Research Square

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Exploring the anti-depression mechanism of Danshen Baoxin Cha in CUMS mice through tryptophane-kynurenine metabolism pathway

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Abstract

Aim of the study: This study investigated whether DBC alleviates depression in a chronic unpredictable mild stress (CUMS) mouse model and explored its mechanism related to the tryptophan-kynurenine (Trp-Kyn) metabolic pathway.

Materials and Methods: CUMS was applied for 4 weeks to establish the model, followed by 2 weeks of DBC treatment. Depression-like behaviors were assessed using open field (OFT), forced swimming (FST), and tail suspension tests (TST). Serum levels of cytokines (IL-6, TNF-α, IFN-γ) and Trp-Kyn pathway metabolites were measured by ELISA. Hippocampal mRNA expression of IL-6, TNF-α, IFN-γ, IDO, 5-HT, and hepatic TDO was analyzed by qRT-PCR. IDO and TDO protein levels were determined by Western blot.

Results: Compared with the model group, DBC treatment significantly improved behavioral outcomes in the OFT, FST, and TST. DBC decreased serum pro-inflammatory cytokines and increased serum TRP and KYNA, while reducing KYN, QUIN, 3-HK, and 3-HAA. The KYN/TRP and KYN/KYNA ratios decreased. Furthermore, DBC downregulated hippocampal mRNA expression of IL-6, TNF-α, IFN-γ, and IDO, as well as hepatic TDO mRNA, and increased hippocampal 5-HT mRNA. Protein levels of hippocampal IDO and hepatic TDO were also reduced.

Conclusion: This study concludes that DBC effectively alleviates depressed-like behaviors in a CUMS mouse model by inhibiting pro-inflammatory cytokines and neurotoxic metabolic pathways, suggesting that DBC can modulate the Trp-Kyn pathway to exert a therapeutic effect on depression.

Introduction

Depression is a common mental disorder, with one of the highest incidences of mental illness worldwide. It is accompanied by low mood, mood disorders, loss of interest, anxiety, insomnia and other symptoms. In severe cases, it can lead to suicidal tendencies [1]. At present, the number of depression patients worldwide has exceeded 350 million, the number has surpassed 100 million in China, accounting for approximately 30% of the total [2–4]. The suicide risk of patients with depression is 30 times higher than that of normal people [5], and approximately 1 million people around the world commit suicide due to depression each year [6]. Depression, as the third leading cause of the global burden of disease, is predicted to become the leading cause by 2030 [7].

Numerous existing studies have shown that depression has become a widespread phenomenon that not only significantly impacts the mental health of patients negatively, but also seriously compromises their physical well-being. Therefore, finding the effective treatments is particularly critical. In recent years, numerous studies have been undertaken to go into the therapeutic mechanisms underlying depression, such as the inflammatory factor hypothesis, HPA axis hypothesis, monoamine neurotransmitter hypothesis, and neuroplasticity hypothesis [8]. Among these, depression triggered by inflammation has garnered increasing attention. The studies have demonstrated that the association between

inflammation and depression may be closely related to the Trp-Kyn metabolic pathway [9]. Tryptophan metabolism primarily occurs via the Kynurenine pathway, producing various metabolites that participate in numerous physiological processes in the body and can affect neuroplasticity. TRP is an essential amino acid in the human body, and its metabolic pathways include the 5-hydroxytryptamine (5-HT) pathway and the KYN pathway. About 95% of TRP is converted into KYN under the action of degrading enzymes, specifically indoleamine 2, 3-dioxygenase (IDO) or tryptophan 2, 3-dioxygenase (TDO). IDO and TDO are rate-limiting enzymes in the Trp-Kyn metabolic pathway, and their levels are positively correlated with depression-like symptoms [10]. IDO and TDO increase or overactivate, leading to increased metabolism of TRP into KYN and resulting in the depletion of a significant amount of TRP. Elevated levels of KYN cause upregulation of downstream factors, aggravating neurotoxicity and disrupting neuroprotective mechanisms, while also inhibiting the neuroprotective effects that are normally mediated by KYN [11].

Currently, the clinical treatment of depression primarily relies on pharmaceuticals, with four main classes of commonly prescribed antidepressants: tricyclic antidepressants such as amitriptyline and maprotiline, selective serotonin reuptake inhibitors like fluoxetine, and serotonin-norepinephrine reuptake inhibitors such as venlafaxine [12]. However, the limitations of pharmaceuticals have become increasingly prominent, such as slow onset, significant side effects, unstable efficacy, and a high recurrence rate [13]. Traditional Chinese medicine (TCM) has shown potential in the prevention and treatment of a wide variety of complex health conditions. Tea therapy, a distinctive aspect of TCM, is utilized for both disease prevention and treatment. Drinking tea may offer a variety of health benefits and serve as a soothing way to boost emotional well-being [14]. Furthermore, it has been proposed that tea may play an auxiliary role in the prevention and management of depressive symptoms [15]. *Danshen Baoxin* Cha (DBC), an over-the-counter medication formulated based on TCM herbal principles, primarily consists of *Salvia miltiorrhiza* Bunge and green tea, integrates multi-target antidepressant mechanisms that are deeply intertwined with the modulation of neuroinflammation and the Trp-Kyn metabolic pathway.. *Salvia miltiorrhiza*, a bitter and slightly cold herb, has the effect of clearing the heart, promoting blood circulation and alleviating pain [16]. Its active compounds, including cryptotanshinone and salvianolic acid B, which exhibit antidepressant effects in animal models by regulating the brain's neurochemical microenvironment [17–19]. Notably, these compounds may inhibit pro-inflammatory cytokines (TNF-α, IL-6) that disrupt the Trp-Kyn balance, shifting metabolism toward the neuroprotective branch (KYNA) while reducing neurotoxic byproducts (QUIN), thereby mitigating neuronal damage and synaptic dysfunction. On the other hand, green tea offers numerous benefits, such as refreshing the mind, combating fatigue, and facilitating digestion [20]. Beyond the above benefits, research has demonstrated that green tea powder can inhibit hippocampal neuronal damage and apoptosis, restore the number of Nissl bodies, and reduce inflammation in mice with CUMS-induced depression [21]. The polyphenols in green tea can also improve depressive-like behaviors through the hippocampal CREB-BDNF pathway or inhibition of the hypothalamic-pituitary-adrenal axis [22, 23], which are critical regulators of the Trp-Kyn pathway. Chronic stress, often associated with HPA hyperactivity, drives excessive Trp catabolism

toward neurotoxic Kyn, while anti-inflammatory interventions may rebalance this pathway, fostering resilience against depressive-like phenotypes.

Our team's previous studies have investigated the preventive and therapeutic effects of DBC on coronary heart disease (CHD) with depression in mice [24] and CHD with cognitive impairment in rats [25]. Building on these findings, the current study endeavors to explore the therapeutic effects and underlying mechanisms of DBC in treating depression, capitalizing on the synergistic actions of *Salvia miltiorrhiza* and green tea. It is hypothesized that the components of DBC may modulate the Trp-Kyn pathway. For instance, the active compounds in *Salvia miltiorrhiza* and green tea polyphenols might influence the activity of key enzymes involved in the Trp-Kyn pathway, such as IDO and TDO, which are responsible for initiating the conversion of tryptophan to kynurenine. By regulating these enzymes, DBC could potentially restore the balance between the production of 5-HT and Kyn, thereby alleviating depressive symptoms. Furthermore, the anti-inflammatory and neuroprotective effects of DBC, as demonstrated by the inhibition of hippocampal neuronal damage and reduction of inflammation, may also be intertwined with the Trp-Kyn pathway. Inflammatory processes can upregulate the activity of IDO and TDO, leading to increased kynurenine production and subsequent neurotoxic effects. Thus, the ability of DBC to modulate inflammation may contribute to the normalization of the Trp-Kyn pathway. In conclusion, by delving into the relationship between DBC and the Trp-Kyn metabolic pathway, this study not only aims to uncover the novel therapeutic mechanisms of DBC in treating depression but also holds the potential to expand the clinical applications of this TCM-based formulation. This exploration could provide valuable insights for the development of innovative depression treatments, integrating the wisdom of traditional medicine with modern scientific understanding of neurobiology and metabolism.

Materials and methods

Chemicals and reagents

DBC (220502) is a Chinese medicine tea drink produced by Daxing'an Lingtian Herbal Industry Co., Ltd.. The implementation standard is the National Drug Standard WS-6007(B-0007)-2014Z issued by the State Food and Drug Administration. The approval number is the national drug approval code B20040008, which designates it as a red label OTC, a class of non-prescription drugs. According to the instructions, DBC soaked concentrated liquid: Each packet (2.5g) is soaked with 200mL boiling water for 5 ~ 10 minutes. After soaking, it is concentrated to a certain concentration by automatic rotary evaporation instrument, and 1.54g/mL liquid is obtained. Store in the refrigerator at -20℃ and heat at 37℃ before administration. Our research group detected 12 batches of DBC in the early stage, and the similarity of its fingerprint ranges from 0.999 to 1.000. A total of 14 common peaks were identified, and 9 components were identified, namely Salvianic acid A, epigallocatechin, protocatehydehyde, caffeine, epicatechin, epigallocatechin gallate, epicatechin gallate, rosmarinic acid and salvianolic acid B. The contents of the above nine components respectively were 2.87 ~ 3.76 mg/g, 4.69 ~ 6.01 mg/g, 0.52 ~ 0.67 mg/g, 10.60 ~ 12.25 mg/g, 1.36 ~ 1.67 mg/g, 11.73 ~ 14.74 mg/g, 3.89 ~ 4.46 mg/g, 0.55 ~ 0.73 mg/g, 5.90 ~ 6.85 mg/g[26].

IL-6 (ml002293), IFN-γ (ml002277), TNF-α (MM-0132M1), TRP (MM-0756M1), KYN (MM-0755M1), KYNA (MM-44717M1), QUIN (MM-0647M1), 3-HK (MM-46400M1) and 3-HAA (MM-47303M1) were measured using an ELISA kit. The mRNA levels of IL-6, TNF-α, IFN-γ, IDO, 5-HT and TDO were determined by qRT-PCR. The primers were purchased from Shenggong Biotech (Shanghai) Co., Ltd. GAPDH (60004-1-lg), IDO (66528-1-lg) and TDO (15880-1-AP) antibodies were purchased from Wuhan Sanying Biotechnology Co., Ltd. Rabbit/Rat Resistant (#S0001 #S0002 1: 2000) were from Affinity Corporation.

Animal CUMS model and drug therapy

Male Kunming (KM) mice (2-month-old, 18-22g) from Guangzhou University of Chinese Medicine (license:SCXK GD 2018-0034) were housed in SPF conditions (23 ± 2℃, 50%-60%RH, 12h light/dark). They were acclimatized for 1 week before experiment. Animal handling was carried out in accordance with Chinese legislation on the use and care of laboratory animals and the "Guiding Principles on the Care and Use of Laboratory Animals" approved by the Laboratory Animal Ethics Committee of Guangzhou University of Chinese Medicine (Approval No. 20230327004).

After adaptive training, KM mice were randomly divided into five groups, with the control group and four experimental groups each consisting of 10 mice. The control group was fed freely and did not receive any stimulation. In contrast, the other four groups were given different stimuli until the final behavioral test. The stressors included eight types of modeling methods [27]: 24 hours of water deprivation, 24 hours of fasting, 5 minutes of tail clip (using a plastic clip held 1cm away from the tip of the mice's tail), 45° tilted cage for 24 hours, wet bedding for 24 hours, day and night reversal, 3 hours of behavioral restraint, and 5 minutes of horizontal shock. To avoid habituation, the same stimulation was not repeated within 3 days. Model group (CUMS); DBC-L group: CUMS model mice treated with low-dose DBC (7.7g/kg/d); DBC-M group: CUMS model mice treated with medium-dose DBC (15.4g/kg/d); DBC-H group: CUMS model mice treated with high-dose DBC (30.8g/kg/d)[28]. Each of these groups was treated until the final behavioral test, ensuring a comprehensive evaluation of the effects of different DBC dosages on the CUMS-induced depressive-like behaviors.Each behavioral test was performed on two days, in the following order: OFT, FST, and TST.

After the successfully establishing the model, low-dose, medium-dose and high-dose DBC were orally administered to the mice via gavage once daily for 2 weeks (stimulation was also performed during drug administration), whereas the control and model group mice received an equal volume of ultrapure water.

Sample collection and preparation

At the end of the behavioral tests, mice were treated with inhaled isoflurane, followed by blood collection from the ocular region. Subsequently, the mice were euthanized through cervical dislocation, and their brain and liver tissues were extracted. Blood samples were centrifuged at 3500 r/min for 15 min. The supernatant was then transferred to a clean EP tube and stored in a refrigerator at -80℃ for future

testing. The serum levels of IL-6, TNF-α, IFN-γ and tryptophan metabolites (TRP, KYN, KYNA, QUIN, 3-HK, 3-HAA) were detected by ELISA. A part of the hippocampus was preserved in 4% paraformaldehyde and stored at -80°C for HE staining and Nissl's staining. The remaining brain and liver tissues were stored in a freezer at -80℃ for real-time fluorescence quantitative PCR to detect the mRNA expression levels of IL-6, TNF-α, IFN-γ, IDO, 5-HT, and TDO. Western blot was used to analyze the protein expression of IDO in hippocampus and TDO in liver.

Behavioral Studies

Open Field Test (OFT)

The OFT can detect spontaneous motor ability and exploration behavior of mice [29]. Before the test, mice were placed in the laboratory for 60 minutes to adapt to the laboratory environment. During the observation, a single mouse was placed in the center of a clean open box measuring 50cm×50cm×40cm. The bottom of the box was divided into 25 squares, with 5 squares in each row and column, and 9 squares in the center constituted the central area. The horizontal movement distance of the mouse in the open box within 5 minutes was observed, and the total distance of the mouse's spontaneous movement as well as the distance traveled within the central region were analyzed.

Forced Swimming Test (FST)

The FST is often used to assess depression-like behavior in model animals. The FST was conducted based on the original method, with slight modifications [30]. The mice were put into a cylindrical swimming pool with a height of 30cm and a diameter of 20cm, filled with water to a level of 15cm and maintained at a temperature of 23–25 ℃. The mice were placed in the water for 6 minutes. After allowing them to adapt to the environment for the first 2 minutes, the time of immobility during the last 4 minutes was recorded. The duration of immobility in the last 4 minutes for each mouse was analyzed and calculated.

Tail Suspension Test (TST)

The TST is often used to assess depression-like behavior in model animals. The TST was carried out according to the conventional method [31]. The mouse's tail was wrapped with tape cut in advance and hung on an "S"-shaped small iron hook, so that it was suspended upside down, with its head approximately 5 to 10cm away from the bottom of the suspension box. The tension on the hook changed as the mouse struggled, and the immobility time of the mouse was recorded. Behavioral analysis equipment software was used to collect and record the immobile behavior of mouse over a period of 6 minutes, and to analyze and calculate the duration of immobility in the last 4 minutes.

Histopathology

The hippocampus brain tissue of mice was fixed with 4% paraformaldehyde for 24 hours, gradually dehydrated and cleared, and then embedded in paraffin. Sections were sliced to a thickness of 5μm. For HE staining: The prepared sections were dewaxed, stained with hematoxylin and eosin, and subsequently dehydrated with anhydrous ethanol. After staining, they were cleared with xylene and mounted with glycerin gelatin. The results were observed under a microscope. For Nissl staining: The prepared sections were dewaxed, stained with toluidine blue solution, and then dehydrated with anhydrous ethanol. After staining, they were cleared with xylene and glycerin gelatin. The results were observed under a microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

First, prepare ELISA reagents, samples and standards. Incubate prepared sample and standard product at 37℃ for 30 minutes. Wash plate 5 times, then add enzyme-labeled reagent and incubate again at 37℃ for another 30 minutes. After 5 more washes, add chromogenic substrates A and B, and develop color at 37℃ for 10 minutes. Finally, add the termination solution, detect OD within 15 minutes post-addition to calculate sample concentration.

qRT-PCR method

Mouse hippocampal and liver tissues were cut and added to TRIzol for routine extraction of total RNA. cDNA was synthesized using reverse transcription kit. Using cDNA as a template, the expression levels of IL-6, TNF-α, IFN-γ, IDO, 5-HT and TDO were detected using fluorescence quantitative kit, with GAPDH as the internal reference. The relative expression levels of each gene were analyzed by the 2-ΔΔCt method. The specific primers used are listed in Table 1.

Table 1
Sequences of the primers used for qPCR.

Sequence name	Primers' sequence (5' to 3')
IL-6	F: GAGCATACCACTCCCAACAGACC
	R: AAGTGCATCATCGTTGTTCATACA
TNF-α	F: GCCAGTGAGTGAAAGGGACAGAAC
	R: GGACTAGCCAGGAGGGAGAACAG
IFN-γ	F: GGCTCTGGAGGCTGGAGGAAG
	R: TGATAGGCGGTGAGGCTACAAGG
IDO	F: TGTGTGAATGGTCTGGTCTCTGTG
	R: TTTGAGGGCTCTTCCGACTTGTC
5-HT	F: AAGCCAAAGCAGAGGAGGAGATC
	R: GATGAGGAGCAGGGTGGGTAAATAG
TDO	F: GGCTATCATTACCTGCGTTCAACTG
	R: ACTCGGCTGTGTAAAGGAATTTGTG

Western Blot (WB)

Total protein was extracted from hippocampus by adding cold RIPA protein lysate, mixing with magnetic beads, homogenizing, and allowing it to stand on ice for 30 minutes to ensure complete lysis. The protein concentration was determined using the BCA method after centrifugation at 4℃ and 12000 rpm for 10 minutes. The total protein samples of 40μg were separated by SDS-PAGE electrophoresis. The gel was then removed, and the proteins were transferred to a PVDF membrane, which was blocked with 5% skim milk powder for 4 hours. After washing three times, the primary antibody diluent (1: 5000) was added, and the membrane was incubated overnight at 4℃. The membrane was washed three times again, and the secondary antibody diluent (1: 2000) was added. The membrane was incubated on a shaker at room temperature for 1 hour, and then the secondary antibody diluent was removed. The memrane was washed three times with TBST. After evently adding ECL chemiluminescence solution, exposure, development, and photography were performed. Using GAPDH as internal reference protein, the gray ratio between target protein and internal reference protein was calculated using ImageLab software from the chemiluminescence imaging developer.

The total protein from the liver was extracted, with the exception 25μg of total protein, and all other operations remained the same as described above.

Statistical analysis

The experimental results were all represented as Mean ± SD, and were statistically analyzed by SPSS26.0 software. Values with $P < 0.05$ were considered statistically significant. If the data conform to normal distribution, typically, the single factor analysis of variance (One-Way analysis of variance, ANOVA) is adopted, with comparsons between two groups using the LSD or T3 test. Nonparametric tests are used if the data do not conform to a normal distribution. The final results were plotted using GraphPad prism 8.0.

Results

Influence of DBC on depression-like behavior of CUMS depression model mice

The effectiveness of DBC as an antidepressant was evaluated using the OFT, FST, and TST. Figure 1a depicts the flowchart outlining the entire experimental procedure. The behavioral results following administration are presented in Fig. 1, with Fig. 1b displaying the open field trajectory diagram of each group. In the OFT (Fig. 1c and d), compared with the control group, the total distance traveled and central distance of the model group were reduced ($P < 0.01$). In contrast, the mice in the three treatment groups exhibited a dose-dependent trend towards the control group in terms of both total distance and central distance ($P < 0.01$). The findings of the OFT indicate that the administration of DBC increased autonomous and exploratory behavior and decreased tension levels in the unknown area for depressed mice. In the FST and TST (Fig. 1e and f), the immobility time of mice in the model group was increased compared to that of the control mice ($P < 0.01$). Conversely, the immobility time of mice in the three dosing groups treated with DBC decreased. The above behavioral analyses show that DBC can, to some extent, better regulate depression-like behaviors in depressed mice and exert an antidepressant effect.

Effects of DBC on depression-like hippocampus of CUMS depression mice

We conducted hippocampus pathological staining to determine the anti-depression effect of DBC (Supplementary Materials 1). The results of HE staining (Fig. 2a) showed that the number of neurons cells in the hippocampal CA1 region of mice in the control group was large; they had regular and complete morphological structures, orderly arrangements, clear hierarchies, and uniform distributions, with no neuronal lesions. In the model group, the number of neurons cells in the hippocampal CA1 of mice decreased significantly, the cells and nuclei showed swelling, shrinkage, shedding and necrosis, the cell gap increased, the hierarchy was unclear and the arrangement was disordered. Compared with the model group, the DBC-L group showed a certain improvement, particularly in the slightly reduced intrecellular space. Compared with the model, the number of neuronal cells in the hippocampal CA1 of mice was increased in the DBC-M group and the DBC-H group, the cells were arranged neatly, the layers were clear, the nuclei were not deeply stained, the cell space was normal, and the morphology was most similar to that of the control group. The results of Nissl staining (Fig. 2b) showed that neuron cells in the hippocampal CA1 region of mice in the control group were abundant in quantity, complete in morphology

and structure, arranged neatly and stratified clearly, and the cytoplasm is rich in blue-purple Nissl bodies, which are evenly arranged. While the neuron cells in the hippocampal CA1 region of mice in the model group had a large number of nissl bodies loss, and the nuclei were deeply stained and constricted, and the cell arrangment was disordered. Compared with the model group, the degree of Nissl body loss, nuclear hyperstaining, and contraction in neurons in the hippocampal CA1 region of mice in each administration group was alleviated to a certain extent. Among them, the number of Nissl bodies in neurons in hippocampal CA1 region of mice in DBC-M group and DBC-H group were significantly increased, arranged and distributed neatly and evenly, and only a small amount of nuclear hyperstaining and pyretic contraction were observed. The apoptosis of hippocampal neurons was significantly improved, and the morphology of hippocampal tissue was most similar to that of the control group.

Effects of DBC on serum and hippocampal pro-inflammatory cytokines of CUMS mice

As shown in Fig. 3a, b, and c, compared with the control group, the contents of serum pro-inflammatory cytokines IL-6, TNF-α and IFN-γ in the model group were significantly increased ($P < 0.05$, $P < 0.01$). In contrast to the model group, the contents of serum pro-inflammatory cytokines IL-6, TNF-α and IFN-γ in mice were significantly decreased in all treatment groups ($P < 0.05$, $P < 0.01$), and there was no statistical difference in the IFN-γ index in the DBC-M group. As shown in Fig. 3d, e, and f, compared with the control group, the mRNA expression levels of pro-inflammatory cytokines IL-6, TNF-α, and IFN-γ in hippocampus of mice were significantly increased in the model group ($P < 0.01$). Compared with the model group, the mRNA expression levels of pro-inflammatory cytokines IL-6, TNF-α and IFN-γ in the hippocampus of mice in all treatment groups were significantly decreased ($P < 0.01$). The findings indicate that DBC can lower levels of inflammatory factors and alleviate depressive symptoms in mice, while the model group is associated with increased inflammation in mice.

Effects of DBC on serum Trp-Kyn pathway in CUMS mice

To explore the role of imbalanced Trp-Kyn metabolism in depression, as shown in Fig. 4a-k, we compared serum levels of various metabolites between the control group and the model group. In the model group, serum TRP and KYNA contents were significantly decreased ($P < 0.01$), while KYN, QUIN, 3-HK and 3-HAA levels were increased ($P < 0.01$). Furthermore, the KYN/TRP and KYN/KYNA ratios increased ($P < 0.01$), whereas the KYN/QUIN, KYNA/QUIN, and KYNA/3-HK ratios decreased ($P < 0.01$). Compared with the model group, all treatment groups shows increased serum levels of TRP, KYN, and KYNA ($P < 0.05$, $P < 0.01$), and decreased levels of QUIN, 3-HK and 3-HAA ($P < 0.01$). Additionally, the KYN/TRP and KYN/KYNA ratios decreased ($P < 0.01$). while the KYN/QUIN, KYNA/QUIN, and KYNA/3-HK ratios increased ($P < 0.05$, $P < 0.01$). Notably, the KYN/QUIN ratio did not significantly differ in the DBC-H group. However, the KYNA/QUIN ratio in the DBC-L group showed a significant difference and an increasing trend compared to the model group. These findings suggest that the Trp-Kyn metabolism is disrupted in the serum and brain of CUMS mice, with a greater involvement in the Kyn pathway. Importantly, DBC exerts a certain improvement effect on depression via the Trp-Kyn pathway.

Effects of DBC on related enzymes in hippocampus and liver of CUMS mice

IDO and TDO are the rate-limiting enzymes in the Trp-Kyn pathway. IDO is primarily found in the brain, whereas TDO is mainly located in the liver. An increase in both IDO and TDO leads to an elevation in KYN and a decrease in 5-HT in the brain, which can exacerbate depression. We evaluated the mRNA and protein levels of IDO in hippocampal and TDO in liver tissues of CUMS mice. As shown in Fig. 5a, b, and c, qRT-PCR was used to detect the mRNA levels of these enzymes in the hippocampus and liver tissues of mice. Compared with the control group, the mRNA expression levels of IDO in the hippocampus and TDO in the liver were significantly increased in the model group ($P < 0.01$), while the 5-HT levels in the hippocampus were significantly decreased ($P < 0.05$). In contrast, compared with the model group, the mRNA expression levels of IDO in the hippocampus and TDO in the liver were significantly decreased in all treatment groups ($P < 0.05$, $P < 0.01$), and 5-HT levels in the hippocampus were significantly increased ($P < 0.01$). Notably, there was no statistical difference in 5-HT levels in DBC-L group, but an increasing trend was observed. The results suggest that DBC induces a decrease in TRP flow toward KYN, but in the branching pathway of KYN, increases the KYNA pathway, which acts for neuroprotection, while promoting the 5-HT pathway, thereby alleviating depression.

As depicted in Fig. 5d, e, f,g and Supplementary Materials 2, Western blot analysis was performed to detect the proteins levels of these enzymes in the hippocampus and liver tissues of mice. Compared with the control group, the protein levels of IDO in the hippocampus and TDO in the liver were significantly increased in the model group ($P < 0.05$, $P < 0.01$). In comparison to the model group, the protein levels of IDO in the hippocampus and TDO in the liver were significantly decreased in each administration group ($P < 0.05$, $P < 0.01$). Therefore, DBC can modulate the activity of IDO and TDO.

Discussion

The present research investigated the potential antidepressant effects of DBC on mice with CUMS-induced depression-like symptoms and explored the underlying mechanisms. The CUMS depression model used in this study is a well-established and widely applied method both nationally and internationally [32]. After subjecting the mice to continuous mild unpredictable stress for 4 weeks, we confirmed the successful establishment of the CUMS model. Behavioral tests, including OFT, FST, and TST, were conducted to assess the validity of the model. Results revealed a significant reduction in the total distance traveled and central distance in the OFT for mice in the CUMS model group, indicating depressive-like behaviors. Moreover, the FST and TST showed an increase in immobility time among mice in the model group, consistent with typical depression symptoms. Following a 2-week DBC treatment, significant improvements were noted in both the total distance traveled and central distance in the OFT. Furthermore, DBC administration reduced the immobility time during FST and TST, suggesting an improvement in depressive-like behavior in mice.

At present, the common clinical antidepressant pharmaceuticals such as fluoxetine and paroxetine, may cause side effects like nausea and insomnia upon long-term use, thereby affecting patients' medication compliance [33, 34]. TCM, with syndrome differentiation as its core, emphasizes the overall physical and mental well-being, and its multi-component and multi-target pharmacological effects demonstrate advantages in treating depression. Additionally, Chinese herbal tea therapy, characterized by its health benefits and emotional relief, possesses unique advantages in preventing and treating depression. DBC, an over-the-counter medicine formulated based on traditional Chinese herbal medicine theory, primarily contains *Salvia miltiorrhiza* and green tea. Numerous studies have shown that *Salvia miltiorrhiza* and green tea exhibit antidepressant effects. In addition, our research group used HPLC fingerprint spectra to identify 9 components of DBC, which were Salvianic acid A, epigallocatechin, protocatehydehyde, caffeine, epicatechin, epigallocatechin gallate, epicatechin gallate, rosmarinic acid, and salvianolic acid B [26]. We analyzed DBC's components, including those closely related to the pathogenesis of depression, such as Salvianic acid A, Salvianolic acid B and Caffeine. The behavior of depressed mice treated with tanshensu sodium was significantly improved, suggesting that the antidepressant effect of tanshensu is played by increasing the content of monoamine neurotransmitters in the hippocampus and modulating the CREB/BDNF signaling pathway [19]. Salvianolic acid B has been shown to have anti-inflammatory effects in studies [35], alleviating depressive-like behavior in mice, significantly shortening the duration of immobility in FST and TST, and having antidepressant effects [36]. Caffeine partially ameliorates behavioral changes in depressive model of CUMS in mice [37]. Due to the previous findings, the antidepressant mechanism of DBC can be further explored, and the expression level of the Trp-Kyn metabolic pathway-related indexes closely related to depression can be detected, which is also the innovation of this paper.

TRP is an essential amino acid in the human body, and its decomposition pathway include 5-HT pathway and KYN pathway. Approximately 95% of TRP is converted into KYN under the action of IDO or TDO. SchferA et al. found that when under pressure, TRP levels decreases while KYN production increases [16]. When IDO and TDO activation are induced by inflammation, KYN is further metabolized, leading to a significant reduction of TRP. This depletion results in a decrease in the amount of TRP available for the production of 5-HT, causing insufficient 5-HT production [38]. Since 5-HT cannot efficiently cross the blood-brain barrier, its deficiency affects the function of the amygdala, leading to excessive excitation of the cerebral cortex and the manifestation of depression-like symptoms such as emotional anxiety, irritability, and insomnia [39]. While the amygdala plays a crucial role in emotion regulation, the hippocampus is equally important in depression research. The hippocampus not only mediates neuroendocrine responses to stress but also participates in learning, memory, and emotional responses. It has become one of the most studied brain regions in understanding the pathogenesis of depression and the effects of antidepressant drugs [40, 41]. Therefore, in this study, the hippocampus is selected as the primary site for detection .

KYN can be metabolized through two distinct pathways, Trp-Kyn-3-HAA and Trp-Kyn-KYNA, catalyzed by different enzymes. The Trp-Kyn-3-HAA metabolic pathway involves KYN being converted to 3-HK by Kynurenine hydroxylase, and then, 3-HK is further metabolized to 3-HAA through a series of enzymatic

reactions. Finally, QUIN, picolinic carboxylic acids, and nicotinamide adenine dinucleotides (NAD) are generated by a series of multistage enzymatic reactions and participate in various physiological processes in vivo. The metabolic pathway of Trp-Kyn-KYNA is that KYN generates KYNA under the action of Kynurenine aminotransferase (KAT). QUIN is an endogenous agonist of NMDA receptor. The increase of QUIN in the body will produce excitatory neurotoxic effects, and the elevated level of QUIN may damage the axons of neurons and cause neurodegenerative changes. KYNA as an endogenous antagonist of NMDA receptor, can counteract the neurotoxic effects of quinolinic acid, exhibiting neuroprotective effects [42].

TDO and IDO are key rate-limiting enzymes that catalyze the metabolism of tryptophan into the Kynurenine pathway, which can reduce TRP and KYN content. The KYN/TRP ratio serves as an indicator of IDO and TDO activity [43]. A large ratio corresponds to greater activity of IDO and TDO, whereas a lower ratio indicates lower activity. In this experiment, the KYN/TRP ratio of mice in the model group was increased, and DBC could reduced this ratio, indicating inhibition of IDO and TDO activities. Clinical studies have demonstrated that during stress and other conditions, immune activation and the activity of IDO, kynurenase and 3-HK increase, leading to elevated levels of neurotoxic KYN metabolites and the reduced neuroprotective products, which promote neuronal damage and degeneration. Therefore, the KYN/KYNA and KYN/QUIN ratios can indirectly determine the relationship between neuroprotection and toxicity in the Trp-Kyn pathway [44, 45]. In this experiment, the serum KYN/KYNA ratio of mice in the model group was increased, while the KYN/QUIN ratio was decreased; the results in the drug-dosing groups were opposite to those in the model group. The findings suggest that DBC inhibited pro-inflammatory cytokines IL-6, TNF-α and IFN-γ, suppressed IDO activity in the hippocampus and TDO activity in the liver, promoted the production of neuroprotective metabolites such as TRP and KYNA, inhibited the generation of neurotoxic metabolites KYN, 3-HK, 3-HAA, and QUIN, decreased KYN/TRP and KYN/KYNA ratios, increased KYN/QUIN, KYNA/QUIN and KYNA/3-HK ratios, and enhanced the expression of 5-HT in the hippocampus, leading to an improvement in depressive symptoms. Thus, the Trp-Kyn metabolic pathway may play a pivotal role in ameliorating depression.

Despite the interesting findings, our study still has limitations. First, the same group of mice underwent three behavioral tests during the experiment, which may have influenced the animal behaviors due to their previous experience and body condition. Second, the tryptophan metabolic pathway was primarily involved in this study; other mechanisms may also be involved but were not involved in this study. Finally, the potential regulatory role of DBC in other brain regions, such as prefrontal cortex and amygdala, which collectively contribute to neuroinflammation and neuronal function. These limitations in the present study should be addressed in our further research to ensure the accuracy of the results.

Conclusion

DBC significantly ameliorated depressive effects in mice. This is shown in Fig. 6, DBC could successfully prevent and control the development of depression by inhibiting pro-inflammatory cytokines, downregulating expression of IDO and TDO, suppressing Trp-Kyn metabolic pathways, and shifting

metabolism towards TRP-5-HT pathways. In conclusion, this study elucidated the therapeutic mechanism of DBC involving Trp-Kyn metabolism, thereby providing a reliable and effective therapeutic strategy for the further treatment of depression.

Abbreviations

DBC
Danshen Baoxin Cha
CUMS
Chronic Unpredictable Mild Stress
Trp-Kyn
Tryptophan-Kynurenine
OFT
Open field test
FST
Forced swimming test
TST
Tail suspension test
HE
Hematoxylin-eosin
ELISA
Enzyme-linked immunosorbent assay
IL-6
Interleukin-6
TNF-α
Tumor necrosis factor α
IFN-γ
Interferon-γ
TRP
tryptophan
KYN
kynurenine
KYNA
Kynurinic acid
QUIN
Quinolinic acid
3-HK
3-hydroxy-L-kynurenine
3-HAA
3-hydroxyanthranilic acid

IDO
Indoleamine 2, 3-dioxygenase
5-HT
5-hydroxytryptamine
TDO
Tryptophan 2, 3-dioxygenase
qRT-PCR
Quantitative real-time polymerase chain reaction
WB
Western blot analysis
TCM
Traditional Chinese medicine
NAD
Nicotinamide adenine dinucleotides
KAT
Kynurenine aminotransferase.

Declarations

Supplementary Information

The online version contains supplementary material available at

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Not applicable.

Author contributions

All authors contributed to the study conception and design. WL, HP, LZ and LJ participated in the design of the study, reviewed and revised the manuscript. WC participated in the design of the study, conducted the experiments and drafted the manuscript. YC conducted the experiments and drafted the manuscript. SW and LR contributed to the data collection. DN, GZ and KZ cperformed the statistical analysis. LY helped revise the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Ethical approval

All in vivo interventions were accepted by the Animal Ethics Committee of Guangzhou University of Chinese Medicine (No. 20230327004).

Clinical trial number

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

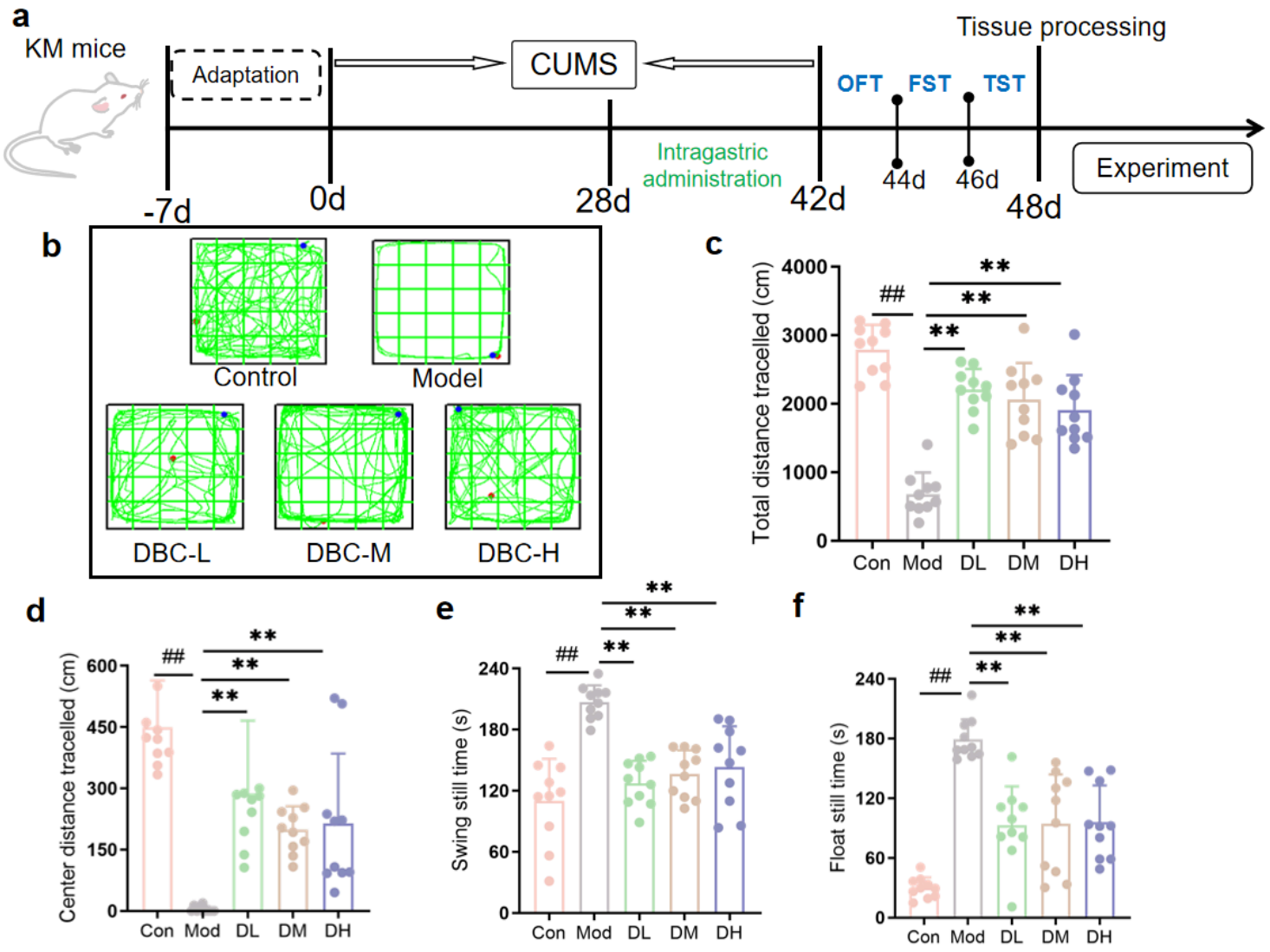


Figure 1

DBC improves OFT, FST and TST behavior in CUMS depression model mice. **a** Schematic

timeline of experiment procedure. **b** The track plot of the OFT. OFT: **c** the total activity distance and **d** the central activity distance in each group. FST: **e**the floating still time in each group. TST: **f** the swinging still time in each group. Data are shown as mean±SEM, n=10 mice per group. ##p<0.01 vs. the control group. **p<0.01 vs. the model group. Con, control group; Mod, model group; DL, low dose of DBC group; DM, medium dose of DBC group; DH, high dose of DBC group. One-way ANOVA, followed by LSD method multiple comparisons tests (**d**) , followed by Dunnett's T3 method multiple comparisons tests (**e** and **f**) or non-parametric independent samples Kruskal–Wallis test (**c**)

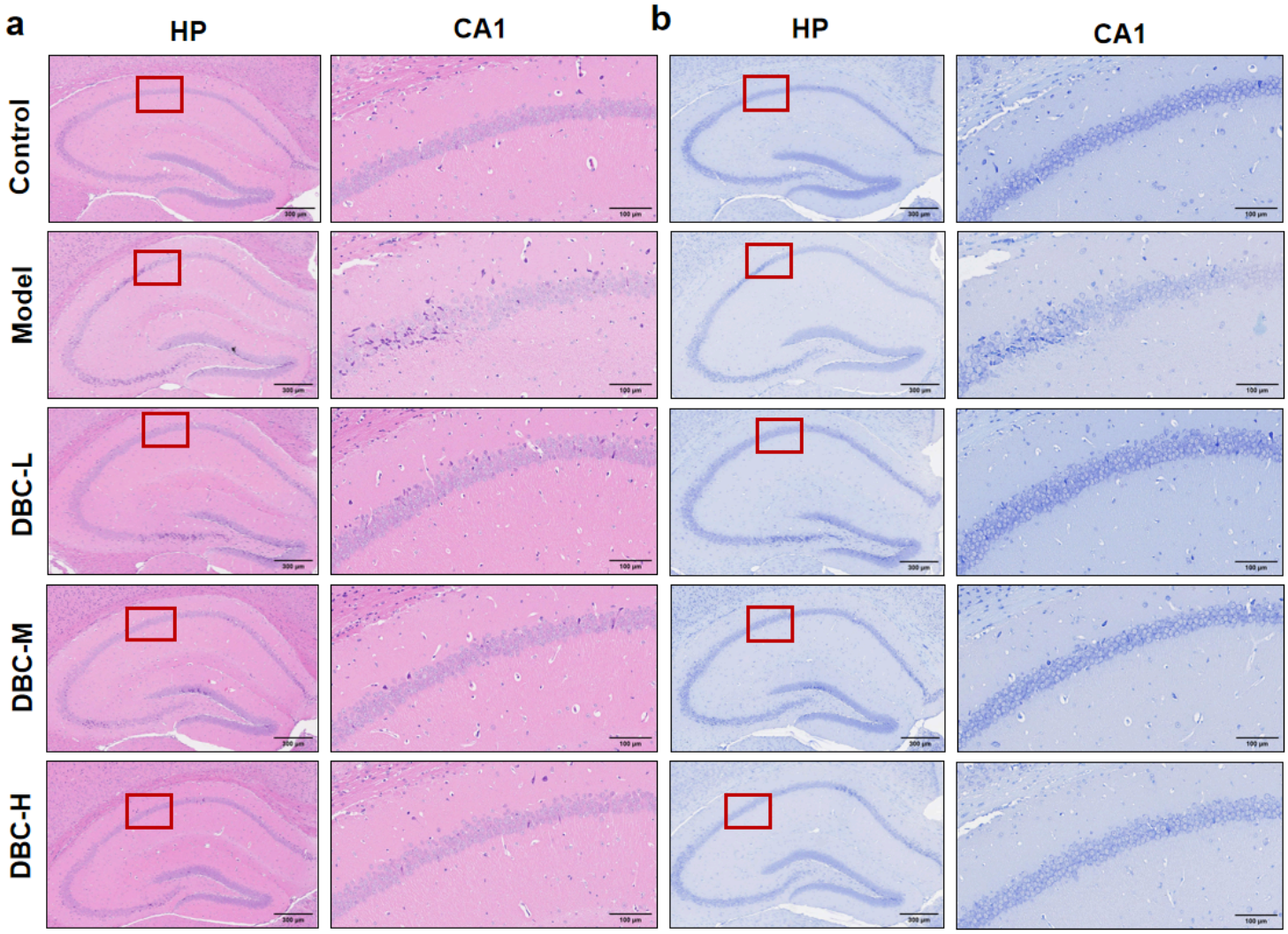


Figure 2

Effect of DBC on hippocampal neuropathology of CUMS depression mice. **a** Images of haematoxylin-eosin (HE) staining of the CA1 in the hippocampus of different groups. **b** Images of Nissl's staining of the CA1 in the hippocampus of different groups. Control, control group; Model, model group; DBC-L, low dose of DBC group; DBC-M, medium dose of DBC group; DBC-H, high dose of DBC group.

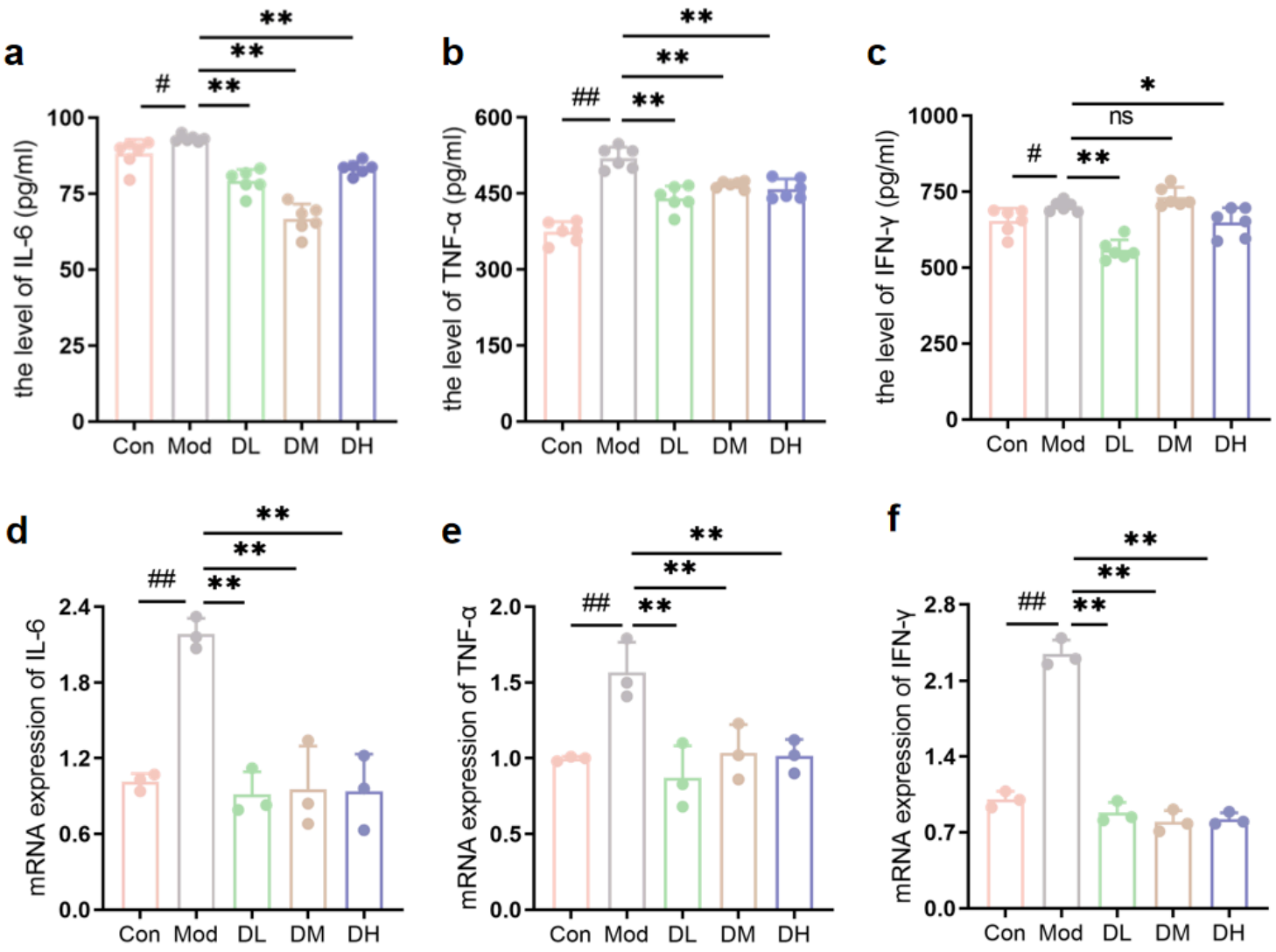


Figure 3

Effect of DBC on serum and hippocampal pro-inflammatory cytokines of CUMS depression model mice. Serum: **a** IL-6 levels in mice. **b** TNF-α levels in mice. **c** IFN-γ levels in mice. Data are presented as mean±SEM, n=6 mice per group. Hippocampus: **d** IL-6 levels in mice. **e** TNF-α levels in mice. **f** IFN-γ levels in mice. Data are presented as mean±SEM, n=3 mice per group. #p<0.05, ##p<0.01 vs. the control group. *p<0.05, **p<0.01 vs. the model group, ns, no significant difference. Con, control group; Mod, model group; DL, low dose of DBC group; DM, medium dose of DBC group; DH, high dose of DBC group. One-way ANOVA, followed by LSD method multiple comparisons tests (**a**, **b**, **c**, **d**, **e**, and **f**)

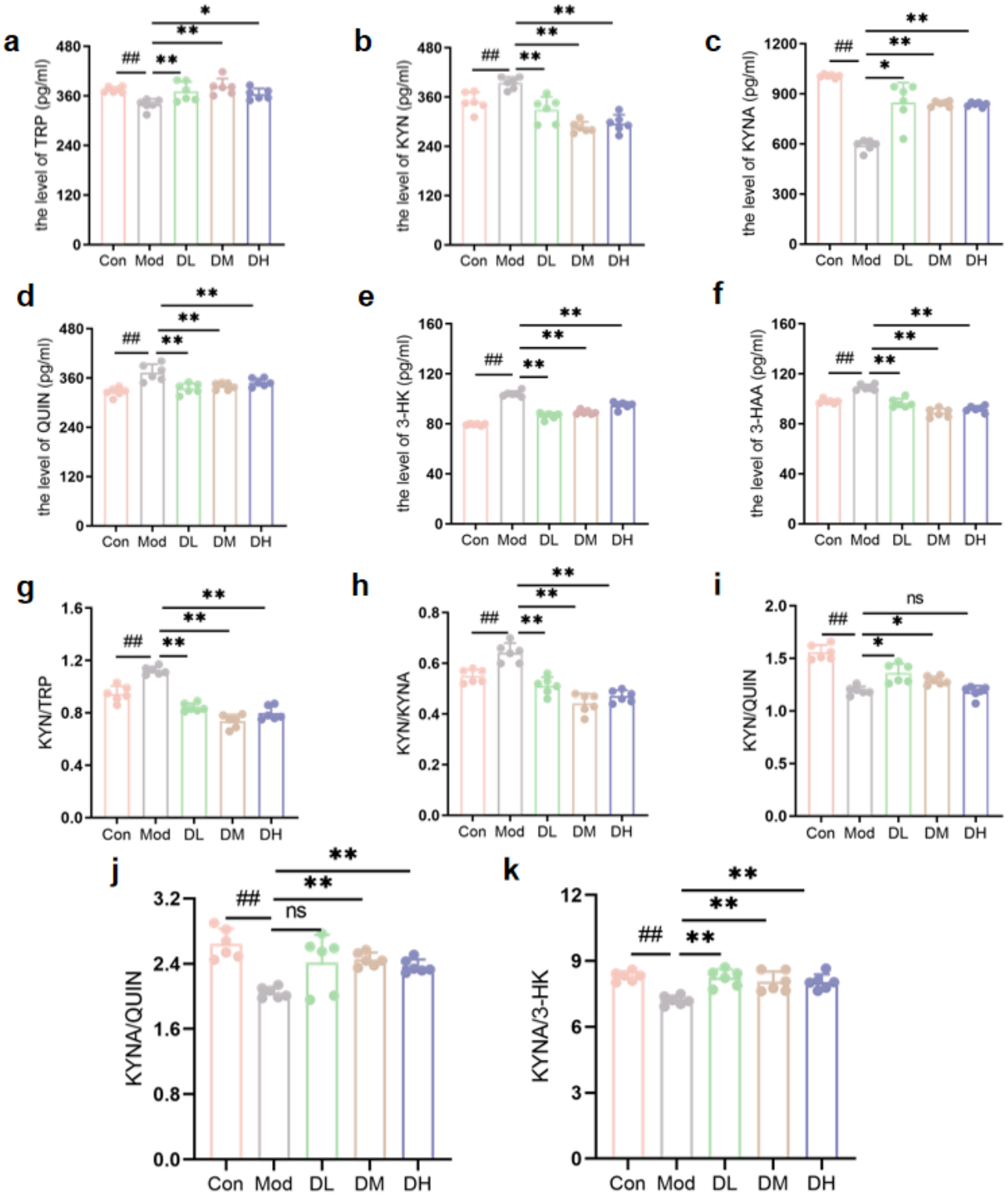


Figure 4

Effect of DBC on serum Trp-Kyn pathway in CUMS depression mice. **a** TRP levels in mice. **b** KYN level in mice. **c** KYNA levels in mice. **d**QUIN level in mice. **e** 3-HK level in mice. **f** 3-HAA levels in mice. **g** KYN/TRP levels in mice. **h** KYN/KYNA levels in mice. **i**KYN/QUIN level in mice. **j** KYNA/QUIN level in mice. **k** KYNA/3-HK level in mice. Data are presented as mean±SEM, n=6 mice per group. ##p<0.01 vs. the control group. *p<0.05, **p<0.01 vs. the model group, ns, no significant difference. Con, control

group; Mod, model group; DL, low dose of DBC group; DM, medium dose of DBC group; DH, high dose of DBC group. One-way ANOVA, followed by LSD method multiple comparisons tests (**a**, **d**, **e**, **f**, **g**, **h**, and **k**) or followed by or Dunnett's T3 method test (**b**, **c**, **i**, and **j**)

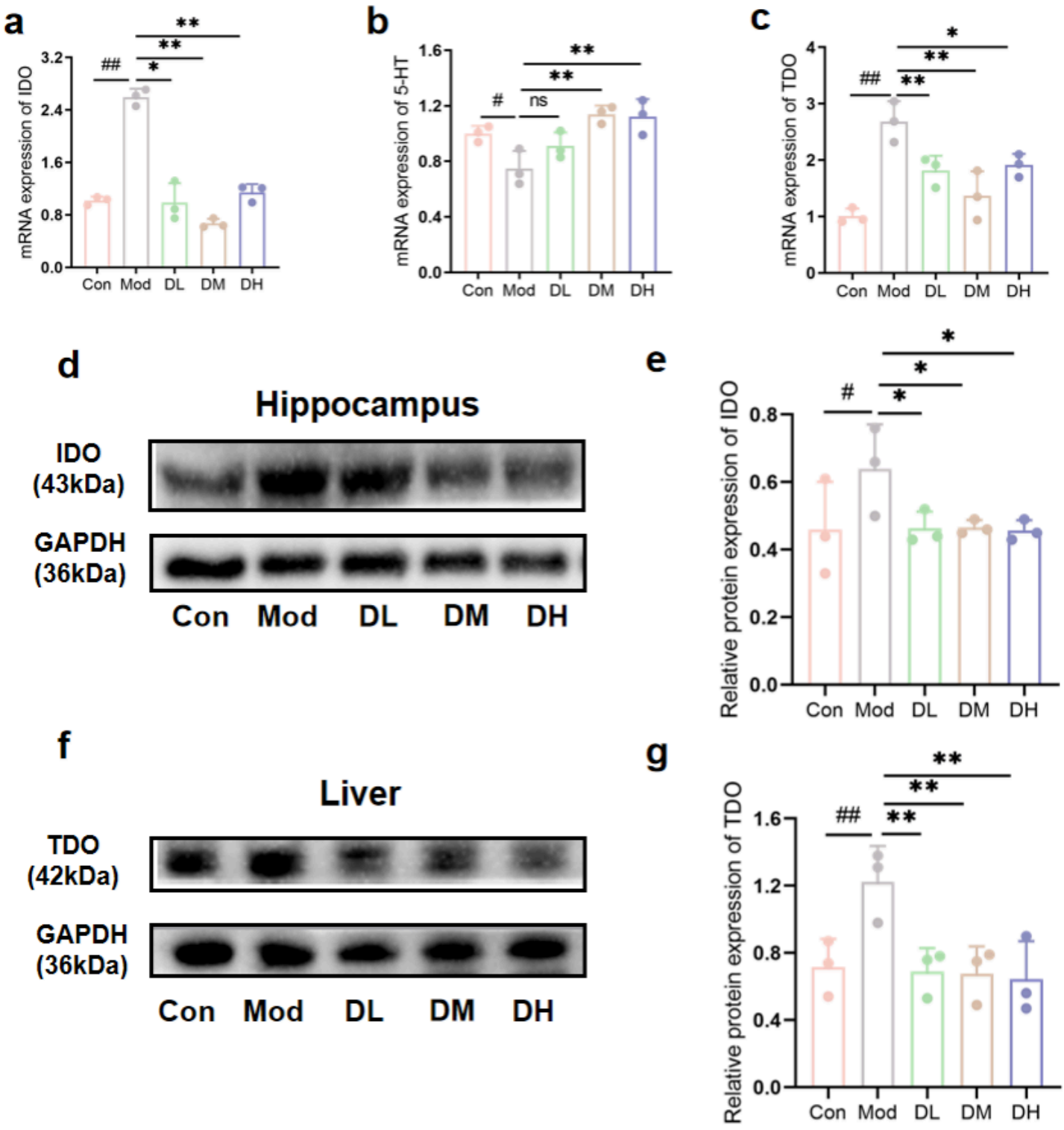


Figure 5

Effects of DBC on hippocampal and liver related enzymes in CUMS depression mice. **a-b** The mRNA level of IDO and 5-HT in the hippocampus of mice. **c** The mRNA levels of TDO in the liver of mice. **d-g**Protein

expression levels of IDO in the hippocampus (full-length gels are presented in Supplementary Materials 2 Figure [**A**]) and TDO in the liver (full-length gels are presented in Supplementary Materials 2 Figure [**D**]) by western blot analysis. Data are shown as mean±SEM, n=3 mice per group. #p<0.05, ##p<0.01 vs. the control group. *p<0.05, **p<0.01 vs. the model group, ns, no significant difference. One-way ANOVA, followed by LSD method multiple comparisons tests (**b**, **c**, **e** and **g**) or Dunnett's T3 method test (**a**)

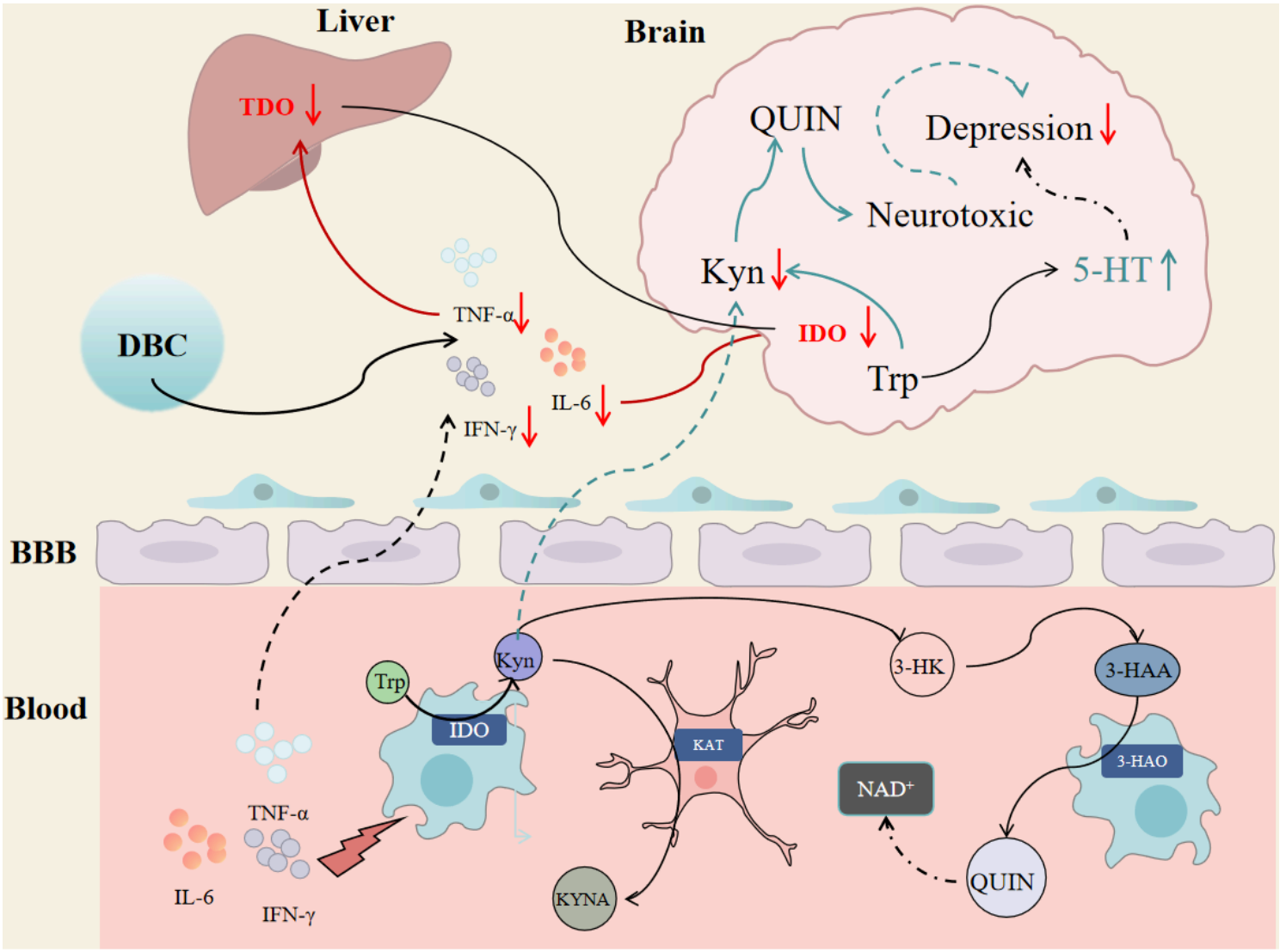


Figure 6

The Trp-Kyn metabolism mechanism of depression

Supplementary Files

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