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Ferulic acid alleviates lipopolysaccharide-induced depression-like behavior by inhibiting inflammation and apoptosis

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ABSTRACT

Objective: To identify the anti-depressive effect of ferulic acid (FA) in mice exposed to lipopolysaccharide (LPS) and explore its molecular mechanisms.

Methods: The mice were divided into 5 groups as follows: Control, LPS, LPS + SP, LPS + FA, and LPS + FA + anisomycin. The LPS + FA and LPS + FA + anisomycin groups were administered with FA (100 mg/kg, *i.p.*) once daily continuously for 7 days, and the other groups received an equivalent volume of saline. On the 7th day, LPS (0.1 mg/mL, *i.p.*) was injected in all mice except the control group 30 min after FA or saline administration. The LPS + SP and LPS + FA + anisomycin groups were intravenously administered with SP600125 [c-Jun *N*-terminal kinase (JNK) inhibitor] (100 μL/site, *i.v.*) and anisomycin (JNK activator) (100 μL/site, *i.v.*) 15 min before LPS, respectively. The depressive behaviors were assessed by open field test, sucrose preference test, and forced swimming test at 24 h post-LPS administration. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels in plasma were measured by ELISA. The levels of phospho-JNK, TNF-α, IL-1β, Bcl-2, Bax, cytochrome c and caspase-3 were evaluated by Western blotting.

Results: FA alleviated depression symptoms caused by LPS in mice, including increasing sucrose water consumption in sucrose preference test and reducing the immobility time in forced swimming test. FA could inhibit upregulated levels of phospho-JNK, TNF-α, and IL-1β. FA also markedly decreased Bax, caspase-3, and cytochrome c, and increased Bcl-2 levels. Besides, SP600125 showed neuroprotective effect similar to FA which was attenuated by anisomycin.

Conclusions: FA attenuates inflammation and apoptosis by inhibiting LPS-induced activation of JNK to alleviate depression-like behaviors.

KEYWORDS: Ferulic acid; Lipopolysaccharide; Depression; JNK; Inflammation; Apoptosis

1. Introduction

Depression is a neurological disorder characterized by reduced self-activity, low mood, and anhedonia, and it affects nearly 20% of the world's total population[1]. In recent years, researchers find that the standard antidepressants have many side effects such as sexual dysfunction, low blood pressure, and drowsiness[2]. Therefore, it is of great significance to investigate the mechanisms of depression and explore natural antidepressants with low toxicity.

Inflammation plays a crucial role in the pathogenesis of depression. The clinical researches found increased levels of inflammatory cytokines in the peripheral blood of patients who suffer from depression[3]. The elevated expression of inflammation-related genes is also found in the prefrontal cortex (PFC) in major depressed patients[4]. Previous studies have shown that lipopolysaccharide (LPS) injection in mice results in depression-like behaviors, such as increasing immobility time in forced

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swim and tail suspension tests at 24 h post-injection. Hence, the injection of LPS can establish an inflammation-related animal model of depression[5]. LPS plays pivotal roles in the activation of inflammatory response and microglial activation[6]. Specifically, LPS binds to its cognate receptors to regulate the c-Jun *N*-terminal kinase (JNK) signaling pathway. Activated JNK is involved in the release of inflammatory cytokines [*e.g.*, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β)][7,8]. These increased molecules reach the brain and elicit depressive symptoms[9,10]. Therefore, the inhibition of overproduction of proinflammatory cytokines may be a strategy to treat these disorders.

Cell apoptosis is a "programmed" cell death, which includes mitochondrial-dependent and non-mitochondrial-dependent pathways. The mitochondrial apoptosis pathway is directly controlled by pro- and anti-apoptotic molecules[11]. Previous studies show that the mitochondrial apoptosis pathway is associated with the pathological process of depression and serves as a target for the antidepressant action[12].

Ferulic acid (FA) (4-hydroxy-methoxy cinnamic acid, Supplementary Figure 1A), is a hydroxycinnamic acid derivative originally found in grains, vegetables, and fruits. FA is found to relieve depression-like behaviors by promoting energy metabolism and inhibiting inflammation[13,14]. Several studies indicate that supplementation with FA can ameliorate depressive symptoms[15]. However, the effect of FA on LPS-induced depression and its relevant mechanism has not yet been investigated. Hence we explored the neuroprotective effects and mechanisms of FA on LPS-induced depression in mice in this study.

2. Materials and methods

2.1. Animals

Seven-week-old male ICR mice weighing (20 ± 5) g were purchased from the Laboratory Animal Center of Xuzhou Medical University (Xuzhou, China). The mice were allowed to acclimatize to the experimental conditions (humidity of 50%-60%, the ambient temperature of 20-25℃, and 12 h light/dark cycles) for a week and with free access to food and water.

2.2. Materials

FA with 98% purity was purchased from Zhengzhou Lion Biological Technology Co. Ltd (Zhengzhou, China). LPS, from *Salmonella enterica* (serotype typhimurium), was purchased from Sigma (St. Louis, MO, USA). SP600125 (JNK inhibitor) and anisomycin (JNK activator) were purchased from Merck Chemical Technology Co. Ltd (Shanghai, China). The primary antibodies of JNK, p-JNK, TNF-α, IL-1β, B-cell lymphoma-2 (Bcl-2), Bcl-2 associated X protein (Bax), caspase-3, and cytochrome c (Cyt-c), β-actin and all secondary antibodies were purchased from Santa Cruz Biotechnology Co. Ltd (Santa, USA).

2.3. Experimental procedures

The powder of LPS and FA was dissolved in normal saline to final concentrations of 0.06 mg/mL (LPS), 0.1 mg/mL (LPS), and 10 mg/mL (FA), and administered intraperitoneally. The SP600125 and anisomycin were dissolved in normal saline to final concentrations of 6 and 5 mg/mL, respectively, and given by intravenous injection (100 μL/site, *i.v.*)[16]. All the drugs were freshly prepared before use. Initially, two different concentrations (0.6 and 1.0 mg/kg) of LPS were tested to determine the better one for injection in all assays and LPS at 1.0 mg/kg was selected.

The mice were randomly divided into five groups (control, LPS, LPS + SP, LPS + FA, LPS + FA + anisomycin) consisting of 12 mice in each group. Mice in the LPS + FA group were intraperitoneally administered with FA (100 mg/kg, *i.p.*) once a day continuously for 7 d, whereas the other four groups were administered with an equal volume of saline. At day 7, 15 min after the last injection of FA or saline, the LPS + SP group was administered with SP600125 by intravenous injection (100 μL/site, *i.v.*), and the LPS + FA + anisomycin group was administered with anisomycin (100 μL/site, *i.v.*). Thirty minutes after the last injection of FA or saline, the LPS, LPS + SP, LPS + FA and LPS + FA + anisomycin groups were administered with LPS (1.0 mg/kg, *i.p.*). The control group only received an equal volume of saline.

Depression behaviors were assessed 24 h after LPS injection. After behavioral tests, the mice were anesthetized and sacrificed using 1% pentobarbital sodium, and blood or tissue samples were immediately collected for subsequent experiments. The experimental procedure was depicted in Supplementary Figure 1B.

2.4. Sucrose preference test (SPT) and determination of body weight

Sucrose preference is an effective indicator to assess the depression-like behavior in mice. We carried out the SPT according to the method described in previous studies[17]. Briefly, each mouse was placed in one cage with a clean water bottle (water) and a sucrose water bottle (sucrose solution, 1% w/v) after injection of LPS, and the original weight of each bottle was recorded. The two bottles were respectively weighed again 24 h later. The consumption of water and sucrose solution was measured. The sucrose preference (%)=sucrose water consumption/(sucrose water consumption + clean water consumption). Moreover, body weight is also an effective indicator to evaluate the depressive behavior in mice, and it was measured

before and 24 h after LPS treatment.

2.5. Open field test (OFT)

The OFT was performed according to the methods in previous studies[18]. Briefly, a black plastic open box (50 cm × 50 cm × 40 cm) was used and its bottom was divided into 9 equal squares, of which the center square was considered as the center area. The mouse was placed in the center square and adapted for 3 min. The locomotive activity was recorded for 5 min with the ZH-ZFT video analysis system (Huaibei Zhenghua Biological Instrument and Equipment Co. Ltd., Huaibei, China). The distance traveled and crossing numbers (crossing the central square) were analyzed by the Any-maze system software[19].

2.6. Forced swimming test (FST)

Each mouse was randomly placed in a round transparent swimming bucket filled with 15 cm of water [temperature (25 ± 1)℃; 30 cm in height; 11 cm in radius] for 2 min to adapt. When a mouse passively floated in the water, keeping its head or nose above the water, it was judged to be immobile. The process of the FST was recorded with the ZH-QPT video analysis system (Huaibei Zhenghua Biological Instrument and Equipment Co. Ltd., Huaibei, China) for 5 min. The Any-maze system software was used to analyze the accumulated immobility time[19].

2.7. Sample preparation for ELISA and Western blotting

Six mice from each group were sacrificed under anesthesia, using 1% pentobarbital sodium. The peripheral blood was collected and centrifuged at 4℃, 2000 ×*g* for 15 min. The supernatant was collected and plasma TNF-α and IL-1β levels were measured using an ELISA kit (Beyotime, Shanghai, China).

The whole brain was dissected to obtain the PFC as described by Kikusui *et al*[20]. Briefly, the PFC was isolated from the frontal part of the superior frontal gyrus immediately after the decapitation according to the Mouse Brain Anatomy Atlas, immediately frozen in liquid nitrogen and saved at −80℃ until use.

The proteins except Cyt-c were extracted from the PFC of a mouse as the following methods: the tissues were homogenized with ice-cold protein lysis buffer and centrifuged at 12000 ×*g* for 15 min at 4℃ to collect the supernatant for Western blotting. The expression of Cyt-c was assessed according to the method reported by Li *et al*[21]. Briefly, the tissues were centrifuged (2000 ×*g*, 15 min, 4℃) to collect the supernatant which was centrifuged again (14000 ×*g*, 15 min, 4℃), then the cytosolic fractions (the supernatant) and mitochondrial fractions (the pellet) were obtained. The protein concentration was determined with a BCA protein assay kit (Beyotime, Shanghai, China).

2.8. Western blotting analysis

Samples containing 50 μg total proteins were separated by 10%-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 100 V) and transferred onto polyvinylidene fluoride membranes (40 mA per membrane). The membranes were blocked with 3% bovine serum albumin for 2 h and incubated with the primary antibodies (β-actin, JNK, p-JNK, TNF-α, IL-1β, Bax, Bcl-2, caspase-3, and Cyt-c) for one night at 4℃. After washed three times with TBST (Tris-buffer with 0.1% Tween-20), the membranes were incubated with fluorescently labeled anti-mouse/rabbit secondary antibodies (1:1000) in a dark room for 2 h. The membranes were washed three times again with TBST and scanned. Densitometry analysis of the bands was performed with ImageJ 1.47v (National Institute Health, USA). Taking the control group as base 1.00, the relative ratio between the control group and other groups was calculated.

2.9. Statistical analysis

GraphPad Prism 7 software was used for statistical analysis of all data which were presented as mean ± SD. Multiple comparisons were conducted with one-way ANOVA analysis followed by Sidak's multiple comparisons test. P-value < 0.05 was considered statistically significant.

2.10. Ethical statement

All experimental procedures in this study complied with the rules of the NIH for the animal ethics of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Xuzhou Medical University [SYXK (SU) 2015-0030].

3. Results

3.1. Effects of LPS on depression behaviors in mice

To choose the optimal concentration of LPS that could induce depression in mice, two doses (0.6 and 1.0 mg/kg) were examined. The behavior tests were performed at 6 or 24 h after LPS injection. As shown in Figure 1, treatment with LPS in mice showed a significant increase in the immobility time at the dosage of 1.0 mg/kg compared with the control (0 mg/kg, $P < 0.05$, Figure 1A). Therefore, we chose 1.0 mg/kg LPS as the optimal concentration for the subsequent experiments. Moreover, both 0.6 and 1.0 mg/kg LPS injection could cause a body weight loss in mice ($P < 0.05$) (Figure 1B). It was also observed that the locomotor activity measured by

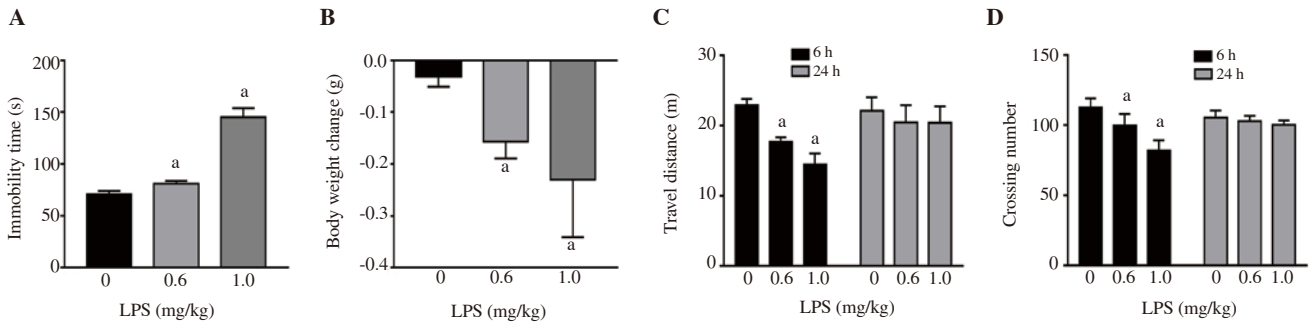

Figure 1. Depression-like behaviors induced by LPS in mice. Mice were injected with 0.6 or 1.0 mg/kg (*i.p.*) LPS. Immobility time for 5 min in forced swimming test (A), body weight (B), travel distance (C), and the crossing number for 5 min in open field test (D) were measured (mean ± SD, n =12). [a]$P < 0.05$ *vs.* the control group. LPS: lipopolysaccharide.

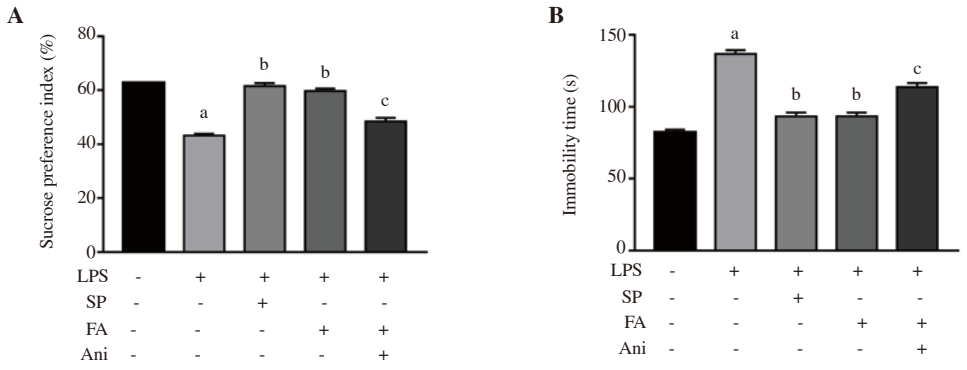

Figure 2. Effects of FA on LPS-induced depression-like behaviors in mice. Twenty-four hours after LPS injection, the sucrose preference was measured by sucrose preference test (A). The immobility time was measured for 5 min by forced swimming test (B) (mean ± SD, n = 12). [a]$P < 0.05$ *vs.* the control group; [b]$P < 0.05$ *vs*. the LPS group; [c]$P < 0.05$ *vs.* the LPS + FA group. LPS: lipopolysaccharide; SP: SP600125; FA: ferulic acid; Ani: anisomycin.

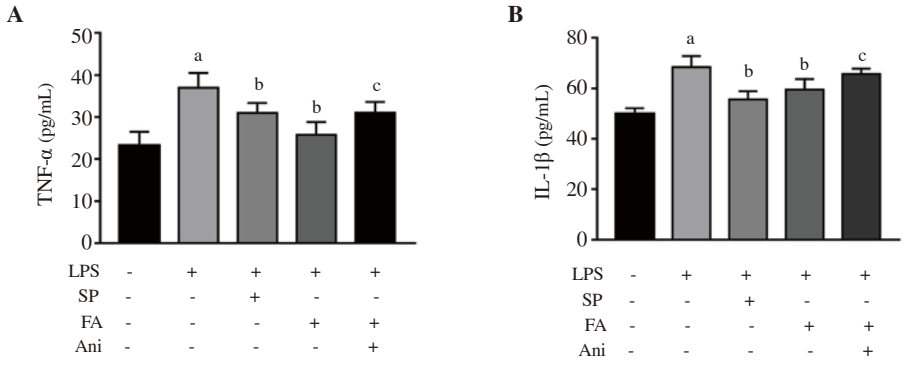

Figure 3. Effects of FA on plasma TNF-α and IL-1β levels induced by LPS. (A) Plasma TNF-α and (B) IL-1β levels were measured by ELISA (mean ± SD, n = 6). [a]$P < 0.05$ *vs.* the control group; [b]$P < 0.05$ *vs.* the LPS group; [c]$P < 0.05$ *vs.* the LPS + FA group. LPS: lipopolysaccharide; SP: SP600125; FA: ferulic acid; Ani: anisomycin.

the travel distance and crossing numbers were reduced in mice 6 h post-LPS administration ($P < 0.05$, Figure 1C and 1D). However, there was no significant difference between different concentrations 24 h after LPS injection.

3.2. Effects of FA on depression-like behaviors in mice

Effects of FA pretreatment on LPS-induced depression behaviors in mice were assessed by the SPT and FST. Results showed that sucrose preference in the LPS group was significantly reduced compared with the control group [(43.22 ± 1.70)% *vs.* (62.68 ± 2.07)%] ($P < 0.05$), while pretreatment with FA [(59.70 ± 2.06)%] reversed the decrease as in the LPS + SP group [(61.50 ± 2.74)%] ($P < 0.05$). The sucrose preference in the LPS + FA + anisomycin group [(48.52 ± 3.25)%] was lower than that in the LPS + FA group ($P < 0.05$) (Figure 2A).

We also examined the effect of FA on the immobility time in the FST. Compared with the control group [(82.17 ± 4.93) s], the immobility time was increased significantly in the LPS group [(136.70 ± 6.59) s]. FA and SP600125 [(93.48 ± 6.32) s and (93.30 ± 6.89) s] reversed LPS-induced change ($P < 0.05$). However, anisomycin [(113.60 ± 7.00) s] treatment abolished the ameliorative effect of FA ($P < 0.05$) (Figure 2B). Thus, behavior tests presented the antidepressant effect of FA on inflammation-induced depression.

3.3. Effects of FA on TNF-α and IL-1β levels in plasma

We used ELISA to measure two representative inflammatory cytokines (TNF-α and IL-1β) levels in plasma. LPS elevated plasma TNF-α and IL-1β levels, while FA and SP600125 decreased the levels of these two inflammatory factors ($P < 0.05$). Anisomycin abolished the inhibitory effect of FA on inflammatory factors ($P < 0.05$) (Figure 3).

3.4. Effects of FA on the expression of p-JNK and inflammatory cytokines in the PFC of mice

The expression levels of JNK and p-JNK were detected by Western blotting. As shown in Figure 4, the p-JNK/JNK ratio was significantly increased in the LPS group compared with the control group. In contrast, the p-JNK/JNK ratio induced by LPS was downregulated by SP600125 and FA ($P < 0.05$). However, an increased ratio of p-JNK/JNK in the PFC of depressive mice was detected in the LPS + FA + anisomycin group ($P < 0.05$, Figure 4A and B).

In addition, LPS markedly increased TNF-α and IL-1β levels ($P < 0.05$). FA and SP600125 administration suppressed the increase. The suppressive effect of FA on the TNF-α and IL-1β levels was diminished by anisomycin ($P < 0.05$) (Figure 4C-E). The results suggest that FA significantly inhibited inflammation through

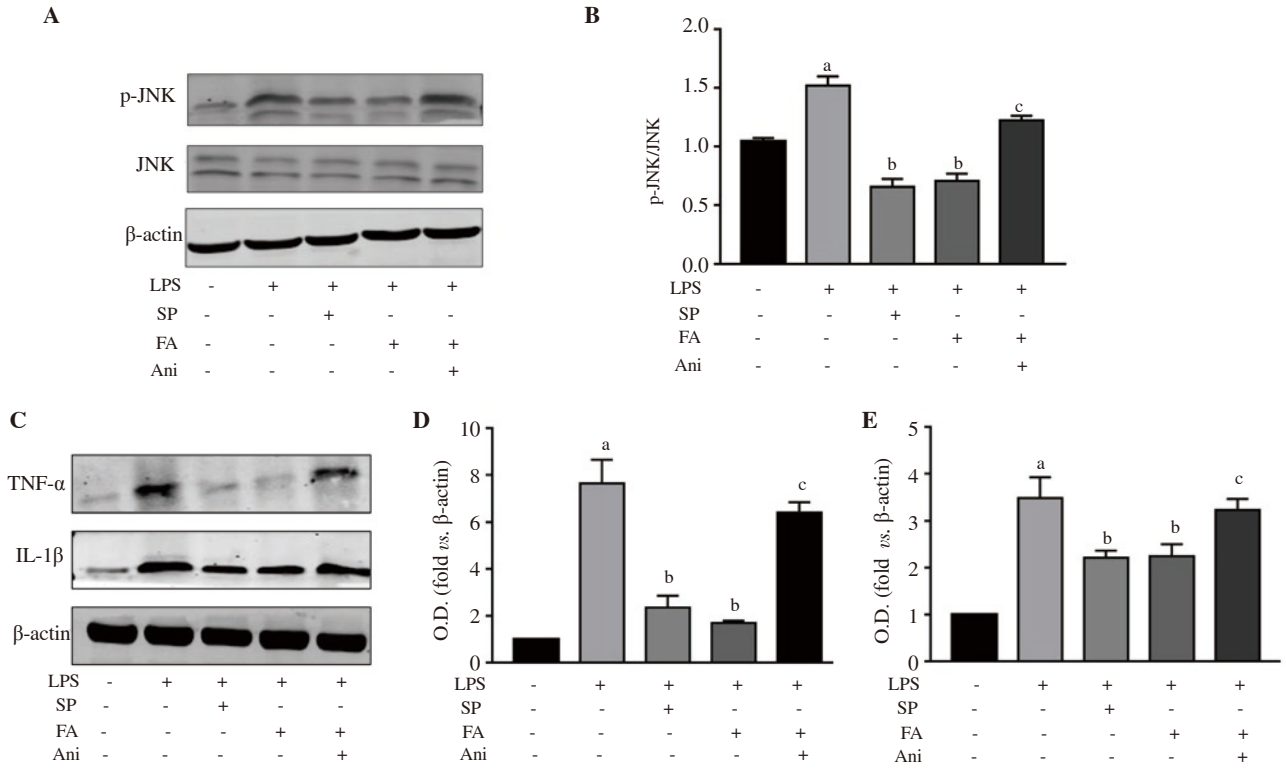

Figure 4. Effects of FA on the expression of p-JNK and inflammatory cytokines in the mouse PFC. (A) Representative bands of p-JNK and JNK are shown. (B) Relative ratio of p-JNK/JNK. (C) Representative bands of TNF-α and IL-1β are shown. (D) Optical density (O.D.) of TNF-α. (E) O.D. of IL-1β (mean ± SD, $n = 6$). [a]$P < 0.05$ *vs.* the control group; [b]$P < 0.05$ *vs.* the LPS group; [c]$P < 0.05$ *vs.* the LPS + FA group. LPS: lipopolysaccharide; SP: SP600125; FA: ferulic acid; Ani: anisomycin.

inhibition of JNK activation.

3.5. Effects of FA on the expression of apoptotic factors in mice

The increased levels of Bax, caspase-3, and Cyt-c were observed in the LPS group, along with decreased Bcl-2 level. These changes were significantly reversed in the LPS + SP and LPS + FA groups. The decreased levels of Bax, caspase-3, Cyt-c and increased level of Bcl-2 were suppressed in the LPS + FA + anisomycin group ($P < 0.05$, Figure 5).

4. Discussion

Peripheral infection, such as intraperitoneal injection of LPS, can induce the release of peripheral inflammatory factors that act on the brain and cause sickness behavior[22]. Hence, intraperitoneal administration of LPS can be used for preparing the animal model of depression, which is an acute model simulating depression in infectious diseases, and it could well mimic the clinical symptoms of depression[23,24]. In this study, the LPS-treated mice exhibited significant depression-like behaviors, which were independent of its motor-depressing effects[25]. Our results showed that locomotor activity (travel distance and crossing numbers) was impaired in both doses (0.6 and 1.0 mg/kg) at 6 h but not 24 h post-LPS administration. Depressive behaviors were assessed in the SPT and FST at 24 h post-treatment with LPS, a time-point when locomotor activity returns to normal and depressive behaviors emerge. Therefore, we identified that the enhancement of immobility in the FST was a result of a depressive phenotype and not an impairment of locomotor activity[22,26]. Pretreatment with FA could relieve these depressive behaviors. Meanwhile, we used SP600125 and anisomycin to confirm the role of p-JNK in the antidepressant effect of FA. SP600125 could simulate the neuroprotective effect of FA, while anisomycin exerted an opposing effect. The results indicated that the JNK signaling pathway may be involved in the antidepressant effect of FA.

LPS-induced TNF-α and IL-1β inflammatory factors could reach the brain and lead to depression[22]. The role of these cytokines in depression was first proposed by Smith and further confirmed by other researchers[27,28]. In this study, we measured the changes in TNF-α and IL-1β in depression-like behaviors in plasma. The results showed that TNF-α and IL-1β levels were upregulated in mice challenged by LPS, and these cytokines were decreased in FA

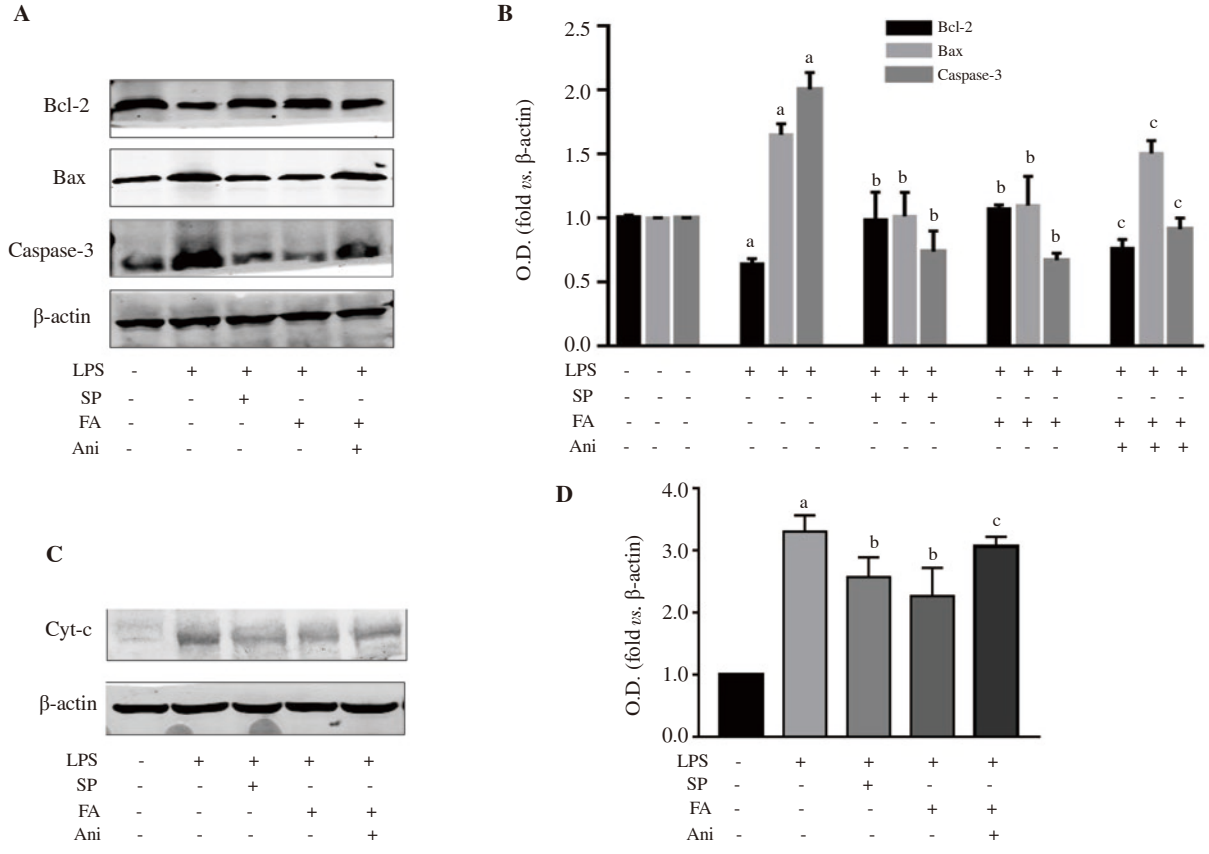

Figure 5. Effects of FA on the expression of Bcl-2, Bax, caspase-3, and Cyt-c. (A) Representative bands of Bcl-2, Bax, and caspase-3 are shown. (B) Optical density (O.D.) of Bcl-2, Bax, and caspase-3. (C) Representative bands of Cyt-c are shown. (D) O.D. of Cyt-c (mean ± SD, $n = 6$). [a]$P < 0.05$ *vs.* the control group; [b]$P < 0.05$ *vs.* the LPS group; [c]$P < 0.05$ *vs.* the LPS + FA group. LPS: lipopolysaccharide; SP: SP600125; FA: ferulic acid; Ani: anisomycin.

treated mice. However, anisomycin, a JNK activator, counteracted the effect of FA. These findings suggest FA could reduce the inflammation induced by LPS *via* inhibition of JNK activation. Previous research has shown that overexpressed immune cytokines could activate microglia, which leads to pro-inflammatory mediator release through JNK phosphorylation to mediate an inflammatory response[29,30]. Activated JNK plays a pivotal role in the pathological processes of depression, including inducing the release of inflammatory cytokines and promoting neuronal cell apoptosis[7-9,31]. Many previous studies have reported that abnormal p-JNK levels exist in the depressive animal PFC and it can be improved by antidepressant treatment[32,33]. Hence, inhibition of JNK phosphorylation can relieve LPS-induced depression-like behaviors. The rodent PFC, one part of the cerebral cortex, includes rostral (pregenual), dorsal, and ventral (subgenual) regions[34]. The PFC is a region in charge of emotions in the brain, and its dysfunction is associated with depression. Therefore, we studied whether LPS-induced depression-like behaviors were dependent on the PFC. Our data showed the expression levels of p-JNK, TNF-α, and IL-1β were increased in the PFC of mice challenged by LPS while the levels of these factors were downregulated by FA. Moreover, it is interesting that SP600125 administration showed the similar anti-inflammatory effects to FA, and anisomycin administration after FA treatment could partially block the anti-inflammatory effects of FA. These data indicated that the suppression of increased inflammatory factors by FA was mediated by inhibition of JNK phosphorylation, eventually attenuating depression-like behaviors. Activation of microglia by LPS is crucial for inducing inflammatory response and results in depression in mice[35,36]. Recent research shows that FA plays an anti-inflammatory role by targeting microglia[37]. In this process, the microglia-mediated neuronal inflammatory response requires the toll-like receptor 4/myeloid differentiation protein-2 complex formation, whereas FA interferes with the complex binding site, resulting in the inhibition of JNK phosphorylation and decreasing levels of inflammatory factors[37,38]. Our findings were consistent with these results. In future studies, we will evaluate whether microglia are the target cells of FA in the LPS-induced depression-like behaviors to gain more insight into the antidepressant effect of FA.

Some studies indicate that dysfunction of mitochondria is crucial in the development of depression, and neuronal apoptosis occurs along with this illness[39-41]. Bcl-2 inhibits the mitochondrial membrane permeability transition pore (mPTP, a group of a protein complex found in membranes of the mitochondria) opening, then prevents the pro-apoptotic factors in the mitochondrial membrane space to enter into the cytoplasm, while Bax has reverse effects[42], and an elevated ratio of Bax/Bcl-2 occurs during apoptosis cell death. When the Bax/Bcl-2 ratio is elevated, mitochondrial mPTP opens, Cyt-c is transferred from the mitochondrial membrane space to the cytoplasm, followed by activation of caspase-3 and ultimately leads to cell apoptosis[43]. Consistent with this, our studies showed that Bcl-2 expression was upregulated, while Bax, Cyt-c, and caspase-3 expression levels were decreased in FA pretreated mice. Hence, the ratio of Bax/Bcl-2 was decreased, leading to the inhibition of caspase-3 activity. Cyt-c is a protein that is specifically expressed by mitochondria and is rarely detected in normal cytoplasm. The release of Cyt-c from mitochondria into the cytoplasm induced by LPS can further support the activation of the apoptosis pathway. Moreover, previous studies suggest that the expression of apoptosis-associated proteins is regulated by the JNK signaling pathway[44]. The results of this present study showed that SP600125 administration possessed the anti-apoptotic effects similar to FA, while anisomycin administration after FA treatment could partially abolish these effects of FA. Our study demonstrates that FA could inhibit mitochondrial apoptosis *via* inhibition of the JNK pathway. The role of mitochondria in the development of new antidepressants has attracted intensive intention. Mitochondrial transplantation may be a new therapeutic strategy for depression treatment[45].

In conclusion, FA suppressed inflammatory response and apoptosis by inhibiting JNK activation, eventually attenuating depression-like behaviors. Moreover, anti-inflammatory/apoptotic effects of FA could be partially counteracted by anisomycin (JNK activator). However, the antidepressant mechanism of FA requires more in-depth molecular studies, and LPS-induced depression is only an acute inflammatory response, which cannot summarize all the processes of depression. Despite all this, FA can be developed as a potential antidepressant with low toxicity to manage inflammation-related depression.

Conflict of interest statement

All authors declare no conflicts of interest.

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Authors' contributions

Both BXM and YRB conceptualized this study and designed the experiments. BXM, YRB and DSJ performed the experiments. BXM and YRB analyzed the data and wrote the manuscript which was further reviewed by HL. HL and ZRL supervised the project.

References

[1] Nabavi SM, Daglia M, Braidy N, Nabavi SF. Natural products, micronutrients, and nutraceuticals for the treatment of depression: A short review. *Nutr Neurosci* 2017; **20**(3): 180-194.

[2] Chung C. New perspectives on glutamate receptor antagonists as antidepressants. *Arch Pharm Res* 2012; **35**(4): 573-577.

[3] Motivala SJ, Sarfatti A, Olmos L, Irwin MR. Inflammatory markers and sleep disturbance in major depression. *Psychosom Med* 2005; **67**(2): 187-194.

[4] Shelton RC, Claiborne J, Sidoryk-wegrzynowicz M, Reddy R, Aschner M, Lewis DA, et al. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry* 2011; **16**(7): 751-762.

[5] O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry* 2009; **14**(5): 511-522.

[6] Wilms H, Sievers J, Rickert U, Rostami-Yazdi M, Mrowietz U, Lucius R. Dimethylfumarate inhibits microglial and astrocytic inflammation by suppressing the synthesis of nitric oxide, IL-1beta, TNF-alpha and IL-6 in an *in-vitro* model of brain inflammation. *J Neuroinflammation* 2010; **7**: 30. Doi: 10.1186/1742-2094-7-30.

[7] Choi Y, Lee MK, Lim SY, Sung SH, Kim YC. Inhibition of inducible NO synthase, cyclooxygenase-2 and interleukin-1beta by torilin is mediated by mitogen-activated protein kinases in microglial BV2 cells. *Br J Pharmacol* 2009; **156**(6): 933-940.

[8] Wang MJ, Huang HY, Chen WF, Chang HF, Kuo JS. Glycogen synthase kinase-3beta inactivation inhibits tumor necrosis factor-alpha production in microglia by modulating nuclear factor kappaB and MLK3/JNK signaling cascades. *J Neuroinflammation* 2010; **7**: 99. Doi: 10.1186/1742-2094-7-99.

[9] Zeng KW, Fu H, Liu GX, Wang XM. Icariin attenuates lipopolysaccharide-induced microglial activation and resultant death of neurons by inhibiting TAK1/IKK/NF-kappaB and JNK/p38 MAPK pathways. *Int Immunopharmacol* 2010; **10**(6): 668-678.

[10]Miller AH, Maletic V, Raison CL. Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009; **65**(9): 732-741

[11]Tricarico PM, Crovella S, Celsi F. Mevalonate pathway blockade, mitochondrial dysfunction and autophagy: A possible link. *Int J Mol Sci* 2015; **16**(7): 16067-16084.

[12]Gong W, Zhou Y, Gong W, Qin X. Coniferyl ferulate exerts antidepressant effect *via* inhibiting the activation of NMDAR-CaMKII-MAPKs and mitochondrial apoptotic pathways. *J Ethnopharmacol* 2020; **251**: 112533. Doi: 10.1016/j.jep.2019.112533.

[13]Sasaki K, Iwata N, Ferdousi F, Isoda H. Antidepressant-like effect of ferulic acid *via* promotion of energy metabolism activity. *Mol Nutr Food Res* 2019; **63**: e1900327. Doi: 10.1002/mnfr. 201900327.

[14]Yabe T, Hirahara H, Harada N, Ito N, Nagai T, Sanagi T, et al. Ferulic acid induces neural progenitor cell proliferation *in vitro* and *in vivo*. *Neuroscience* 2010; **165**(2): 515-524.

[15]Singh T, Kaur T, Goel RK. Ferulic acid supplementation for management of depression in epilepsy. *Neurochem Res* 2017; **42**(10): 2940-2948.

[16]Li J, Xu B, Chen Z, Zhou C, Liao L, Qin Y, et al. PI3K/AKT/JNK/p38 signalling pathway-mediated neural apoptosis in the prefrontal cortex of mice is involved in the antidepressant-like effect of pioglitazone. *Clin Exp Pharmacol Physiol* 2018; **45**(6): 525-535.

[17]Zhou D, Zhang Z, Liu L, Li C, Li M, Yu H, et al. The antidepressant-like effects of biperiden may involve BDNF/TrkB signaling mediated BICC1 expression in the hippocampus and prefrontal cortex of mice. *Pharmacol Biochem Behav* 2017; **157**(6): 47-57.

[18]Fu YY, Zhang F, Zhang L, Liu HZ, Zhao ZM, Wen XR, et al. Mangiferin regulates interleukin-6 and cystathionine-b-synthase in lipopolysaccharide-induced brain injury. *Cell Mol Neurobiol* 2014; **34**(5): 651-657.

[19]Yang N, Ren Z, Zheng J, Feng L, Li D, Gao K, et al. 5-(4-hydroxy-3-dimethoxybenzylidene)-rhodanine (RD-1)-improved mitochondrial function prevents anxiety- and depressive-like states induced by chronic corticosterone injections in mice. *Neuropharmacology* 2016; **105**: 587-593. Doi: 10.1016/j.neuropharm.2016.02.031.

[20]Kikusui T, Kanbara N, Ozaki M, Hirayama N, Ida K, Tokita M, et al. Early weaning increases anxiety *via* brain-derived neurotrophic factor signaling in the mouse prefrontal cortex. *Sci Rep* 2019; **9**(1): 3991.

[21]Li Y, Ge X, Liu X. The cardioprotective effect of postconditioning is mediated by ARC through inhibiting mitochondrial apoptotic pathway. *Apoptosis* 2009; **14**(2): 164-172.

[22]Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat Rev Neurosci* 2008; **9**(1): 46-56.

[23]Yang S, Chen X, Xu Y, Hao Y, Meng XF. Effects of metformin on lipopolysaccharide-induced depressive-like behavior in mice and its mechanisms. *Neuroreport* 2020; **31**(4): 305-310.

[24]Kubera M, Curzytek K, Duda W, Leskiewicz M, Basta-Kaim A, Budziszewska B, et al. A new animal model of (chronic) depression induced by repeated and intermittent lipopolysaccharide administration for 4 months. *Brain Behav Immun* 2013; **31**: 96-104. Doi: 10.1016/j.bbi.2013.01.001.

[25]Frenois F, Moreau M, O'Connor J, Lawson M, Micon C, Lestage J, et al. Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology* 2007; **32**(5): 516-531.

[26]Custódio CS, Mello BSF, Cordeiro RC, de Araújo FYR, Chaves JH, Vasconcelos SMM, et al. Time course of the effects of lipopolysaccharide on prepulse inhibition and brain nitrite content in mice. *Eur J Pharmacol* 2013; **713**(1-3): 31-38.

[27]Smith RS. The macrophage theory of depression. *Med Hypotheses* 1991;

35(4): 298-306.

[28]Barua CC, Haloi P, Saikia B, Sulakhiya K, Pathak DC, Tamuli S, et al. *Zanthoxylum alatum* abrogates lipopolysaccharide-induced depression-like behaviours in mice by modulating neuroinflammation and monoamine neurotransmitters in the hippocampus. *Pharm Biol* 2018; **56**(1): 245-252.

[29]Santa-Cecília FV, Socias B, Ouidja MO, Sepulveda-Diaz JE, Acuña L, Silva RL, et al. Doxycycline suppresses microglial activation by inhibiting the p38 MAPK and NF-κB signaling pathways. *Neurotox Res* 2016; **29**(4): 447-459.

[30]Kang SM, More SV, Park JY, Kim BW, In PJ, Yoon SH, et al. A novel synthetic HTB derivative, BECT inhibits lipopolysaccharide-mediated inflammatory response by suppressing the p38 MAPK/JNK and NF-κB activation pathways. *Pharmacol Rep* 2014; **66**(3): 471-479.

[31]Li D, Li X, Wu J, Li J, Zhang L, Xiong T, et al. Involvement of the JNK/FOXO3a/Bim pathway in neuronal apoptosis after hypoxic-ischemic brain damage in neonatal rats. *PLoS One* 2015; **10**(7): e0132998.

[32]Wu Y, Fu Y, Rao C, Li W, Liang Z, Zhou C, et al. Metabolomic analysis reveals metabolic disturbances in the prefrontal cortex of the lipopolysaccharide-induced mouse model of depression. *Behav Brain Res* 2016; **308**: 115-127. Doi: 10.1016/j.bbr.2016.04.032.

[33] Yu H, Zou Z, Zhang X, Peng W, Chen C, Ye Y, et al. Inhibition of phosphodiesterase 4 by FCPR03 alleviates lipopolysaccharide-induced depressive-like behaviors in mice: Involvement of p38 and JNK signaling pathways. *Int J Mol Sci* 2018; **19**(2): E513.

[34]Laubach M, Amarante LM, Swanson K, White SR. What, if anything, is rodent prefrontal cortex? *eNeuro* 2018; **5**. Doi: 10.1523/ENEURO.0315-18.2018.

[35]Zhao W, Xie W, Le W, Beers DR, He Y, Henkel JS, et al. Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism. *J Neuropathol Exp Neurol* 2004; **63**(9): 964-977.

[36]Zhong X, Cao W, Zhao H, Chen L, Cao J, Wei L, et al. MicroRNA-32-5p knockout eliminates lipopolysaccharide-induced depressive-like behavior in mice through inhibition of astrocyte overactivity. *Brain Behav Immun* 2020; **84**: 10-22. Doi: 10.1016/j.bbi.2019.11.001.

[37]Rehman SU, Ali T, Alam SI, Ullah R, Zeb A, Lee KW, et al. Ferulic acid rescues LPS-induced neurotoxicity *via* modulation of the TLR4 receptor in the mouse hippocampus. *Mol Neurobiol* 2019; **56**(4): 2774-2790.

[38]Yin P, Zhang Z, Li J, Shi Y, Jin N, Zou W, et al. Ferulic acid inhibits bovine endometrial epithelial cells against LPS-induced inflammation *via* suppressing NK-κB and MAPK pathway. *Res Vet Sci* 2019; **126**: 164-169. Doi: 10.1016/j.rvsc.2019.08.018.

[39]Schamne MG, Mack JM, Moretti M. The gender-biased effects of intranasal MPTP administration on anhedonic- and depressive-Like behaviors in C57BL/6 mice: The role of neurotrophic factors. *Neurotox Res* 2018; **34**(4): 808-819.

[40]Zhang T, Hong J, Di T, Chen L. MPTP impairs dopamine D1 receptor-mediated survival of newborn neurons in ventral hippocampus to cause depressive-like behaviors in adult mice. *Front Mol Neurosci* 2016; **9**: 101. Doi: 10.3389/fnmol.2016.00101.

[41]Gardner A, Johansson A, Wibom R, Nennesmo I, von Dobeln U, Hagenfeldt L, et al. Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J Affect Disord* 2003; **76**(1-3): 55-68.

[42]Degli EM, Dive C. Mitochondrial membrane permeabilisation by Bax/Bak. *Biochem Biophys Res Commun* 2003; **304**(3): 455-461.

[43]Pradelli LA, Bénéteau M, Ricci JE. Mitochondrial control of caspase-dependent and -independent cell death. *Cell Mol Life Sci* 2010; **67**(10): 1589-1597.

[44]Wang X, Xie Y, Zhang T, Bo S, Bai X, Liu H, et al. Resveratrol reverses chronic restraint stress induced depression-like Behavior: Involvement of BDNF level, ERK phosphorylation and expression of Bcl-2 and Bax in rats. *Brain Res Bull* 2016; **125**: 134-143. Doi: 10.1016/j.brainresbull.2016.06.014.

[45]Wang Y, Ni J, Gao C, Xie L, Zhai L, Cui G, et al. Mitochondrial transplantation attenuates lipopolysaccharide-induced depression-like behaviors. *Prog Neuropsychopharmacol Biol Psychiatry* 2019; **93**: 240-249. Doi: 10.1016/j.pnpbp.2019.04.010.

Original article

Ferulic acid alleviates lipopolysaccharide-induced depression-like behaviors by inhibiting inflammation and apoptosis

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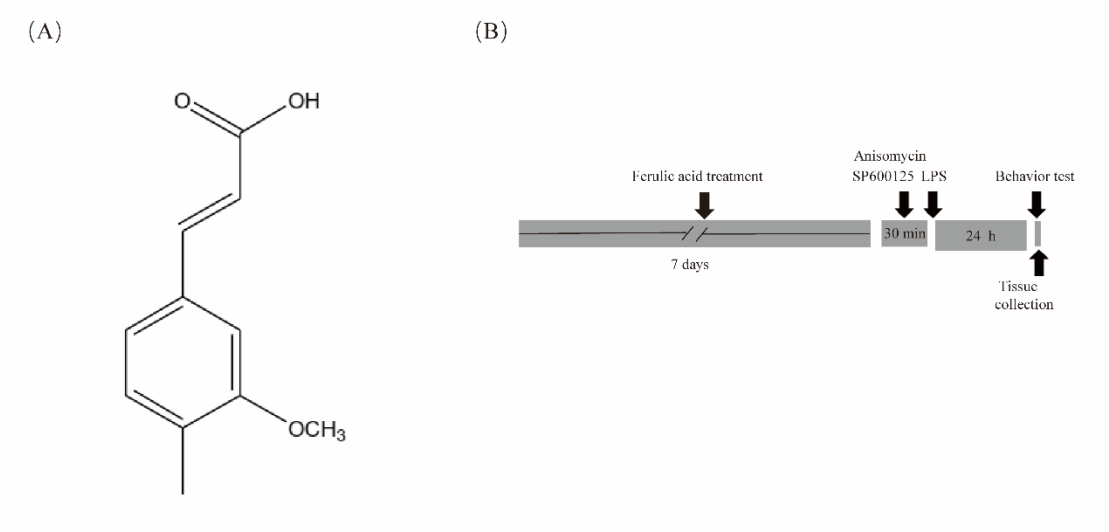

Supplemntary Figure 1. The chemical structure of ferulic acid (A) and experimental design (B).