNF, PSD95 and Arc (Figures 4A-4C). Golgi staining showed Tan IIA increased the number of dendritic spines in the DG region of CUMS mice (p=0.0029) (Figures 4D-4E). And the length of synapses has also been improved by Tan IIA (Figures 4F-4G).

DISCUSSION

This study aims to explore the mechanism by which Tan IIA ameliorates depression. We find Tan IIA can inhibit depression-like behaviors, which may be related to neuroinflammation and synaptic plasticity mediated by TNF-α/TNFR1 pathway and PI3k/Akt pathway in the hippocampus. And the imbalance of neurotransmitters has also been alleviated.

Imbalance of neurotransmitters can induce depression.[31] Fluoxetine is the most common antidepressant in clinic and the effect is achieved by preventing the reuptake of 5-HT,[36] while the antidepressant effect of duloxetine is achieved by preventing the reuptake of NE.[37] And we found Tan IIA promoted the normalization of neurotransmitter levels in CUMS mice, thereby exerting antidepressant effects.

Neuroinflammation has been considered as a significant mechanism of many neurological diseases, including depression, PTSD, AD, PD.[38-41] The burst is often accompanied by over-activation of glial cells and both the over-activation of microglia and astrocytes are believed to mediate the release of cytokines that contribute to depression.[30,42] Tan IIA is an active ingredient of *Salvia miltiorrhiza* and has an exact role in treating some neurological diseases and the realization of this effect may be related to inflammation.[43-45] And previous evidence has also confirmed Tan IIA improves neuroinflammation by TLR4/NF-κB pathway in the hypoxic-ischemic encephalopathy mice.[46] Meanwhile, Tan IIA attenuates neuroinflammation by RAGE/NF-κB pathway, thereby improving memory.[47] Moreover, the over-activation of microglia can be prevented by Tan IIA.[48] Based on these, we believe that the molecular mechanism of Tan IIA's antidepressant effect may be achieved by inhibiting inflammation. And as expected, we also find Tan IIA prevented the massive release of TNF-α and IL-1β. Meanwhile, administration of Tan IIA reduced the number of GFAP- and Iba1-positive cells. Since network pharmacology suggested that TNF-α/TNFR1 pathway plays a major role in the treatment of depression with Tan IIA and Tan IIA suppresses the expression of TNFR1 in the hippocampus.

Depression can be considered a microglia-related disease. As the most common and important type of glial cell, the functions of microglia have garnered significant attention. In the past, a substantial amount of research has clearly demonstrated that microglia can regulate neuroinflammation.[49] And now, its function in regulating the formation of neural networks and synaptic plasticity has also been confirmed.[50] Therefore, synaptic plasticity damage is induced by neuroinflammation[51] and which is closely associated with depression.[52] And in a previous study, we found that the levels of synaptic plasticity-related proteins were decreased in CUMS and LPS mice.[8] Excitingly, Tan IIA has the ability to improve synaptic plasticity, which contributes to attenuate fear memory and anxiety.[53] Moreover, Tan IIA promotes the level of BDNF.[21] And our findings also corroborate the view that Tan IIA can enhance synaptic plasticity in the hippocampus, along with the length of dendrites and the number of dendritic spines. This evidence supports the notion that Tan IIA's improvement of depression is associated with synaptic changes.

The PI3K/Akt pathway is an essential pathway in regulating emotional disorders and has also been confirmed to regulate depression.[27,54] Previous evidence indicates PI3K/Akt pathway can induce NF-κB nuclear translocation, then pro-inflammatory cytokines are released.[55,56] And neuroinflammation is often accompanied by impairment of synaptic plasticity,[57] this finding is consistent with our previous results.[58] Moreover, PI3K/Akt pathway may alleviate depression-like behaviors by improving neuroplasticity.[54,59,60] And in this study, our results indicate PI3K/Akt pathway may be an essential pathway for the treatment of depression with Tan IIA. We find Tan IIA promotes the phosphorylation of PI3K and Akt in the hippocampus of CUMS mice. Meanwhile, Tan IIA inhibits inflammation and improves synaptic plasticity. Under the circumstances we have every reason to suspect that the anti- inflammation and synaptic plasticity-enhancing effects of Tan IIA may be achieved through the PI3K/Akt pathway.

Previous studies have identified that both the PI3K/Akt pathway and the TNF-α/TNFR1 pathway are crucial for the regulation of depression by traditional Chinese medicine monomers.[27,41] Tanshinone IIA has been shown to ameliorate neurological diseases through these pathways. Ma *et al.*'s findings confirmed that Tanshinone IIA can regulate synaptic plasticity of hippocampal neurons via the PI3K/Akt pathway,[61] and its improvement in cognitive dysfunction is also linked to this pathway.[62] Additionally, Tanshinone IIA appears to have the potential to inhibit the TNF-α/TNFR1 pathway, thereby mitigating neuronal damage.[63] There is an interaction between the PI3K/Akt and TNF-α/TNFR1 pathways, likely affecting inflammatory responses, apoptosis, cell survival and proliferation. Phosphorylation of PI3K generally

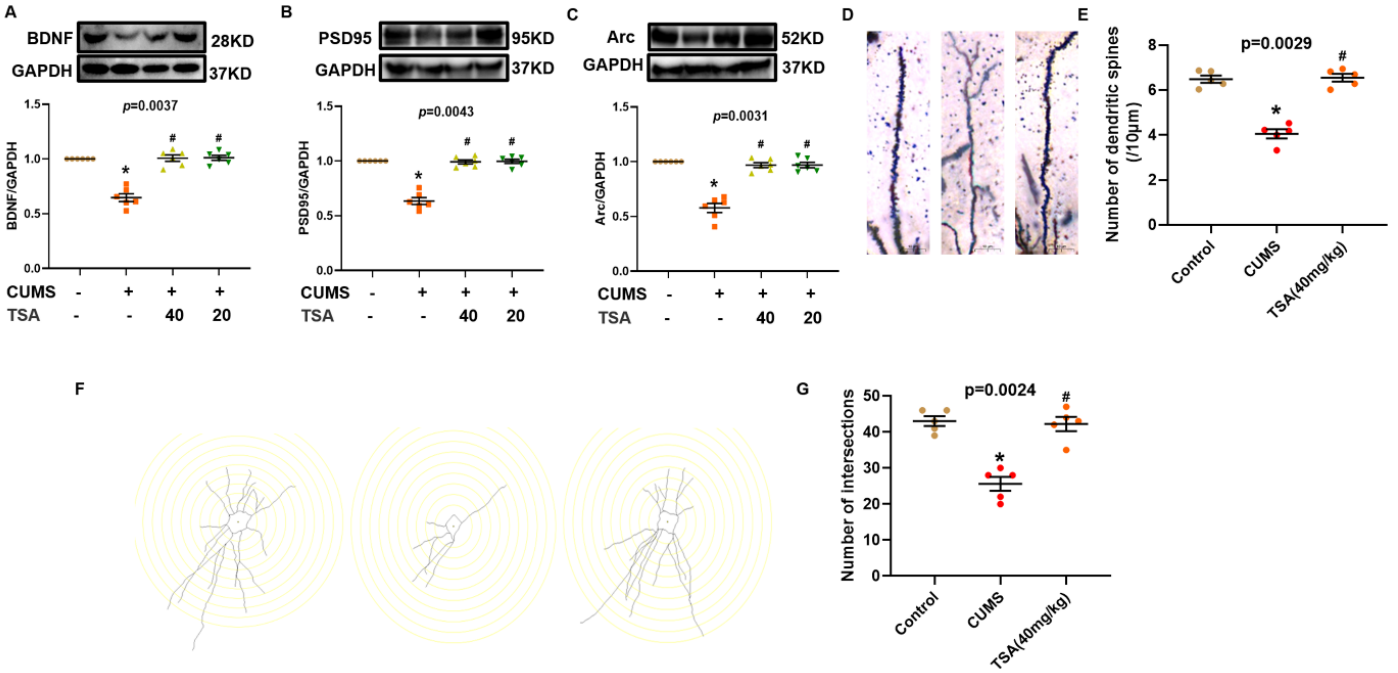

Figure 4: Tan IIA improved synaptic plasticity in the hippocampus of CUMS mice. (A) The level of BDNF was analyzed (*n*=6); (B) The level of PSD95 was analyzed (*n*=6); (C) The level of Arc was analyzed (*n*=6); (D-E) The number of spines in DG; (F-G) Length of synapses in DG. Data were presented as the mean±SEM. $^{*}p<0.05$ vs Control, $^{\#}p<0.05$ vs CUMS.

coincides with inflammation suppression. However, in disease states, inhibition of PI3K phosphorylation leads to nuclear translocation of NF-κB, promoting excessive activation of M1 microglia and the release of pro-inflammatory cytokines, including TNF-α. These TNF-α molecules bind to TNFR1 on cell membranes, further enhancing neuroinflammation.[64] The surge of neuroinflammation can further inhibit the phosphorylation of PI3K and Akt. Additionally, TNF-α can modulate synaptic function at multiple levels, from fine-tuning neurotransmission to regulating synapse numbers and influencing overall neuronal networks and complex behaviors.[65] In this study, although we have identified that the antidepressant mechanism of Tan IIA may involve the regulation of PI3K/Akt and TNF-α/TNFR1 pathways, we lack direct evidence of a high-speed bidirectional relationship between these pathways, which will be the focus of our future research.

CONCLUSION

Tan IIA can improve depression-like behaviors, possibly by inhibiting glial cell activation, improving synaptic plasticity and reducing inflammation. And this study provides new perspectives for treating depression.

ABBREVIATIONS

5-HT: 5-hydroxytryptamine; **NE:** Norepinephrine; **Arc:** Activity-regulated cytoskeleton-associated protein; **PSD95:** Postsynaptic density 95; **BDNF:** Brain-derived neurotrophic factor; **CUMS:** Chronic unpredictable mild Stress; **FST:** Forced swimming test; **TST:** Tail suspension test; **SPT:** Sucrose preference test; **GFAP:** Glial fibrillary acidic protein; **Iba1:** Ionized calcium binding adapter molecule 1; **IL-1β:** Interleukin 1β; **TNF-α:** Tumor necrosis factor-α; **TNFR1:** Tumor necrosis factor receptor 1; **PI3K:** Phosphatidylinositol 3-kinase; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **PPI:** Protein-protein interaction.

FUNDING

This work was financially supported by the Administration of Traditional Chinese Medicine of Zhejiang Province, China (2018ZQ048).

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

ETHICAL STANDARDS

Experimental procedures were approved by the Ethics Committee of Zhejiang University of Chinese Medicine (Hangzhou, China: Approval No.: IACUC-20210719-05).

AUTHOR CONTRIBUTIONS

ZJ-S designed the research; Q-L and GQ-Z performed the research; Q-L and YC-J analyzed the data; ZJ-S and SW-W provided suggestions for the experimental design and manuscript; Q-L, YC-J and GQ-Z wrote the manuscript.

SUMMARY

Depression is a neuropsychiatric disorder that affects 15-20% of people, with a lifetime prevalence of 10%-15% and has developed into one of the most significant disability factors. Although previous studies have confirmed the pathogenesis of depression is related to neurotransmitters, HPA axis, neuroinflammation, synaptic plasticity, oxidative stress, programmed cell death and gut microbiota, the specific pathogenesis of depression is still unclear and antidepressants are generally associated with poor efficacy, high side effects and short duration of action. However, many active ingredients of herbs have been proven to effectively alleviate depression with fewer adverse reactions. Tan IIA is a lipophilic diterpenoid compound extracted from *Salvia miltiorrhiza* and some researchers argue that it can alleviate neuroinflammation, apoptosis and oxidative stress in the CNS. But the mechanism by which Tan IIA intervenes in depression remains unknown. And this study was intended to investigate the effects of Tan IIA on depression and determine potential mechanisms. The current findings of this study reveal Tan IIA administration improves depression-like behaviors in CUMS mice and this effect may be mediated through inhibiting neuroinflammation, increasing synaptic plasticity and improving neurotransmitter levels in the hippocampus. Therefore, it is clear that Tan IIA can be a promising salutary candidate to treat depression.

REFERENCES

1. Hasin DS, Sarvet AL, Meyers JL, Saha TD, Ruan WJ, Stohl M, *et al.* Epidemiology of adult DSM-5 major depressive disorder and its specifiers in the United States. JAMA Psychiatry. 2018; 75(4): 336-46. doi: 10.1001/jamapsychiatry.2017.4602, PMID 29450462.
2. Levey DF, Polimanti R, Cheng Z, Zhou H, Nuñez YZ, Jain S, *et al.* Genetic associations with suicide attempt severity and genetic overlap with major depression. Transl Psychiatry. 2019; 9(1): 22. doi: 10.1038/s41398-018-0340-2, PMID 30655502.
3. Solem S, Hagen R, Wang CE, Hjemdal O, Waterloo K, Eisemann M, *et al.* Metacognitions and mindful attention awareness in depression: a comparison of currently depressed, previously depressed and never depressed individuals. Clin Psychol Psychother. 2017; 24(1): 94-102. doi: 10.1002/cpp.1983, PMID 26450662.
4. Jacobson L. Glucocorticoid receptor deletion from locus coeruleus norepinephrine neurons promotes depression-like social withdrawal in female but not male mice. Brain Res. 2019; 1710: 82-91. doi: 10.1016/j.brainres.2018.12.026, PMID 30576626.
5. Seo JS, Wei J, Qin L, Kim Y, Yan Z, Greengard P. Cellular and molecular basis for stress-induced depression. Mol Psychiatry. 2017; 22(10): 1440-47. doi: 10.1038/mp.2016.118, PMID 27457815.
6. Shen F, Song Z, Xie P, Li L, Wang B, Peng D, et al. *Polygonatum sibiricum* polysaccharide prevents depression-like behaviors by reducing oxidative stress, inflammation and cellular and synaptic damage. J Ethnopharmacol. 2021; 275: 114164. doi: 10.1016/j.jep.2021.114164, PMID 33932516.
7. Juruena MF, Bocharova M, Agustini B, Young AH. Atypical depression and non-atypical depression: is HPA axis function a biomarker? A systematic review. J Affect Disord. 2018; 233: 45-67. doi: 10.1016/j.jad.2017.09.052, PMID 29150144.
8. Song Z, Shen F, Zhang Z, Wu S, Zhu G. Calpain inhibition ameliorates depression-like behaviors by reducing inflammation and promoting synaptic protein expression in the hippocampus. Neuropharmacology. 2020; 174: 108175. doi: 10.1016/j.neuropharm.2020.108175, PMID 32492450.
9. Lindqvist D, Dhabhar FS, James SJ, Hough CM, Jain FA, Bersani FS, *et al.* Oxidative stress, inflammation and treatment response in major depression. Psychoneuroendocrinology. 2017; 76: 197-205. doi: 10.1016/j.psyneuen.2016.11.031, PMID 27960139.
10. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, *et al.* The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 2019; 4(4): 623-32. doi: 10.1038/s41564-018-0337-x, PMID 30718848.
11. Li D, Wang J, Sun D, Gong X, Jiang H, Shu J, *et al.* Tanshinone IIA sulfonate protects against cigarette smoke-induced COPD and down-regulation of CFTR in mice. Sci Rep. 2018; 8(1): 376. doi: 10.1038/s41598-017-18745-5, PMID 29321495.
12. Liu HC, Liu HH. [Adverse reactions of tanshinone II(a) sodium sulfonate injection in treating 18 cases: an analysis of clinical features]. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2013; 33(9): 1287-89. PMID 24273991.
13. Zhou L, Zuo Z. Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics and clinical use. J Clin Pharmacol. 2005; 45(12): 1345-59. doi: 10.1177/0091270005282630, PMID 16291709.
14. Chen J, Wang F, Lee FS, Wang X, Xie M. Separation and identification of water-soluble salvianolic acids from *Salvia miltiorrhiza* Bunge by high-speed counter-current chromatography and ESI-MS analysis. Talanta. 2006; 69(1): 172-79. doi: 10.1016/j.talanta.2005.09.041, PMID 18970550.
15. Ren Y, Houghton PJ, Hider RC, Howes MJ. Novel diterpenoid acetylcholinesterase inhibitors from *Salvia miltiorhiza*. Planta Med. 2004; 70(3): 201-04. doi: 10.1055/s-2004-815535, PMID 15114495.
16. Wang X, Morris-Natschke SL, Lee KH. New developments in the chemistry and biology of the bioactive constituents of Tanshen. Med Res Rev. 2007; 27(1): 133-48. doi: 10.1002/med.20077, PMID 16888751.
17. Hamidpour M, Hamidpour R, Hamidpour S, Shahlari M. Chemistry, pharmacology and medicinal property of Sage (Salvia) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease and cancer. J Tradit Complement Med. 2014; 4(2): 82-8. doi: 10.4103/2225-4110.130373, PMID 24860730.
18. Imanshahidi M, Hosseinzadeh H. The pharmacological effects of Salvia species on the central nervous system. Phytother Res. 2006; 20(6): 427-37. doi: 10.1002/ptr.1898, PMID 16619340.
19. Li J, Wen PY, Li WW, Zhou J. Upregulation effects of tanshinone IIA on the expressions of NeuN, Nissl body and IkappaB and downregulation effects on the expressions of GFAP and NF-kappaB in the brain tissues of rat models of Alzheimer's disease. NeuroReport. 2015; 26(13): 758-66. doi: 10.1097/WNR.0000000000000419, PMID 26164608.
20. Dai C, Liu Y, Dong Z. Tanshinone I alleviates motor and cognitive impairments via suppressing oxidative stress in the neonatal rats after hypoxic-ischemic brain damage. Mol Brain. 2017; 10(1): 52. doi: 10.1186/s13041-017-0332-9, PMID 29137683.
21. Lu J, Zhou H, Meng D, Zhang J, Pan K, Wan B, *et al.* Tanshinone IIA improves depression-like behavior in mice by activating the ERK-CREB-BDNF signaling pathway. Neuroscience. 2020; 430: 1-11. doi: 10.1016/j.neuroscience.2020.01.026, PMID 32014436.
22. Bartsch T, Wulff P. The hippocampus in aging and disease: from plasticity to vulnerability. Neuroscience. 2015; 309: 1-16. doi: 10.1016/j.neuroscience.2015.07.084, PMID 26241337.
23. Qiao H, Li MX, Xu C, Chen HB, An SC, Ma XM. Dendritic spines in depression: what we learned from animal models. Neural Plast. 2016; 2016: 8056370. doi: 10.1155/2016/8056370, PMID 26881133.
24. Li W, Ali T, He K, Liu Z, Shah FA, Ren Q, *et al.* Ibrutinib alleviates LPS-induced neuroinflammation and synaptic defects in a mouse model of depression. Brain Behav Immun. 2021; 92: 10-24. doi: 10.1016/j.bbi.2020.11.008, PMID 33181270.
25. Sekio M, Seki K. Lipopolysaccharide-induced depressive-like behavior is associated with alpha(1)-adrenoceptor dependent downregulation of the membrane GluR1 subunit in the mouse medial prefrontal cortex and ventral tegmental area. Int J Neuropsychopharmacol. 2014; 18(1): pyu005. doi: 10.1093/ijnp/pyu005, PMID 25539502.
26. Zhong Y, Zhu Y, He T, Li W, Li Q, Miao Y. Brain-derived neurotrophic factor inhibits hyperglycemia-induced apoptosis and downregulation of synaptic plasticity-related proteins in hippocampal neurons via the PI3K/Akt pathway. Int J Mol Med. 2019; 43(1): 294-304. doi: 10.3892/ijmm.2018.3933, PMID 30365051.
27. Guo LT, Wang SQ, Su J, Xu LX, Ji ZY, Zhang RY, *et al.* Baicalin ameliorates neuroinflammation-induced depressive-like behavior through inhibition of toll-like receptor 4 expression via the PI3K/AKT/FoxO1 pathway. J Neuroinflammation. 2019; 16(1): 95. doi: 10.1186/s12974-019-1474-8, PMID 31068207.
28. Liu C, Wu Y, Zha S, Liu M, Wang Y, Yang G, *et al.* Treatment effects of tanshinone IIA against intracerebroventricular streptozotocin induced memory deficits in mice. Brain Res. 2016; 1631: 137-46. doi: 10.1016/j.brainres.2015.11.040, PMID 26656068.
29. Liu C, Gu JY, Han JH, Yan FL, Li Y, Lv TT, *et al.* Enriched environment combined with fluoxetine ameliorates depression-like behaviors and hippocampal SYP expression in a rat CUS model. Brain Res Bull. 2017; 135: 33-9. doi: 10.1016/j.brainresbull.2017.09.009, PMID 28939473.
30. Leng L, Zhuang K, Liu Z, Huang C, Gao Y, Chen G, *et al.* Menin deficiency leads to depressive-like behaviors in mice by modulating astrocyte-mediated neuroinflammation. Neuron. 2018; 100(3): 551-563.e7. doi: 10.1016/j.neuron.2018.08.031, PMID 30220511.
31. Nutt DJ. Relationship of neurotransmitters to the symptoms of major depressive disorder. J Clin Psychiatry. 2008; 69 Suppl E1: 4-7. PMID 18494537.
32. Dantzer R. Dantzer R. Cytokine, sickness behavior and depression. Neurol Clin. 2006; 24(3): 441-60. doi: 10.1016/j.ncl.2006.03.003, PMID 16877117.
33. Goshi N, Morgan RK, Lein PJ, Seker E. A primary neural cell culture model to study neuron, astrocyte and microglia interactions in neuroinflammation. J Neuroinflammation. 2020; 17(1): 155. doi: 10.1186/s12974-020-01819-z, PMID 32393376.
34. Norden DM, Trojanowski PJ, Villanueva E, Navarro E, Godbout JP. Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1

or GFAP immunoreactivity following systemic immune challenge. Glia. 2016; 64(2): 300-16. doi: 10.1002/glia.22930, PMID 26470014.

35. Gerhard DM, Wohleb ES, Duman RS. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity. Drug Discov Today. 2016; 21(3): 454-64. doi: 10.1016/j.drudis.2016.01.016, PMID 26854424.
36. Alboni S, van Dijk RM, Poggini S, Milior G, Perrotta M, Drenth T, *et al.* Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol Psychiatry. 2017; 22(4): 552-61. doi: 10.1038/mp.2015.142, PMID 26645631.
37. Bidari A, Moazen-Zadeh E, Ghavidel-Parsa B, Rahmani S, Hosseini S, Hassankhani A. Comparing duloxetine and pregabalin for treatment of pain and depression in women with fibromyalgia: an open-label randomized clinical trial. Daru. 2019; 27(1): 149-58. doi: 10.1007/s40199-019-00257-4, PMID 30877484.
38. Cheng J, Dong Y, Ma J, Pan R, Liao Y, Kong X, *et al.* Microglial Calhm2 regulates neuroinflammation and contributes to Alzheimer's disease pathology. Sci Adv. 2021; 7(35): eabe3600. doi: 10.1126/sciadv.abe3600, PMID 34433553.
39. Dong Y, Li S, Lu Y, Li X, Liao Y, Peng Z, *et al.* Stress-induced NLRP3 inflammasome activation negatively regulates fear memory in mice. J Neuroinflammation. 2020; 17(1): 205. doi: 10.1186/s12974-020-01842-0, PMID 32635937.
40. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, *et al.* Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell. 2016; 167(6): 1469-1480.e12. doi: 10.1016/j.cell.2016.11.018, PMID 27912057.
41. Xu X, Piao HN, Aosai F, Zeng XY, Cheng JH, Cui YX, *et al.* Arctigenin protects against depression by inhibiting microglial activation and neuroinflammation via HMGB1/TLR4/NF-kappaB and TNF-alpha/TNFR1/NF-kappaB pathways. Br J Pharmacol. 2020; 177(22): 5224-45. doi: 10.1111/bph.15261, PMID 32964428.
42. Fenn AM, Gensel JC, Huang Y, Popovich PG, Lifshitz J, Godbout JP. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. Biol Psychiatry. 2014; 76(7): 575-84. doi: 10.1016/j.biopsych.2013.10.014, PMID 24289885.
43. Hu KB, Lu XM, Wang HY, Liu HL, Wu QY, Liao P, *et al.* Effects and mechanisms of tanshinone IIA on PTSD-like symptoms. Phytomedicine. 2023; 120: 155032. doi: 10.1016/j.phymed.2023.155032, PMID 37611463.
44. Subedi L, Gaire BP. Tanshinone IIA: a phytochemical as a promising drug candidate for neurodegenerative diseases. Pharmacol Res. 2021; 169: 105661. doi: 10.1016/j.phrs.2021.105661, PMID 33971269.
45. Xie X, Xu Y, Zhou X, Su P, Jiang X, Jin Z. The protective effect of an extract of *Salvia miltiorrhiza* Bunge (Danshen) on cerebral ischemic injury in animal models: a systematic review and meta-analysis. J Ethnopharmacol. 2023; 317: 116772. doi: 10.1016/j.jep.2023.116772, PMID 37400004.
46. Fang C, Xie L, Liu C, Fu C, Ye W, Liu H, *et al.* Tanshinone IIA improves hypoxic ischemic encephalopathy through TLR-4-mediated NF-kappaB signal pathway. Mol Med Rep. 2018; 18(2): 1899-908. doi: 10.3892/mmr.2018.9227, PMID 29956801.
47. Ding B, Lin C, Liu Q, He Y, Ruganzu JB, Jin H, *et al.* Tanshinone IIA attenuates neuroinflammation via inhibiting RAGE/NF-kappaB signaling pathway *in vivo* and *in vitro*. J Neuroinflammation. 2020; 17(1): 302. doi: 10.1186/s12974-020-01981-4, PMID 33054814.
48. Wang S, Jing H, Yang H, Liu Z, Guo H, Chai L, *et al.* Tanshinone I selectively suppresses pro-inflammatory genes expression in activated microglia and prevents nigrostriatal dopaminergic neurodegeneration in a mouse model of Parkinson's disease. J Ethnopharmacol. 2015; 164: 247-55. doi: 10.1016/j.jep.2015.01.042, PMID 25666429.
49. Zhou X, Zhao R, Lv M, Xu X, Liu W, Li X, *et al.* ACSL4 promotes microglia-mediated neuroinflammation by regulating lipid metabolism and VGLL4 expression. Brain Behav Immun. 2023; 109: 331-43. doi: 10.1016/j.bbi.2023.02.012, PMID 36791893.
50. Wang H, He Y, Sun Z, Ren S, Liu M, Wang G, *et al.* Microglia in depression: an overview of microglia in the pathogenesis and treatment of depression. J Neuroinflammation. 2022; 19(1): 132. doi: 10.1186/s12974-022-02492-0, PMID 35668399.
51. Liu Y, Zhang Y, Zheng X, Fang T, Yang X, Luo X, *et al.* Galantamine improves cognition, hippocampal inflammation and synaptic plasticity impairments induced by lipopolysaccharide in mice. J Neuroinflammation. 2018; 15(1): 112. doi: 10.1186/s12974-018-1141-5, PMID 29669582.
52. Bijata M, Bączyńska E, Müller FE, Bijata K, Masternak J, Krzystyniak A, *et al.* Activation of the 5-HT7 receptor and MMP-9 signaling module in the hippocampal CA1 region is necessary for the development of depressive-like behavior. Cell Rep. 2022; 38(11): 110532. doi: 10.1016/j.celrep.2022.110532, PMID 35294881.
53. Jiang YL, Wang XS, Li XB, Liu A, Fan QY, Yang L, *et al.* Tanshinone IIA improves contextual fear- and anxiety-like behaviors in mice via the CREB/BDNF/TrkB signaling pathway. Phytother Res. 2022; 36(10): 3932-48. doi: 10.1002/ptr.7540, PMID 35801985.
54. Guo N, Wang X, Xu M, Bai J, Yu H, Le Zhang PI. PI3K/AKT signaling pathway: molecular mechanisms and therapeutic potential in depression. Pharmacol Res. 2024; 206: 107300. doi: 10.1016/j.phrs.2024.107300, PMID 38992850.
55. Sun Y, Zhang H, Wu Z, Yu X, Yin Y, Qian S, *et al.* Quercitrin rapidly alleviated depression-like behaviors in lipopolysaccharide-treated mice: the involvement of PI3K/AKT/NF-kappaB signaling suppression and CREB/BDNF signaling restoration in the hippocampus. ACS Chem Neurosci. 2021; 12(18): 3387-96. doi: 10.1021/acschemneuro.1c00371, PMID 34469122.
56. Xie Y, He Q, Chen H, Lin Z, Xu Y, Yang C. Crocin ameliorates chronic obstructive pulmonary disease-induced depression via PI3K/Akt mediated suppression of inflammation. Eur J Pharmacol. 2019; 862: 172640. doi: 10.1016/j.ejphar.2019.172640, PMID 31491407.
57. Zheng ZH, Tu JL, Li XH, Hua Q, Liu WZ, Liu Y, *et al.* Neuroinflammation induces anxiety- and depressive-like behavior by modulating neuronal plasticity in the basolateral amygdala. Brain Behav Immun. 2021; 91: 505-18. doi: 10.1016/j.bbi.2020.11.007, PMID 33161163.
58. Zhang Z, Song Z, Shen F, Xie P, Wang J, Zhu AS, *et al.* Ginsenoside Rg1 prevents PTSD-like behaviors in mice through promoting synaptic proteins, reducing Kir4.1 and TNF-alpha in the hippocampus. Mol Neurobiol. 2021; 58(4): 1550-63. doi: 10.1007/s12035-020-02213-9, PMID 33215390.
59. Kitagishi Y, Kobayashi M, Kikuta K, Matsuda S. Roles of PI3K/AKT/GSK3/mTOR pathway in cell signaling of mental illnesses. Depress Res Treat. 2012; 2012: 752563. doi: 10.1155/2012/752563, PMID 23320155.
60. Yang C, Ni HY, Yin JJ, Zhou T, Gu QX, Chen TT, *et al.* Atorvastatin ameliorates depressive behaviors via regulation of alpha7nAChR expression by PI3K/Akt-BDNF pathway in mice. Biochem Biophys Res Commun. 2022; 593: 57-64. doi: 10.1016/j.bbrc.2022.01.034, PMID 35063770.
61. Ma M, Hua X, Jia C, Xiao N, Zhang L, Wei L, *et al.* Tanshinone IIA regulates synaptic plasticity in Mg(2+)-free-induced epileptic hippocampal neurons via the PI3K/Akt signaling pathway. J Integr Neurosci. 2024; 23(3): 61. doi: 10.31083/j.jin2303061, PMID 38538223.
62. Li ZH, Cheng L, Wen C, Ding L, You QY, Zhang SB. Activation of CNR1/PI3K/AKT pathway by tanshinone IIA protects hippocampal neurons and ameliorates sleep deprivation-induced cognitive dysfunction in rats. Front Pharmacol. 2022; 13: 823732. doi: 10.3389/fphar.2022.823732, PMID 35295327.
63. Quan YZ, Wang JH, Zhang SH, Jin GN, Lu JM, Liu YM, *et al.* The intervention mechanism of tanshinone IIA in alleviating neuronal injury induced by HMGB1 or TNF-alpha-mediated microglial activation. Toxicol *In Vitro*. 2024; 101: 105950. doi: 10.1016/j.tiv.2024.105950, PMID 39357688.
64. Li L, Jiang W, Yu B, Liang H, Mao S, Hu X, *et al.* Quercetin improves cerebral ischemia/reperfusion injury by promoting microglia/macrophages M2 polarization via regulating PI3K/Akt/NF-kappaB signaling pathway. Biomed Pharmacother. 2023; 168: 115653. doi: 10.1016/j.biopha.2023.115653, PMID 37812891.
65. Zipp F, Bittner S, Schafer DP. Cytokines as emerging regulators of centra nervous system synapses. Immunity. 2023; 56(5): 914-25. doi: 10.1016/j.immuni.2023.04.011, PMID 37163992.

Cite this article: Li Q, Jin Y, Wu Y, Zhu M, Wu S, Zhang G, *et al.* Tanshinone IIA Ameliorates Chronic Unpredictable Mild Stress Induced Depression-Like Behaviors: Integrating Network Pharmacology and Molecular Mechanism Evaluation. Indian J of Pharmaceutical Education and Research. 2026;60(4):1596-605.