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The effects of EGb761 on lipopolysaccharide-induced depressive-like behaviour in C57BL/6J mice

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Abstract

There is an increasing body of evidence for the involvement of inflammation and brain-derived neurotrophic factor (BDNF) in depression. Ginkgo extract EGb761 possesses anti-inflammatory, anti-oxidative, anti-arteriosclerosis, and neuroprotective activities. But the effect of EGb761 on lipopolysaccharide (LPS)-induced depressive-like behaviours has not been investigated. The present study mainly aimed to examine the antidepressant-like activities of Ginkgo extract EGb761 in mice after lipopolysaccharide administration. C57BL/6J male mice were pretreated with EGb761 or vehicle for 10 days. Then, a single dose of lipopolysaccharide was intraperitoneally administrated to mice to induce depressive-like behaviour. Forced swim test (FST), tail suspension test (TST), and sucrose preference test were performed to evaluate the depressive-like behaviours of the mice. Locomotor activity was examined by open field test. Levels of brain-derived neurotrophic factor, TNF-α, IL-1β, IL-6, IL-17A, and IL-10 in hippocampus tissue homogenate were measured using ELISA kits. We found that LPS administration induced significant depressive-like behaviours, higher levels of tumour necrosis factor α (TNF-α), interleukin (IL) 1β, IL-6, and IL-17A, but lower levels of BDNF and IL-10 in hippocampus tissue homogenate of the mice from the vehicle group compared to the control mice. Pretreatment with middle dose (100 mg/kg/day) and high dose (150 mg/kg/day) of EGb761 significantly attenuated depressive-like behaviours without affecting spontaneous locomotor activity, and inhibited the changes of hippocampal cytokines and BDNF induced by LPS administration. We conclude that EGb761 has antidepressant-like activities in mice with LPS-induced depressive-like behaviours.

Key words: *EGb761, depressive-like behaviour, lipopolysaccharide, inflammation, brain-derived neurotrophic factor.*

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Introduction

Depression is a serious psychological disorder affecting 20% of the population worldwide [1]. Besides psychotherapeutic methods, drugs posing antidepressant activity targeting different pathogenic factors are necessary for the majority of depressive patients.

There is a growing body of evidence supporting the link between inflammation and the aetiology of depression [2]. Some cytokines are believed to be important contributors to the pathogenesis of depression. Preclinical and clinical research, including our previous study, have documented that subjects with depression usually have increased levels of inflammatory cytokines such as interleukin (IL) 1β, tumour necrosis factor α (TNF-α) and IL-6 [2-5]. These cytokines can both directly impact neuron function and indirectly stimulate the production of neuroactive molecules associated with depression. Evidence also demonstrates that many antidepressant medications can affect levels of those cytokines [6, 7], and that the therapy directly targeted at controlling the over-expression of those cytokines has antidepressant effects [2]. Recently, Th17 cells, a new sub-group of T help cells, and their cytokine IL-17, have been shown to have a positive role in the pathogenesis of depression in recent literature, including our previous study [8-11]. But regulatory T (T_{reg}) cell-associated cytokine IL-10 was negatively correlated with depressive behaviours and showed antidepressant effects in some animal studies [12]. Considering these findings,

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medications targeted at alleviating inflammation seem to be potential treatments for depression.

Recently, it has been noted that neurotrophic factors play critical roles in mediating the behavioural responses to antidepressants, although the mechanisms are not clear [13]. Brain-derived neurotrophic factor (BDNF) is believed to be involved in the pathogenesis of depression [14]. Animal and clinical studies, including our previous findings, show that BDNF levels are decreased in individuals with untreated depression [15], and that antidepressant management could restore the decreased BDNF levels [16]. Studies also show that levels of BDNF are positively correlated with serotonin. Moreover, central administration of BDNF produces antidepressant-like activity in a rat model of depression, which directly supports the protective role of BDNF in depression [17]. Evidence also shows that inflammatory cytokines could decrease systemic BDNF levels in humans, which integrates the roles of inflammation and BDNF in depression [18, 19].

Traditional Chinese medicine (TCM) has long been used in the treatment of depression [20]. In recent decades, many clinical and preclinical studies have focused on the antidepressant features and underlying mechanisms of TCM and extracts on depression [21]. Ginkgo extract EGb761 has recently shown anti-inflammatory, anti-oxidative, anti-arteriosclerosis, and neuroprotective activities in clinical and animal studies [22, 23]. There is also evidence that EGb761 enhances the expression of BDNF in the brains of ischaemic stroke rats [24]. What is more, Rojas *et al*.'s study found that pretreatment with EGb761 (10 mg/kg, i.p.) daily for 17 days significantly decreased the immobility time in the forced swimming test in BALB/c mice, possibly by inhibiting oxidative stress [25]. However, it is unknown whether EGb761 has antidepressant-like effects in LPS-induced depression, which is a well-established animal model of inflammation-associated depression.

Thus, this study aimed to investigate the antidepressant-like effects of EGb761 in mice after lipopolysaccharide (LPS) administration, and to detect its possible mechanisms.

Material and methods

Animals

C57BL/6J male mice were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China) and acclimatised to the new laboratory for seven days prior to the experiment. Forty mice were randomly divided into: control group, vehicle group, low-dose group, middle-dose group, and high-dose group, with eight mice in each group. The animals were fed under standard laboratory conditions with a 12 : 12 hour light-dark cycle, controlled temperature (20-26°C), and controlled humidity 60 ±5%. Groups of four mice were housed in cages and were provided food and water ad libitum. All the protocols used in the current study complied with the Guide for the Care and Use of Laboratory Animals of National Institutes of Health, and was approved by the Committee on the Ethics of Animal Experiments of Shandong University and Weifang People's Hospital. Efforts were made to minimise the animals' suffering.

Drug administration

Mice in the low-dose group, middle-dose group, and high-dose group were orally treated with different doses of EGb761 (respectively: 50, 100, or 150 mg/kg/day, dissolved in saline) (Dr. Willmar Schwabe Gmbh & Co., KG) from day 1 to day 12; mice in the control group and vehicle group were treated with the same volume of vehicle saline instead. After 10 days of pretreatment, the mice in the vehicle group, low dose group, middle-dose group, and high-dose group were intraperitoneally administrated LPS (0.83 mg/kg, the dose can induce acute sickness response and subsequent depressive-like behaviours, according to literature).

Locomotor activity test

Twenty-four hours after the LPS administration, the spontaneous locomotor activity of the mice was assessed by open field test. Briefly, the mouse was put into the centre of an open field apparatus consisting of a square wooden arena (100 × 100 × 50 cm) which was divided into 25 equal squares, and explored freely for 5 minutes. This apparatus was cleaned with 5% ethyl alcohol between investigations. The exploration was recorded by a camera and numbers of rearings and crossings were counted by two investigators who were blind to the groups.

Forced swim test

We carried out forced swim test 24 hours after the LPS administration. A cylinder (20 cm in diameter × 254 cm tall) containing 10 cm of water at 24 ±1°C was used in the test. The mouse was placed into the cylinder and video-recorded for five minutes. Videos were analysed by two investigators who were blind to the groups to calculate the immobility time (the time that the mouse floated while making only necessary movements to keep its head above water) during the last four minutes.

Tail suspension test

During the tail suspension test, the mouse was suspended 30 cm above the floor by hanging on a fixed hook using adhesive tape (positioned about 3 cm from the tip of the tail) for 6 minutes, and the duration of immobility during the final 5 minutes was determined.

Sucrose preference test

The reduction of sucrose intake can evaluate the anhedonia of the animals. Prior to the test, the mice were trained to consume sucrose. When testing, one bottle of

water and one bottle of 1% sucrose solution were given to each mouse for 24 hours (24 to 48 hours following LPS treatment). The bottles were weighted before and after the test to calculate the consumption of water and sucrose solution. Sucrose consumption percentage was calculated as sucrose intake/total fluid intake × 100.

Cytokine assay

Forty-eight hours after the LPS administration, the mice were sacrificed under deep anaesthesia. The hippocampus was immediately removed from the brain and was used to made tissue homogenate using a homogeniser. After centrifugation (4°C, 3000 rpm, 15 minutes), the supernatant was sub-packaged and stored at –20°C for further measurements. Levels of TNF-α, IL-1β, IL-6, IL-17A, and IL-10 in the supernatant of the hippocampus homogenate were measure using commercially available specific ELISA kits according to the instructions provided by the manufacturers (Cusabio Company, Wuhan, China).

Brain-derived neurotrophic factor assay

Levels of BDNF in hippocampus tissue were also investigated by ELISA using the sub-packaged supernatant of the hippocampus homogenate strictly according to the instructions (Boster Company, Wuhan, China).

Statistical analysis

Results of behavioural parameters, cytokines, and BDNF are presented as mean ±SEM. SPSS 15.0 was used to perform the statistical analysis. The intergroup differences were compared by one-way analysis of variance (ANOVA) followed by Students-Newman-Keuls (SNK) test. Differences were considered significant if the p value was less than 0.05.

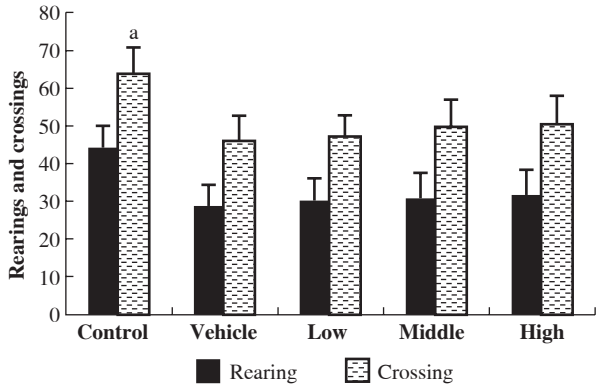
Results

Locomotor activity in the open field test

In order to observe the effects of EGb761 on spontaneous locomotion of the mice, the numbers of rearings and crossings of the mice in the open field test were counted. There were less rearings and crossings in the vehicle group than in the control group (both $p < 0.05$), which indicated that LPS injection reduced the locomotor activity of the mice. There were no significant differences in the numbers rearings and crossings among the vehicle group, low-dose group, middle-dose group, and high-dose group (all $p > 0.05$), suggesting that EGb761 pretreatment had no marked effects on the locomotor activity of the mice (Fig. 1).

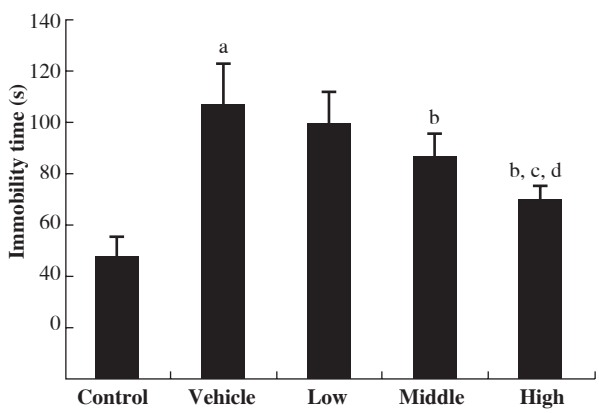
Forced swim test

Immobility time is extensively used to assess the depressive state of rodents. Figure 2 shows the results of the



Locomotor activity of the mice was assessed by open field test. Each bar is the mean ± SD of the numbers of rearings or crossings in each group. Differences between groups were determined by ANOVA procedure and S-N-K test.
[a]$p < 0.05$, vs. control group
[b]$p < 0.05$, vs. vehicle group
[c]$p < 0.05$, vs. low dose group
[d]$p < 0.05$, vs. middle dose group

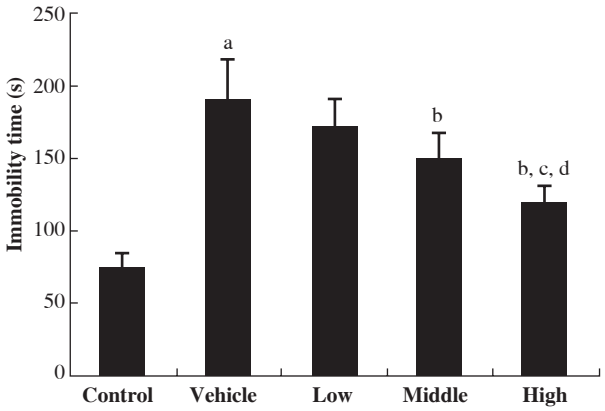
Fig. 1. Effects of EGb761 on locomotor activity of the mice



Each bar is the mean ± SD of the immobility time of the mice in each group in forced swim test. Differences between groups were determined by ANOVA procedure and S-N-K test.
[a]$p < 0.05$, vs. control group
[b]$p < 0.05$, vs. vehicle group
[c]$p < 0.05$, vs. low dose group
[d]$p < 0.05$, vs. middle dose group

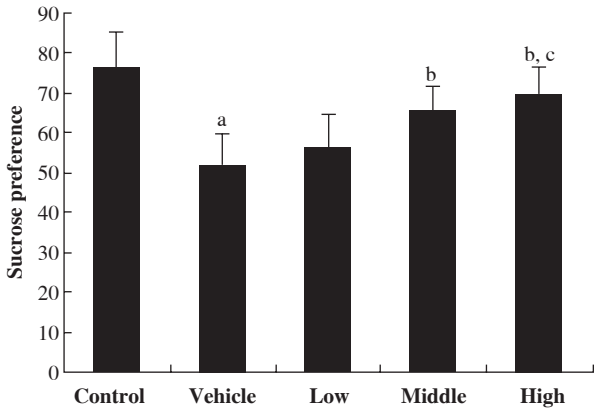
Fig. 2. Effects of EGb761 on immobility time in forced swim test

immobility time in forced swim test (FST). The vehicle group showed markedly longer immobility time than the control group ($p < 0.05$), indicating that LPS injection induced depressive behaviours in mice. However, the middle-dose group and the high-dose group showed shorter immobility time than the vehicle group (both $p < 0.05$). Moreover, the high-dose group's immobility time was much shorter than that of the low-dose group and the middle-dose group (both $p < 0.05$). The results suggest that



Each bar is the mean ± SD of the immobility time of the mice in each group in tail suspension test. Differences between groups were determined by ANOVA procedure and S-N-K test.
[a]$p < 0.05$, vs. control group
[b]$p < 0.05$, vs. vehicle group
[c]$p < 0.05$, vs. low dose group
[d]$p < 0.05$, vs. middle dose group

Fig. 3. Effects of EGb761 on immobility time in tail suspension test (TST)



Each bar is the mean ± SD of the sucrose preference of the mice in each group. Differences between groups were determined by ANOVA procedure and S-N-K test.
[a]$p < 0.05$, vs. control group
[b]$p < 0.05$, vs. vehicle group
[c]$p < 0.05$, vs. low dose group
[d]$p < 0.05$, vs. middle dose group

Fig. 4. Effects of EGb761 on sucrose preference

EGb761 pretreatment had an antidepressant-like effect in mice with LPS injection, and the effect was dose related.

Tail suspension test

Figure 3 shows the results of the immobility time in tail suspension test (TST). The duration of immobility in TST was significantly elevated by LPS injection in the vehicle group, compared to the control animals ($p < 0.05$), and this elevation was significantly reversed by EGb761 pretreatment in the middle-dose group and high-dose group in comparison with the vehicle group ($p < 0.05$). Moreover, the high-dose group's immobility time was much shorter than that of the low-dose group and the middle-dose group (both $p < 0.05$), suggesting a dose-related antidepressant-like effect of EGb761.

Sucrose preference

Anhedonia, which is a common symptom of depression, can be quantified by sucrose preference test. Figure 4 shows the effect of EGb761 pretreatment on the sucrose preference of the mice in the sucrose preference test during 24 to 48 hours following LPS injection. LPS injection significantly decreased the sucrose preference in the vehicle group, compared to the control group ($p < 0.05$). However, sucrose preferences of the middle-dose group and the high-dose group were much higher than the vehicle group (both $p < 0.05$). Moreover, sucrose preference of the high-dose group was higher that of the low-dose group (both $p < 0.05$).

Cytokine assay

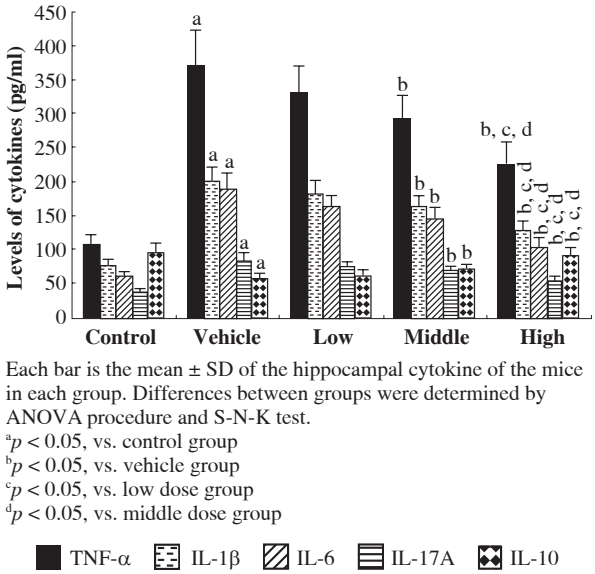
The levels of some inflammatory and anti-inflammatory cytokines involved in depression were measured by ELISA. The vehicle group showed much higher levels of inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-17A, but lower levels of anti-inflammatory cytokine IL-10 in the hippocampus tissue homogenate than in the control group (all $p < 0.05$), indicating that LPS injection induced inflammation in the hippocampus. However, EGb761 pretreatment significantly inhibited the elevation of hippocampal TNF-α, IL-1β, IL-6, and IL-17A and the reduction of IL-10 in the middle-dose group and high-dose group, compared to the vehicle group (all $p < 0.05$). Moreover, the high dose was more potent that the low dose and middle dose (all $p < 0.05$) (Fig. 5).

Brain-derived neurotrophic factor assay

Brain-derived neurotrophic factor level in the hippocampus tissue homogenate was measured by ELISA. Its level declined in the vehicle group significantly more than in the control group ($p < 0.05$). However, EGb761 pretreatment significantly inhibited the reduction of hippocampal BDNF production in the middle-dose group and the high-dose group in comparison with the vehicle group (both $p < 0.05$). Moreover, the high-dose group showed higher levels of BDNF than the low-dose group and the middle-dose group (Fig. 6).

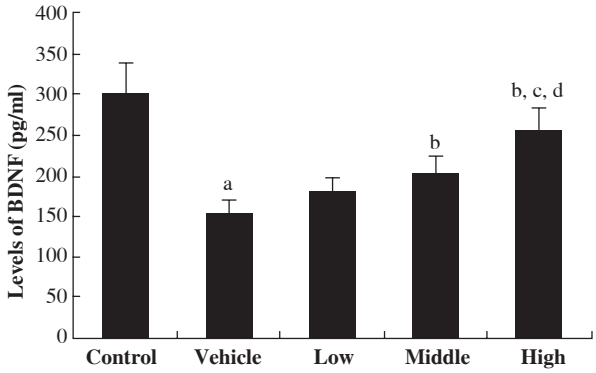
Discussion

The main findings of the current study are that Ginkgo extract EGb761 reversed the depressive-like behaviours and inhibited the changes of hippocampal levels of TNF-α, IL-1β, IL-6, IL-17A, IL-10, and BDNF induced by peripheral LPS injection in mice.



Each bar is the mean ± SD of the hippocampal cytokine of the mice in each group. Differences between groups were determined by ANOVA procedure and S-N-K test.
[a] $p < 0.05$, vs. control group
[b] $p < 0.05$, vs. vehicle group
[c] $p < 0.05$, vs. low dose group
[d] $p < 0.05$, vs. middle dose group

Fig. 5. Effects of EGb761 on levels of hippocampal cytokine



Each bar is the mean ± SD of the hippocampal BDNF of the mice in each group. Differences between groups were determined by ANOVA procedure and S-N-K test.
[a] $p < 0.05$, vs. control group
[b] $p < 0.05$, vs. vehicle group
[c] $p < 0.05$, vs. low dose group
[d] $p < 0.05$, vs. middle dose group

Fig. 6. Effects of EGb761 on levels of hippocampal brain-derived neurotrophic factor (BDNF)

EGb761 has various pharmacological activities [22, 23]. In order to investigate the possible antidepressant-like activities of EGb761, we pretreated the C57BL/6J mice with different doses of EGb761 for 10 days and intraperitoneally injected LPS to the animals in the current study. Peripheral injection LPS can induce some features of inflammation and is extensively used as a model for peripherally-induced systemic inflammation and neuroinflammation. Studies also show that LPS injection successfully induces depressive-like behaviours in animals, and this LPS-induced animal model of depression is extensively used in evaluating antidepressant-like effects of medications [20, 26]. In the current study, we firstly evaluated the depressive-like behaviours of the mice by FST, which is one of the most widely employed behavioural tests for screening depressive-like behaviour and antidepressant efficacy in animals. We found that the vehicle group had significant longer immobility time than the control group, which meant that LPS successfully induced depressive-like behaviours in mice. However, EGb761 pretreatment markedly decreased the immobility time in the middle-dose and the high-dose group compared to the vehicle group, indicating that EGb761 had antidepressant-like activities in the mice. Consistent with the results of the FST, LPS injection also significantly increased the immobility duration in the TST, while middle doses and high doses of EGb761 pretreatment markedly reversed LPS-induced immobility alterations, which again confirmed the antidepressant-like activities of EGb761. Meanwhile, to quantify the anhedonia, which is a common symptom of depression, we subjected mice to the sucrose preference test. We found that LPS injection markedly reduced the sucrose consumption percentage of the mice. However, two doses of EGb761 significantly inhibited the reduction of the sucrose consumption, suggesting that EGb761 could ameliorate the LPS-induced anhedonia in mice. All the behavioural tests demonstrated that EGb761 had antidepressant-like activities in mice after LPS injection, which is partially consistent with Rojas *et al.*'s study, which reported that EGb761 decreased the immobility time of EGb761 in BALB/c mice in the forced swimming test [25].

It is generally accepted that the amelioration of locomotor activity can decrease the immobility time in FST and TST, resulting in false positive results of immobility [27]. In the current study we enrolled the open field test to investigate the possible effects EGb761 on locomotor activity. We found no significant difference in the numbers of rearings and crossings between vehicle groups and the three doses of EGb761 groups, suggesting that EGb761 had no marked effect on the locomotor activity. The results demonstrated that the reduction of immobility time in the in FST and TST was not influenced by locomotor activity, and thus should be specific to the antidepressant effects.

The role of inflammation in the pathogenesis of depression has been well demonstrated in literature [2]. To explore the possible mechanism by which EGb761 exerts its antidepressant activity, we measured hippocampal levels of some inflammatory cytokines. We found that LPS administration induced significant elevation of hippocampal levels of TNF-α, IL-1β, and IL-6 – inflammatory cytokines that have been shown to contribute to the pathogenesis of depression [2-5]. We found that LPS also induced a marked reduction of hippocampal IL-10 in the vehicle group, a prototypical anti-inflammatory cytokine

that is known to inhibit expression of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Our findings were similar to the earlier studies that found hippocampal inflammation in animals after LPS administration. Recently, Th17 cytokines have been shown to play an important role in depression [8-11]. To our knowledge, no study investigated the changes of hippocampal Th17 cytokine after LPS administration. In this study, we found levels of Th17 cytokines IL-17A increased in the vehicle group compared to the control animals. However, pretreatment with middle-dose and high-dose EGb761 markedly inhibited the elevation of hippocampal TNF-α, IL-1β, IL-6, and IL-17A and the reduction of IL-10. These findings indicated that EGb761 simultaneously suppressed the inflammatory response and activated anti-inflammatory response in mice with LPS administration. We supposed that the inhibition of hippocampal inflammation should contribute to the antidepressant-like activities.

There is a great deal of evidence from preclinical and clinical studies, including our previous findings, that BDNF is involved the pathogenesis of depression [14, 15]. Studies have also shown that LPS administration can suppress BDNF production [28], which is negatively correlated with depression [29]. Moreover, antidepressant management could restore the decreased BDNF levels [16]. In this study, we found that LPS markedly inhibited the hippocampal levels of BDNF in the vehicle group, compared to the control animals, similar to the previous studies [28]. Nonetheless, EGb761 pretreatment significantly reduced the reduction of hippocampal BDNF production in comparison with the vehicle group. We supposed that the restoration of BDNF production might contribute to the improvement of depressive-like behaviours induced by LPS in mice.

Taken together, EGb761 could attenuate the LPS induced depressive-like behaviours, suppress the hippocampal production of inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-17A, and restore the hippocampal levels of BDNF and anti-inflammatory cytokine IL-10 in mice with LPS administration.

The authors declare no conflict of interest.

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