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Curcumin relieves depressive-like behaviors via inhibition of the NLRP3 inflammasome and kynurenine pathway in rats suffering from chronic unpredictable mild stress

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ABSTRACT

Increasing evidence suggests that inflammation is related to the pathophysiology of depression. Curcumin (CUR), which is a natural component extracted from the rhizome of *Curcuma longa*, seems to be efficacious in depression treatment. Hence, the present study aims to explore whether the anti-depressive effect of curcumin is connected to its anti-inflammatory features. Twenty-one SD rats were randomly divided into three groups, namely, control, CUMS (chronic unpredictable mild stress), and CUMS + CUR. After stress exposure for four weeks, the CUMS group showed depressive-like behaviors, and the curcumin treatment successfully corrected the depressive-like behaviors in stressed rats. Additionally, the curcumin could effectively decrease mRNA expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and suppress NF-κB activation. Curcumin also inhibited the stressed-induced P2X7R/NLRP3 inflammasome axis activation, along with the reduced transformation of pro-IL-1β to mature IL-1β. The stress-induced activation of indolamine-2, 3-dioxygenase (IDO) and an increased kynurenine/tryptophan ratio were also ameliorated by curcumin supplementation. In conclusion, the study revealed that curcumin relieves a depressive-like state through the inhibition of the NLRP3 inflammasome and kynurenine pathway.

1. Introduction

Depression is a common but serious psychiatric disorder characterized by at least two weeks of a depressive state. According to a report by the World Health Organization from 2012, over 350 million people suffer from depression, which is predicted to be the second leading cause of disability by 2020 [1]. Various management approaches have been introduced to treat depression, such as anti-depressant medication, psychosocial support, and cognitive behavior therapy. However, some patients with depression are partially responsive or resistant to existing treatments [2]. Furthermore, widely used anti-depressive drugs have some side effects that surpass the benefits from treatment in some cases [3]. Thus, it is urgent to discover new therapeutic methods, especially new drugs, to achieve successful treatment.

Since it was first proposed in 1995, growing evidence has supported the hypothesis that inflammation is related to the development of depression [4]. It was reported that the concentration of proinflammatory factors were significantly elevated in the periphery, cerebrospinal fluid, and the hippocampus of patients with depression [5,6]. Furthermore, animal studies showed that proinflammatory cytokines could induce depressive-like behaviors and that models of depression could elevate the levels of proinflammatory cytokines [7,8]. The activation of proinflammatory mediators, such as interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF), can induce an inflammation response in neurons. Subsequently, inflammation in neurons can provoke neuroendocrine and neurochemical alternations that can lead to depression [9].

Proinflammatory cytokines can be controlled by NOD-like receptor protein 3 (NLRP3) inflammasome and NF-κB (nuclear factor-κB). The NLRP3 inflammasome is a kind of intracellular multiprotein complex that is composed of NLRP3, the adaptor protein ASC, and caspase-1

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[10]. The NLRP3 inflammasome plays a key role in the innate immune system and inflammatory response. The NLRP3 inflammasome can be activated by the ATP-gated transmembrane channel P2X7R [11]. Subsequently, activation of the NLRP3 inflammasome results in caspase-1 activation and the transformation of pro-IL-1β to mature IL-1β [12]. NF-κB is an essential factor in immune activation. Once stimulated, the inhibitor of NF-κB (IκBs) would be phosphorylated and degraded, and then the NF-κB would translocate to the nucleus and regulate the expression of inflammatory cytokines, chemokines and adhesion molecules [13].

Proinflammatory cytokines can induce the expression of indolamine-2, 3-dioxygenase (IDO), which is considered to serve as an interface between inflammation and depression [14]. Clinical studies showed that increased IDO activity was positively correlated with the severity of depressive scores [15]. Preclinical studies have shown that IDO activation results in depressive-like symptoms, and the IDO inhibitor can relieve a depressive-like state in mice [14,16]. IDO is a key enzyme that metabolizes tryptophan (TRP) along the kynurenine (KYN) pathway, which leads to tryptophan depletion [17]. Tryptophan is a material of serotonin (5-HT); therefore, the IDO activation will deplete tryptophan and ultimately inhibit 5-HT synthesis [16]. A shortage of 5-HT is an important part of the classic monoamine hypothesis of depression, and selective 5-HT reuptake inhibitors have become the most pivotal antidepressants [18].

Curcumin, which is a natural component discovered in the rhizome of *Curcuma longa*, has been reported to possess anti-inflammatory, antioxidant, and neurotrophic effects [19]. A meta-analysis reviewed six clinical trials including 377 patients who supported the safety and significant clinical efficacy of curcumin in ameliorating depressive symptoms [20]. Although several studies have discussed the potential mechanism of its anti-depressive effect [21,22], the mechanism underlying this effect is still not fully understood. In this study, we used a classic model of depression to explore the following question: Does curcumin exert an anti-depressive effect via inhibition of the NLRP3 inflammasome and kynurenine pathway in rats suffering from chronic unpredictable mild stress?

2. Materials and methods

2.1. Animals

Experiments were conducted on male Sprague-Dawley (SD) rats (210 ± 30 g). All rats were maintained under standard laboratory conditions (12 h light/dark cycles, a temperature of 22 ± 1 °C, relative humidity of 55 ± 10%, food and water ad libitum except for rats kept under a deprivation procedure). The rats were habituated for 7 days before the experiment. The animal experiments were conducted by the ethical standards in the 1964 Declaration of Helsinki and its later amendments, with the approval of the Ethics Committee of the Zhongshan Affiliated Hospital of Zhongshan University. All efforts were made to minimize animal suffering and to reduce the number of animals used in the research.

2.2. CUMS procedure and drug treatment

The SD rats were randomly divided into the following three groups (7 rats in each group): the control, the CUMS (chronic unpredictable mild stress), and the CUMS + CUR group. All the rats were housed individually (cage size: 47.5 cm × 35 cm × 20 cm). The CUMS procedure followed a previous method with minor modifications [23]. Curcumin (Sigma-Aldrich, USA, Fig. 1a) was suspended in 0.5% Tween 80, and then the suspension was administered each day by oral gavage (100 mg/kg) for the CUMS procedure. The CUMS procedure was performed for 4 weeks using the following conditions: food deprivation for 24 h and water deprivation for 24 h; 45° cage tilting for 24 h; damp bedding and group housing for 24 h; restraint for 4 h in an empty water

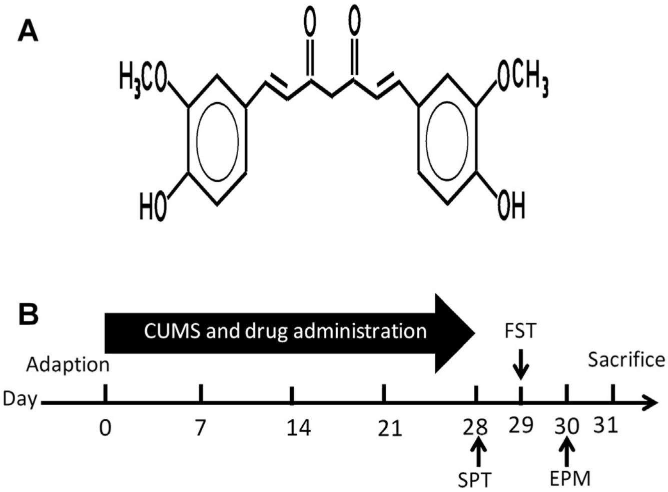

Fig. 1. a. The construction of curcumin. b Timeline of the experimental procedures. SPT, Sucrose preference test; FST, Forced swim test; EPM Elevated plus maze test.

bottle (Wahaha, China); 20 min of noise; 1 min tail clamping; and day-night reversal (12 h/12 h). To make the procedure unpredictable, these protocols were randomly scheduled and changed every week. The rats were subjected to one of these stressors daily.

Behavioral tests, including the sucrose preference test, forced swim test, and elevated plus maze test, were performed to test the animals for depressive-like and anxiety-like states (Fig. 1b). Twenty-four hours after the final behavioral test, the rats were anesthetized with a peritoneal injection of 10% chloral hydrate (0.3 ml/100 g). Hippocampus samples were thoroughly washed with cold physiological saline and then frozen in liquid nitrogen immediately. Finally, the samples were stored at −80 °C until analysis.

2.3. Sucrose preference test (SPT)

The SPT, which is a method to evaluate anhedonia, was induced as described previously [24]. Before the SPT test, the individually housed rats were provided two bottles containing 1% sucrose solution for 48 h to habituate them to the taste of sucrose. After 14 h of water deprivation, the two bottles were weighed and placed in each cage with one containing tap water and another containing 1% sucrose solution. To avoid spatial bias, the side (left and right) the bottles were placed on was randomly selected. One hour later, the two bottles were weighed again, and the weight difference in each bottle was considered the rat intake. The sucrose preference was calculated as a percentage of the consumed sucrose solution relative to the total liquid consumption.

2.4. Forced swim test (FST)

After the SPT test, the FST was conducted according to a classic paradigm with minor modifications [25]. The FST contained two trials. In the first trial, the rats were individually forced to swim for 15 min in a Plexiglas cylinder (45 cm height, 25 cm diameter) filled with water (24 ± 1 °C) to a height of 35 cm. The rats were dried and returned to their cages. In the second trial, the rats were forced to swim again for 5 min 24 h later, and the test session was videotaped. The total duration of immobility in the second trial was measured by an experienced observer who was blinded to the experimental design. A rat was considered immobile when it was floating motionless or making slight movements to keep its head just above water.

2.5. Elevated plus maze test (EPM)

The EPM test was performed according to a previous method [26]. The maze apparatus was shaped like a plus sign and consisted of two opposite open arms (50 × 10 cm) and two opposite closed arms (50 × 10 cm) with 40-cm walls that were connected by a central platform (10 × 10 cm). The apparatus was elevated 50 cm from the floor in a dimly light house. The rats were placed at the center of the platform and pointed in the direction of an open arm. In a 5-min trial, the total number of entries into the open and closed arms, as well as the time spent in each arm, was measured by a video camera fixed vertically above the maze.

2.6. Western blotting analysis

Total protein was prepared from the hippocampus. The protein concentration was determined using the Bradford method. The samples were loaded into precast 12% SDS-PAGE gels with 50 μg of protein in each lane. The proteins in the gels were transferred to a PVDF membrane and then blocked for 1 h in 5% nonfat dry milk in TBS-T (25 mM Tris, 150 mM NaCl, pH 7.5, 0.05% Tween-20). The antibodies and concentrations listed below were used overnight at a temperature of 4 °C: P2X7R (1:300; Abcam; USA), NLRP3 (1:200; Abcam; USA), IDO (1:200; Proteintech; USA), ASC (1:1000; Abcam; USA), caspase-1 (1:1000; Abcam; USA), IL-1β (1:1000; Abcam; USA), β-actin (1:4000; Proteintech; USA), P65 (1:500; Proteintech; USA), IκB (1:1000; Cell Signaling; USA). Subsequently, the membrane was probed with an HRP-conjugated secondary antibody for 40 min. Finally, the film signals, which were digitally scanned and quantified using Image-Pro Plus 6.0 (Media Cybernetics, Baltimore, MD, USA), were normalized to β-actin as an internal standard.

2.7. Quantitative real-time PCR

Total RNA was isolated from the hippocampal homogenates using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The mRNA expression of IL-1β, IL-6, and TNF-α was detected. Quantitative real-time PCR was performed on a Bio-Rad Cx96 Detection System (Biorad, USA) using a SYBR green PCR kit (Applied Biosystems, USA) and gene-specific primers. Oligonucleotide primers specific for rats are listed in Table 1. The 5 ng cDNA samples received 40 cycles of amplification. Each cDNA was tested in triplicate. Relative mRNA expression levels were normalized to β-actin as an internal standard.

2.8. Immunohistochemical examinations

Paraffin-embedded coronal sections of the hippocampus were dewaxed by passing them through a xylol solution. Subsequently, the sections were rehydrated and rinsed in phosphate-buffered saline (PBS) solution. After that, the sections of the hippocampus were blocked with 3% H_2O_2 for 20 min. For antigen retrieval, the sections were boiled on an electric stove in a citric acid buffer (0.01 mol/l, pH 6.0), followed by blocking with 5% goat serum for 1 h at room temperature. After incubation with the anti-P2X7R (1:300; Abcam; USA), anti-NLRP3 (1:200; Abcam; USA), or anti-IDO (1:50; Santa Cruz; USA) overnight at 4 °C, the sections were incubated with a 2-step plus® Poly-HRP Anti-Mouse/Rabbit IgG Detection System (ZSGB-BIO, China) against the primary antibodies. Ultimately, the color reaction was developed with 3,3′-diaminobenzidine (DAB), and the sections were counterstained with hematoxylin.

2.9. Neurochemistry analysis

Following the method established in our previous studies [27], kynurenine, tryptophan, and 5-HT in the hippocampus were detected using high-performance liquid chromatography coupled with tandem mass spectrometry. Briefly, the hippocampus (50 mg) was treated with 1 ml of 85% acetonitrile–water solution and 10 μl of mixed internal standard solution (including 0.12 μg/ml 3,4-dihydroxybenzylamine, 1.41 μg/ml L-aspartic acid-$^{13}C4,^{15}N$, and 0.19 μg/ml 5-hydroxyindole-2-carboxylic acid). Subsequently, the mixtures were homogenized evenly in a Precellys 24 multifunctional homogenizer. The mixtures were vortex-mixed and then centrifuged (15,000 rpm, 4 °C) for 5 min. The supernatant (500 μl) was evaporated to dryness under vacuum conditions. For derivatization, the residue was added with 150 μl of dansyl chloride solution (4 mg/ml in acetonitrile) and 50 μl of 0.1 M Na_2CO_3–$NaHCO_3$ buffer (pH 11.0), and they were reacted at 35 °C for 30 min. The pH of the mixture was adjusted to 7.0 by adding 5 μl of 15% formic acid water solution. Chromatographic analyses were conducted on an ultra-performance liquid chromatography system (Waters, USA) coupled with a Micromass Quattro Premier XE tandem quadruple mass spectrometer (Waters, USA) equipped with an ESI source. We used an Ultimate XB-C8 column (3.0 μm particle size, 2.1 mm × 50 mm, Welch, China) to separate the analytes, and the column temperature was maintained at 40 °C. The mobile phase was a gradient established between solvent A (acetonitrile) and solvent B (water with 20 mM ammonium acetate and 0.1% formic acid), and the flow rate was 0.25 ml/min. The source worked in positive ion mode, and the parameters are listed below: capillary voltage, 3.00 kV; extractor voltage, 3.00 V; source temperature, 120 °C; desolvation temperature, 450 °C; desolvation gas flow, 750 l/h; cone gas flow, 50 l/h. The collision gas (Argon) was sent into the collision cell at a flow rate of 0.16 ml/min. Kynurenine, tryptophan, and 5-HT were quantified relative to the areas of the peaks from internal standards and were calibrated by using standard curves.

2.10. Statistical analysis

The results of the experiment determined as the means ± SD and analyzed using SPSS version 13.0 software. Differences between groups were determined by one-way analysis of variance (ANOVA). When the F ratios were significant, post hoc comparisons were made using the LSD post hoc test. A prior level of significance was established at $P < 0.05$.

3. Results

3.1. Effects of curcumin on behavioral tests

After stress exposure for four weeks, the CUMS group showed decreased weight gain ($P < 0.01$), reduced sucrose preference in SPT

Table 1
Primer sequences used for the qPCR analysis.

Gene	Sense primer (5′–3′)	Antisense primer (5′–3′)	Amplicon length (bp)
IL-1β	AGGTCGTCATCATCCCACGAG	GCTGTGGCAGCTACCTATGTCTTG	119
IL-6	CACAAGTCCGGAGAGGAGAC	ACAGTGCATCATCGCTGTTC	167
TNF-α	GAGAGATTGGCTGCTGGAAC	GAGAGATTGGCTGCTGGAAC	82
β-Actin	CATCCTGCGTCTGGACCTGG	TAATGTCACGCACGATTTCC	116

Table 2
Effects of curcumin supplementation and CUMS on body weight and behavioral changes in elevated plus maze test, forced swim test and sucrose preference test.

Groups	Body weight gain (g)	Time spent on open arms (%)	Open arm entries (n)	Immobility time (s)	% sucrose preference
Control	125.57 ± 11.68	39.57 ± 10.40	11.67 ± 3.56	106.37 ± 13.41	89.71 ± 6.47
CUMS	98.71 ± 12.25**	17.71 ± 5.15**	7.20 ± 2.28*	162.86 ± 13.13**	58.42 ± 10.83**
CUMS + CUR	111.71 ± 16.45	26.43 ± 8.14^{+}	9.71 ± 3.16	129.14 ± 6.35^{++}	82.71 ± 14.44^{++}

Data are the means ± SD (n = 7). *$P < 0.05$, **$P < 0.01$ CUMS group compared to the control group. $^{+}P < 0.05$, $^{++}P < 0.01$ CUMS + CUR group compared to the CUMS group.

($P < 0.01$), and prolonged immobility time ($P < 0.01$) in the FST in compared to the control group. In addition, the CUMS rats displayed a reduction of open arm entries ($P < 0.05$) and in the percentage of time spent on the open arms ($P < 0.01$) in EPM. The treatment of curcumin successfully increased the sucrose preference ($P < 0.01$), decreased immobility time ($P < 0.01$), and increased the percentage of time spent on the open arms ($P < 0.05$) in the CUMS + CUR group compared to the CUMS group (Table 2).

3.2. Curcumin ameliorates stress-induced NF-κB and proinflammatory activation

The protein expression of NF-κB P65 ($P < 0.05$, Fig. 2b) was significantly increased, and IkB ($P < 0.01$, Fig. 2c) was significantly decreased in the CUMS group compared to the control group. The curcumin treatment successfully reduced the protein expression of NF-κB P65 ($P < 0.01$, Fig. 2b) and increased protein expression of IkB ($P < 0.05$, Fig. 2c) in the CUMS + CUR group compared to the CUMS group. The mRNA levels of IL-1β ($P < 0.01$, Fig. 2d), IL-6 ($P < 0.05$, Fig. 2e), and TNF-α ($P < 0.01$, Fig. 2f) increased in the CUMS group compared to the control group, and curcumin treatment significantly prevented the increase of IL-1β ($P < 0.01$, Fig. 2d), IL-6 ($P < 0.05$, Fig. 2e), and TNF-α ($P < 0.01$, Fig. 2f) in the CUMS + CUR group compared to the CUMS group.

3.3. Curcumin inhibits P2X7R-mediated NLRP3 inflammasome activation in CUMS rats

The protein expression of P2X7R ($P < 0.01$, Fig. 3d), NLRP3 ($P < 0.01$, Fig. 3e), ASC ($P < 0.05$, Fig. 3f), and Caspase-1 P20 ($P < 0.01$, Fig. 3g) significantly increased in the CUMS group compared to the control group. The treatment of curcumin successfully reduced the protein expression of P2X7R ($P < 0.01$, Fig. 3d), NLRP3 ($P < 0.05$, Fig. 3e), and Caspase-1 P20 ($P < 0.01$, Fig. 3g) in the CUMS + CUR group compared to the CUMS group. However, curcumin did not reduce ASC protein expression to a significant level. The protein level of pro-IL-1β ($P < 0.01$, Fig. 3h) and mature-IL-1β ($P < 0.01$, Fig. 3i) increased in the CUMS group compared to the control group, and curcumin treatment significantly prevented the increase of pro-IL-1β ($P < 0.05$, Fig. 3 h) and mature-IL-1β ($P < 0.01$, Fig. 3i) in the CUMS + CUR group compared to the CUMS group.

3.4. Curcumin mitigates stress-induced activation of the IDO-kynurenine pathway

The protein expression of IDO was significantly increased in the CUMS group compared to the control group and curcumin treatment markedly decreased IDO expression in the CUMS + CUR group compared to the CUMS group. There is no significant difference in TRP content ($P > 0.05$, Fig. 4c) between the three groups. The KYN content ($P < 0.01$, Fig. 4d) and the KYN/TRP ratio ($P < 0.01$, Fig. 4e) were significantly elevated, whereas the 5-HT content ($P < 0.01$, Fig. 4f) was reduced in the CUMS group compared to the control group. Curcumin treatment significantly restored the change in KYN ($P < 0.01$, Fig. 4d), the KYN/TRP ratio ($P < 0.05$, Fig. 4e), and 5-HT ($P < 0.05$, Fig. 4f) in the CUMS + CUR group compared to the CUMS group.

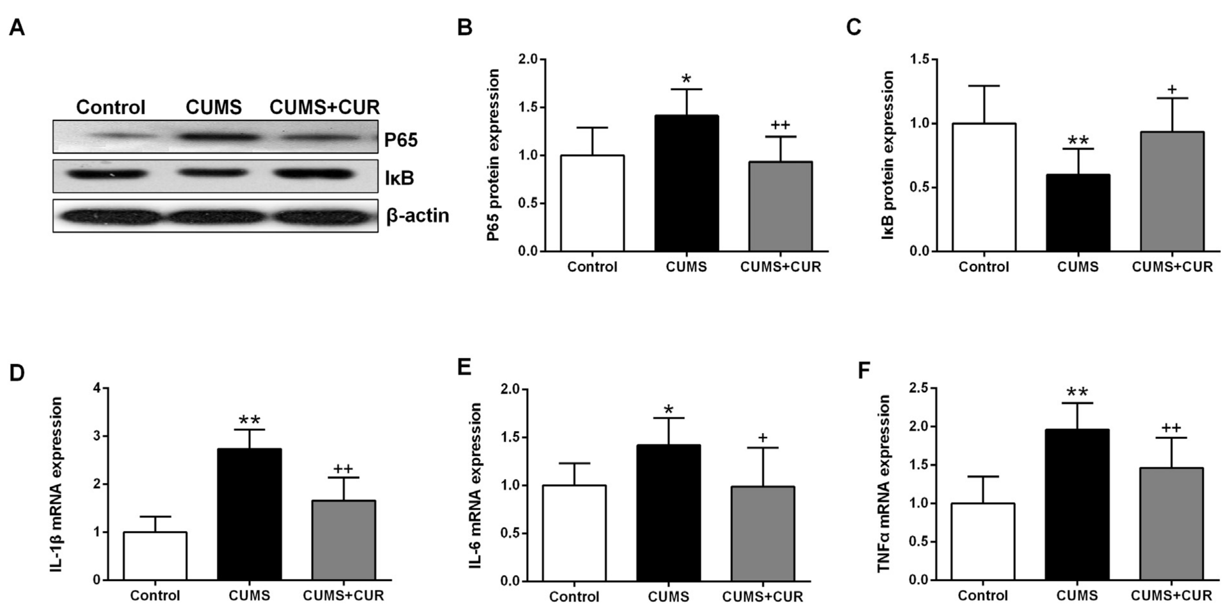

Fig. 2. Curcumin inhibits NF-κB and proinflammatory cytokine expression in the hippocampus of stressed rats.
(a) Representative blots of NF-κB P65 and IkB. Statistical graphs of relative protein expression of NF-κB P65 (b) and IkB (c). Statistical graphs of relative mRNA expression of IL-1β (d), IL-6 (e), and TNF-α (f). Data are the means ± SD (n = 6–7). *$P < 0.05$, **$P < 0.01$ CUMS group compared to the control group. $^{+}P < 0.05$, $^{++}P < 0.01$ CUMS + CUR group compared to the CUMS group.

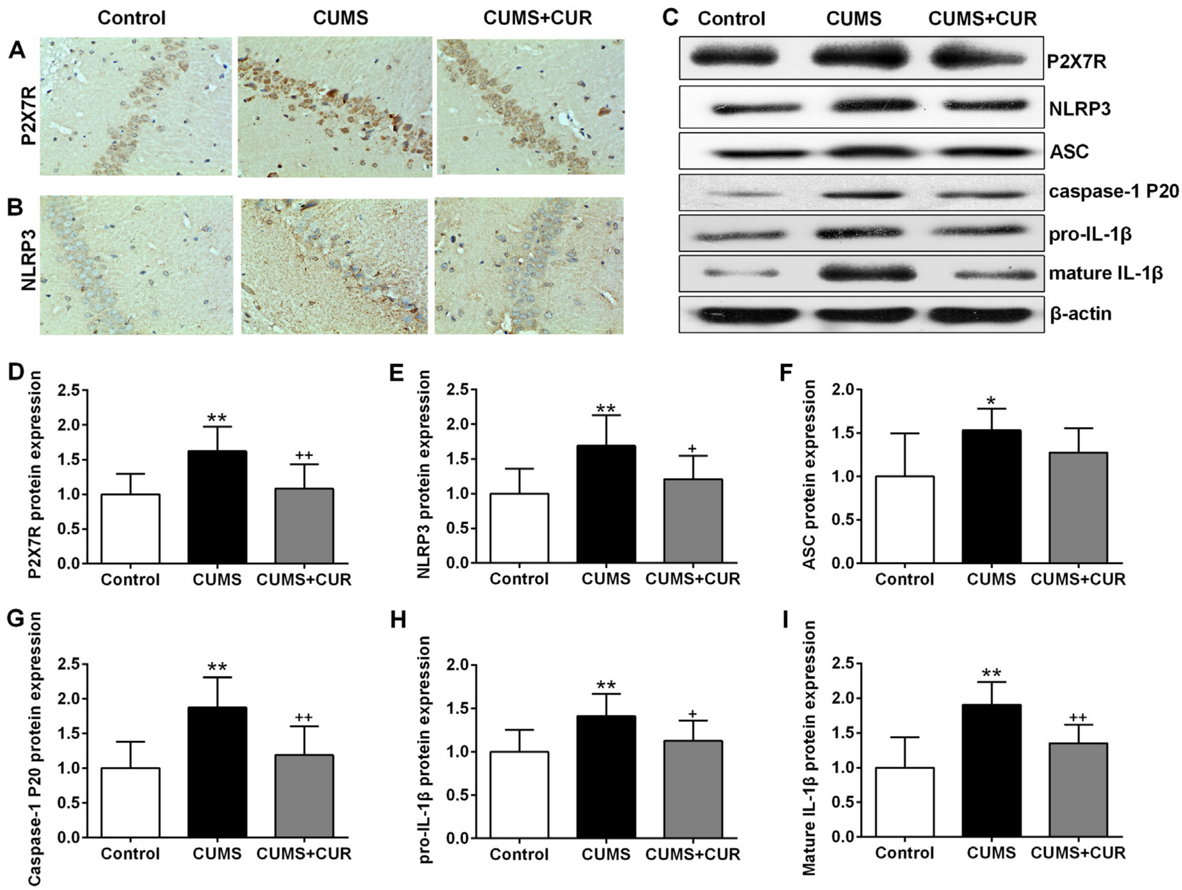

Fig. 3. Curcumin inhibits P2X7R-mediated NLRP3 inflammasome activation and inhibits the transformation of pro-IL-1β to mature IL-1β in the hippocampus of stressed rats. Representative images of immunohistochemical assays of P2X7R (a) and NLRP3 (b) in the hippocampus. Representative blots of P2X7R/NLRP3 inflammasome axis and IL-1β (c). Statistical graphs of relative protein expression of P2X7R (d), NLRP3 (e), ASC (f), caspase-1 P20 (g), pro-IL-1 β (h), and mature IL-1β (i). Data are the means ± SD (n = 6–7). $^{*}P < 0.05$, $^{**}P < 0.01$ CUMS group compared to the control group. $^{+}P < 0.05$, $^{++}P < 0.01$ CUMS + CUR group compared to the CUMS group.

4. Discussion

In this study, the chronic administration of curcumin showed antidepressant activity in CUMS rats. Additionally, the curcumin could effectively decrease mRNA expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and suppress NF-κB activation. Curcumin also inhibited the stressed-induced P2X7R/NLRP3 inflammasome axis activation, along with the reduced transformation of pro-IL-1β to mature IL-1β. The stress-induced activation of indolamine-2, 3-dioxygenase (IDO) and the increased kynurenine/tryptophan ratio were also ameliorated by curcumin supplementation. All these changes might contribute to, at least in part, the antidepressant activity of curcumin (Fig. 5).

The CUMS model is a widely applied animal model of depression. In this study, the CUMS rats showed reduced sucrose preference in SPT and prolonged immobility time in FST, which represented the depressive-like state in rats. Consistent with former findings [21,22], the treatment of curcumin successfully increased sucrose preference and decreased immobility time compared to the CUMS group. The changed behavior represented an anti-depressive effect of curcumin. In line with a previous study [28], the CUMS rats displayed a reduction in open arm entries and in the percentage of time spent in the open arms in EPM, which represents anxiety-like behavior in this model. It is noteworthy that the curcumin also successfully increased in the percentage of time spent on the open arms in the CUMS + CUR group. The result indicates that curcumin has the potential to reduce stress-induced anxiety.

The hippocampus, which is an important part of the limbic system, plays a pivotal role in the development of depression. Hippocampal neuroinflammation has been confirmed in patients with depression and animal models of depression [29,30]. Curcumin could regulate inflammation by not only decreasing proinflammatory cytokines but also increasing anti-inflammatory cytokines [31]. A previous study found that curcumin could effectively inhibit TNF-α and IL-6 expression, as well as the activation of NF-κB in the hippocampus of stressed Wistar rats [32]. In this study, we further found that curcumin treatment dramatically prevented CUMS-induced P2X7R-mediated NLRP3 inflammasome activation and subsequently inhibited the transformation of pro-IL-1β to mature IL-1β. Although TNF-α, IL-6 and IL-1β are both proinflammatory acute-phase response proteins, they may respond differently in depression. A meta-analysis reported significantly higher levels of TNF-α and IL-6 in the bloodstream of depressed subjects but not IL-1β [6]. Interestingly, another meta-analysis showed that antidepressant treatments decreased IL-1β levels and possibly IL-6 but not TNF-a [33]. IL-1β is the most potent proinflammatory cytokine produced by macrophages in the blood and microglia in the brain [34], and IL-1β is a key mediator of stress-related depressive-like behavior [6]. Stimulation of IL-1β resulted in several depressive-like behaviors in animals, and inhibition of IL-1β overexpression showed an anti-depressive-like effect [33]. Thus, the findings indicate P2X7R/NLRP3 inflammasome axis and IL-1β transformation inhibition could be a novel mechanism underlying the anti-depressive effects of curcumin.

It has been shown that proinflammatory cytokines can induce the activity of IDO in the brain [14]. Subsequently, the activation of IDO shifts tryptophan metabolism away from the production of

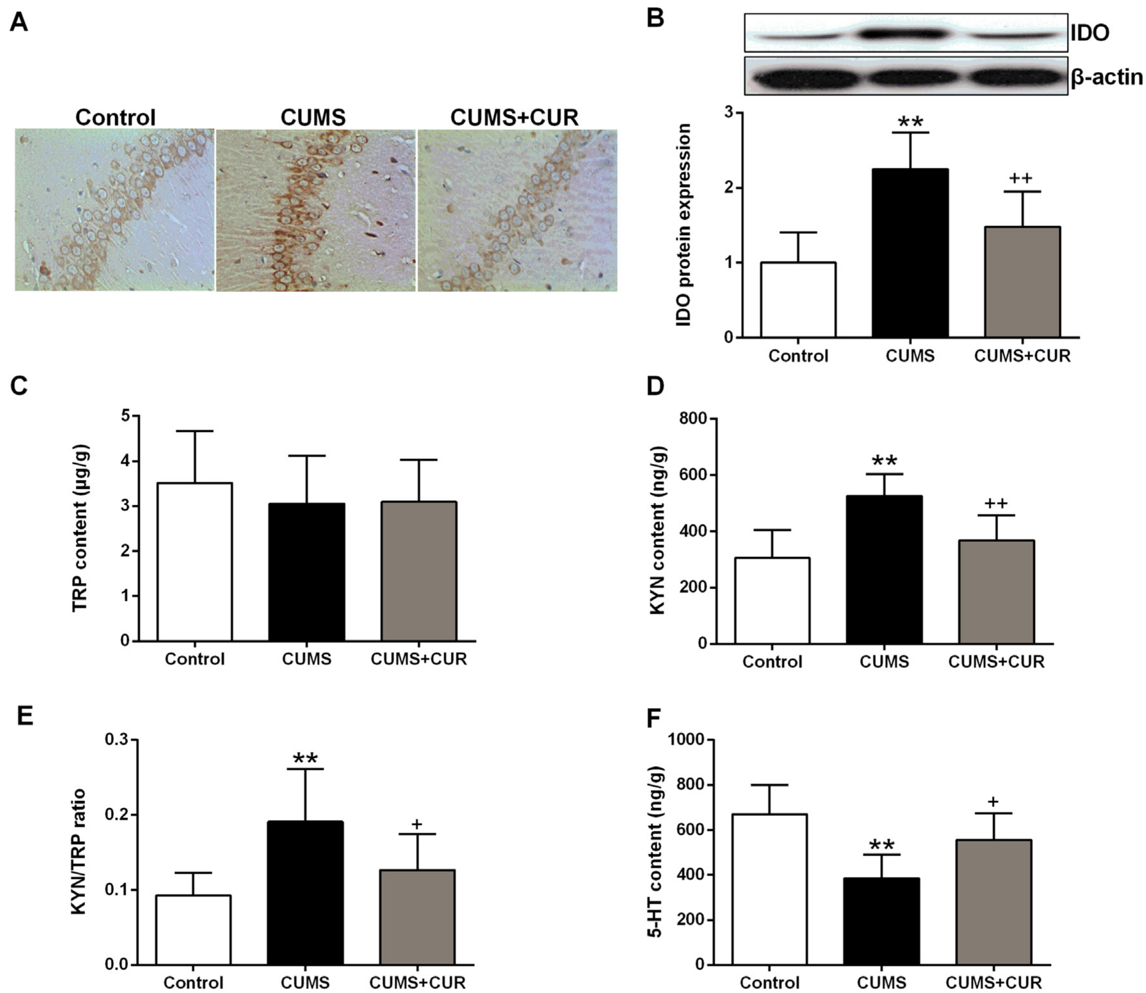

Fig. 4. Curcumin inhibits IDO-kynurenine pathway in the hippocampus of stressed rats.
Representative images of immunohistochemical assays of IDO (a). Representative blots and statistical graphs of IDO (b). Statistical graphs of TRP (c), KYN (d), KYN/TRP ratio (e), and 5-HT (f). Data are the means ± SD (n = 6–7). **P < 0.01 CUMS group compared to the control group. $^{+}P < 0.05$, $^{++}P < 0.01$ CUMS + CUR group compared to the CUMS group.

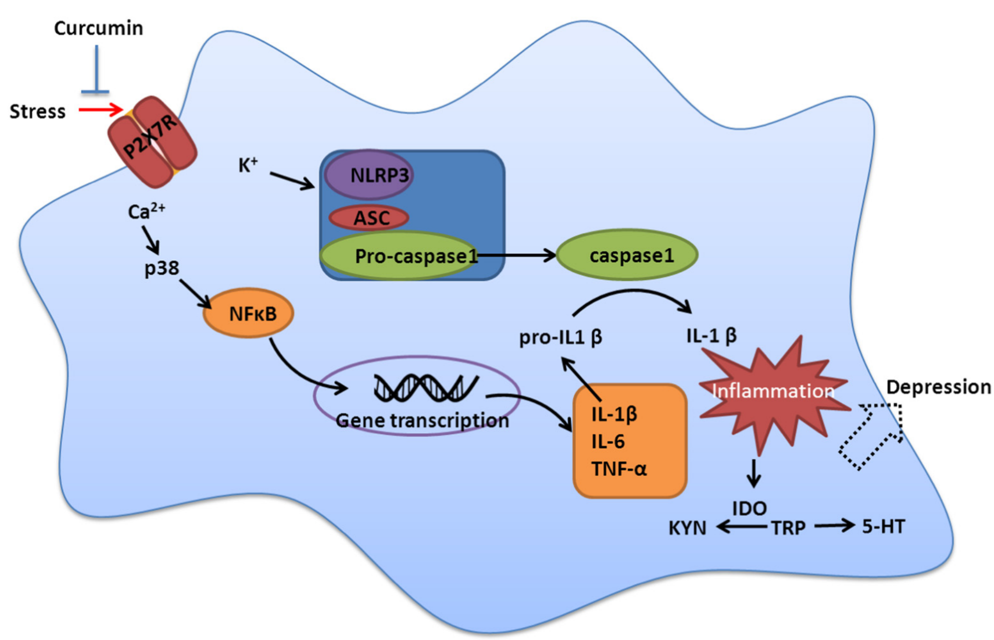

Fig. 5. Schematic diagram summarizing the mechanism of the anti-depressive effect of curcumin in stressed rats.

neurotransmitter 5-HT to neurotoxic KYN, and KYN itself can induce depressive-like behavior [35]. Furthermore, IDO can degrade 5-HT to 5-hydroxykynuramine, which further decreases the level of 5-HT [34]. In the present study, we observed an increase in the KYN/TRP ratio, up-regulated IDO protein expression, and a significant corresponding decrease in the 5-HT level in the hippocampus of CUMS rats, which correlates with the results from previous literature [18]. More importantly, we found that curcumin could effectively restore the elevated IDO expression and decreased 5-HT level in the hippocampus of CUMS rats. The low-level of 5-HT could inhibit the expression of brain-derived neurotrophic factor (BDNF) and dysregulate neuronal survival [36]. Curcumin could prevent the loss of spines and reduction of dendrite length, volume and surface area of the neurons in stressed rats [37]. The results lend more weight to the hypothesis that the anti-depressive effects of curcumin occur through its potent anti-inflammatory effects.

This study also has some limitation. First, we did not test other parts of the brain involved in depression, such as the cerebral cortex. Second, the results in animals cannot be directly translated to humans. Despite these limitations, the conclusion still can be made that treatment with curcumin could ameliorate depressive-like behaviors in CUMS rats. We also provide novel evidence that curcumin could restore changes in proinflammatory cytokines, the P2X7R/NLRP3 inflammasome axis, and the IDO-kynurenine pathway in the hippocampus of CUMS rats, which might ultimately contribute to its anti-depressive like effect.

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Conflicts of interest

The authors declare that they have no competing interests.

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