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ORIGINAL ARTICLE

Fucoxanthin prevents lipopolysaccharide-induced depressive-like behavior in mice via AMPK- NF-κB pathway

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Abstract

Fucoxanthin (FX), a natural carotenoid abundant in edible brown seaweeds, has been shown the great anti-oxidant, anti-inflammatory and anti-diabetic effects in vivo and in vitro. The present study was designed to investigate the effects of FX on lipopolysaccharide (LPS)-induced behavioral defects in mice. In depressive behavior tests, the increased immobility time of forced swimming test and tail suspension test by LPS treatment in mice, which were significantly reversed by FX treatment (200 mg/kg, i.g.). In anxiety behavior tests, LPS injection was neither influence the anxiety-related parameters in marble burying test nor that in elevated plus maze test. Interestingly, anxiolytic effects were observed in single FX treated control and LPS-induced mice groups. FX treatment also reversed LPS-induced body weight loss and food intake decreases. Biochemical analysis indicated that FX inhibited LPS-induced overexpression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α), as well as iNOS and COX-2 in the hippocampus, frontal cortex and hypothalamus, via the modulation of AMPK-NF-κB signaling pathway.

Keywords Fucoxanthin · Lipopolysaccharide (LPS) · Depression · Inflammation · AMPK · NF-κB

Introduction

Major depressive disorder (MDD) is one of the neuropsychiatric disorders, which is characterized by a pervasive and persistent low mood, low energy and loss of interest. About 20% of individuals suffer from the MDD within their lifetime that need the long-term treatment in clinical (Judd et al. 2013; Smith et al. 2013). The pathological mechanisms of MDD is still poorly understood, however, emerging evidence suggested neuroinflammation play an important role in the pathophysiological progression of MDD.

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Increasing evidences have demonstrated that nuclear factor-kappaB (NF-κB) is a central transcriptional factor in the regulation of inflammatory factors. The activated NF-κB affects the transcriptions of many proinflammatory cytokines and neurotoxic mediators, such as IL-1β, IL-6, TNF-α and iNOS (Csaki et al. 2009). Clinical and animal studies have demonstrated that neuroinflammation in MDD patient and depression animal model result from the excess accumulation of proinflammatory cytokines and elicited activation of NF-κB (Monje et al. 2011; Koo et al. 2010). These studies also revealed that LPS-treated mice exhibited depressive-like behavior and followed with the elevated iNOS gene expression and increased nitrite levels in the hippocampus and some other brain regions. In addition, AMP-activated protein kinase (AMPK) has been studied as a key regulator in inflammatory processes. Recent evidences showed that AMPK activator AICAR inhibited lipopolysaccharide (LPS)-induced inflammatory response in different types of cells in vitro (Bai et al. 2010; Giri et al. 2004). However, the role of AMPK in the pathophysiology of MDD is largely unknown.

Fucoxanthin (FX) is a marine carotenoid derived from macroalgae and microalgae. It is demonstrated that FX involve in a variety of physiological process in the body and performs many beneficial properties in the diseases, including

the anti-oxidant and anti-inflammatory and neuroprotective effect (Peng et al. 2011; Miyashita 2009). Recently, the anti-inflammatory and anti-oxidant effects of FX has been expanded. It is reported that FX reversed the oxidative stress and inflammation in amyloid-β42 (Aβ42)-induced BV2 microglia cells, as indicated by the downregulated expressions of pro-inflammatory cytokines and reactive oxygen species (ROS) formation (Pangestuti et al. 2013). Meanwhile, FX significantly suppressed maleic dialdehyde (MDA) in PC12 cells under oxidative stress situation, while the strong anti-inflammatory and anti-oxidant properties of FX was strongly associated with the inhibition of iNOS/NO pathway, accompanied with the suppressions of TNF-a and IL-6 expressions (Tan and Hou 2014). Importantly, kang et al. found FX greatly activated the AMPK signaling pathway as indicated by the increased expressions of the phosphorylation of AMPK, ACC and LKB1 phosphorylation in 3 T3-L1 adipocytes, suggesting FX is a potent regulator in AMPK activation (Kang et al. 2012).

In this study, we aimed to investigate the effect of FX on lipopolysaccharide (LPS)-induced behavior change in mice. The activation of NF-κB and the expressions of brain proinflammatory cytokines, iNOS and COX-2 were tested to detect FX on neuroinflammatory in MDD. In addition, the potential mechanisms of AMPK for FX effects in MDD was also explored.

Materials and methods

Animal

Male ICR mice (4–6 weeks) were obtained from the Animal Center of Shanghai Branch, Chinese Academy of Sciences. The animals were housed at a temperature-controlled room (25 °C) on a reverse 12 h light/dark cycle and allowed them free access to the standard rodent food and water according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Wenzhou Medical University Animal Care and Use Committee. Institutional Animal Ethical clearance number: wydw2017–0134.

Experimental protocols

Fucoxanthin (FX, Sigma-Aldrich) was dissolved in 0.5% sodium carboxymethylcellulose (CMC-Na). Lipopolysaccharide (LPS, Sigma-Aldrich) was dissolved in sterile saline. Mice were randomly divided into 7 groups: (1) control group, (2) control+FX (200 mg/kg, i.g.) group, (3) LPS group (Sigma-Aldrich, dissolved in sterile saline, 1.8 mg/kg, i.p.), (4) LPS + FX (50 mg/kg, i.g.) group, (5) LPS + FX (100 mg/kg, i.g.) group, (6) LPS + FX (200 mg/kg, i.g.) group, and (7) LPS + FX (200 mg/kg, i.g.) + Compound C (Sigma-Aldrich, dissolved in 0.5% DMSO, 10 mg/kg, i.p.) group. Each group were divided into 2 subgroups ($n = 10$ in each subgroup), in which one subgroup was used for depressive behavior test, the other subgroup was used for anxiety behavior test. The experimental procedure was addressed as shown in the Fig.1. In details, all the mice in treated groups were received the FX for consecutive 7 days before LPS injection. The drug is freshly prepared every day. Before administration, FX powder was firstly dissolved in CMC-Na to prepare 5, 10, and 20 mg/ml FX solutions, and every 10 g of body weight is given 0.1 ml drugs by intragastric administration. For Administration by oral gavage, the mice should be gently restrained to immobilize the head but not such that the animal vocalizes or shows other signs of distress. Maintain the mice in an upright (vertical) position and pass the gavage needle along the side of the mouth. Following the roof of the mouth, advance the needle into the esophagus and toward the stomach. If resistance is encountered you may be attempting to enter the trachea and you should alter your needle position. After the needle is passed to the correct length, the compound may be injected. CMC-Na were delivered in mice as control group. At 7th day, control mice were injected with saline, and treated mice were injected with LPS after FX administration for 1 h. After 24 h, the behavior tests of depression and anxiety were assessed. All animals ($n = 140$) received locomotor activity test before behavioral tests. During the experimental study, body weight and food intake were daily recorded. The dose of FX was selected based on previous studies (Zhang et al. 2017; Xiang et al. 2017).

Locomotor activity

The assessment of locomotor activity was carried out as previously described (Yu et al. 2016). Mice was placed in a square chamber which was connected to photoelectric cells with light beams passing through the chamber for 15 min. A training session was performed in the first 5 mins (pre-test), and then locomotion counts were recorded in the following 10 mins.

Forced swimming test

The forced swimming test employed was similar to that described previously (Jiang et al. 2016) with minor modification. Briefly, the mouse was placed in the cylinder (height: 25 cm; diameter: 10 cm; containing 10 cm of water at 24 ± 1 °C) for a 6 min period, the last 4 min of the 6-min test was scored for the immobility time.

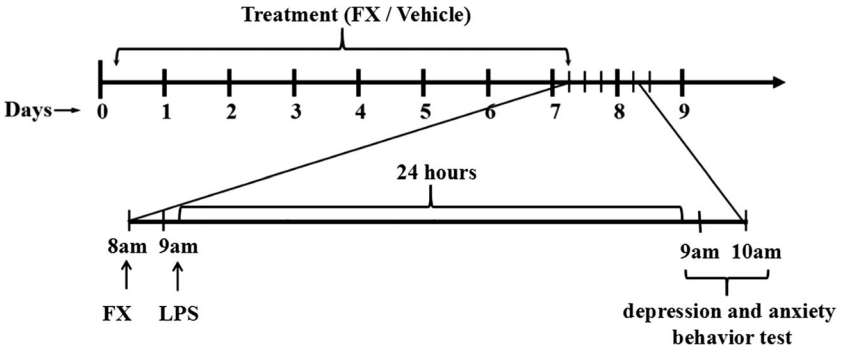

Fig. 1 Experimental protocol schedule. Mice received administration of FX (50, 100, 200 mg/kg, i.g.) or vehicle (carboxymethylcellulose sodium, CMC-Na, i.g.) for 7 days. LPS or vehicle were injected on day 7 after FX treatment, then behavior test were done at 24 h after LPS injection. An other 200 mg/kg FX treated group of mice co-treated compound C (10 mg/kg, i.p.) for 7 days with LPS injection. LPS: lipopolysaccharide, FX: fucoxanthin

Tail suspension test

The tail suspension test (TST) was performed as previous study (Steru et al. 1985). Mice were suspended 50 cm above the floor and stabled with an adhesive tape that placed approximately 1 cm from the tip of the tail. The immobility of mice was recorded at the last 4 mins of 6-min test.

Marble-burying test

Marble-burying test was carried out as previously described (Yu et al. 2015a, b). In brief, mice were placed individually in a polypropylene cage containing 9 clean glass marbles evenly spaced on 5 cm deep sawdust. Ten minutes later, mice were removed, and the number of marbles at least one-half buried in the sawdust was recorded. Mice were submitted to marble-burying test 24 h after LPS injection.

Elevated plus maze test

In brief, the mouse was placed on the apparatus of an elevated platform, which consists of two open arms, two closed arms and a central platform. At the beginning of the test, the mouse was located on the central platform facing open arm. The number of entries and the total time spent in the closed and open arms, respectively, were quantified during a 10-min period using video tracking software (JZZ98; Chinese Academy of Medical Sciences, China) (Jiang et al. 2017).

Animal euthanasia

Decapitation is quick and is presumed to be a relatively painless form of death. Animal used in neurochemical studies are euthanized rapidly by decapitation, without using anesthesia in order to exclude its potential interference with expected measures. Our study will test the changes of neurotransmitters, related enzymes and signal molecules in brain, the drugs for euthanasia such as anesthetics or exposure to CO2 will induce different neurochemical and biological changes. This will induce the misunderstanding of the target drug effects. The method for decapitation: the researcher holds the animal securely, and place it on the stage at the entrance to the guillotine and place the animal's head through the guillotine opening. Once the head is in position, rapidly push the guillotine lever all the way down. Guillotine blades are kept sharp at all times by sharpening them every twelve months or every 250 animals of decapitation. A spare guillotine is available in case the potential turn around times for blade sharpening.

Western blot

Mice were euthanized after behavior test and their brains were collected quickly to distinguish the hippocampus, frontal cortex and hypothalamus. The brain regions of each mouse were examined separately. The tissue samples were weighed, lysed into RIPA lysis buffer (Upstate Chemicon, Temecula, CA, USA) (50 μL lysate was added to every 10 mg tissue) and centrifuged at 13,000 rpm for 30 min at 4 °C. Total protein concentrations of the supernatants were assessed using a BCA assay kit. Proteins in lysate were resolved using 10% sodium dodecyl sulfate polyacrylamide gel and transferred onto polyvinylidene difluoride membranes. Blots were then incubated in blocking buffer for 2 h at room temperature, washed in Tris-buffered saline with 0.1% Tween 20 (TBST), and incubated with the appropriate primary antibodies over night at 4 °C (anti-pAMPK, 1:1000, EPR5683; anti-AMPK, 1:1000, ab80039; anti-β-actin, 1:1000, ab8227 from Abcam; anti-iNOS, 1:400, sc-7271; anti-COX-2, 1:400, sc-1747 from Santa Cruz Biotechnologies). After washing with TBST, the blots were incubated with the secondary antibodies (1:10,000) for 1 h at room temperature. The detection quantification of specific bands was carried out using a

fluorescence scanner (Odyssey Infrared Imaging System, LI-COR Biotechnology, South San Francisco, CA, USA) at 700 and 800 nm wavelengths.

Determination of IL-1β, IL-6, NF-κB p65and TNF-α levels

IL-1β, IL-6, NF-κB p65 and TNF-α levels were measured by using specific ELISA kits (R & D system Inc. Minneapolis, MN, USA). Briefly, serial dilutions of protein standards and samples were added to 96-well ELISA plates, followed by biotinylated anti-antibody. Then the prepared solution of avidin, horseradish peroxidase-conjugated complex was added after rinsing with wash buffer. The reaction was stopped by the stopping solution. The concentrations of IL-1β, IL-6 and TNF-α were determined by a spectrophotometer at 450 nm, respectively. The optical density of NF-κB p65 was detected at the absorbance of 405 nm.

Statistical analysis

Results were presented as the mean ± S.E.M. All data were carried out by one-way analysis of variance (ANOVA), followed by post hoc Tukey test. Differences were considered statistically significant at $P < 0.05$.

Results

Effects of FX on locomotor activity, body weight and food intake of mice

As shown in Fig. 2a, the locomotor activities in mice had no significant difference between control group and LPS treat group. Meanwhile, FX treatment did not significant change the locomotor activities of mice under LPS condition when compared to the LPS only group. Within 24 h after LPS injections, body weight and food intake of LPS treated mice were markedly decreased as compared with control mice ($P < 0.01$, Fig. 2b, c), which were significantly reversed by FX pretreatment at 200 mg/kg ($P < 0.05$). However, co-treatment with the compound C greatly abolished the beneficial effect of FX on body weight and food intake of mice ($P < 0.05$).

FX reverses LPS-induced depressive-like behaviors on mice

Effects of FX on depressive-like behavior in LPS treated mice was assessed by force swimming test and tail suspension test. In force swimming test, LPS induced a significant increase of immobility time, as compared to the control mice ($P < 0.001$, Fig. 3a). Whereas the increased concentrations of FX

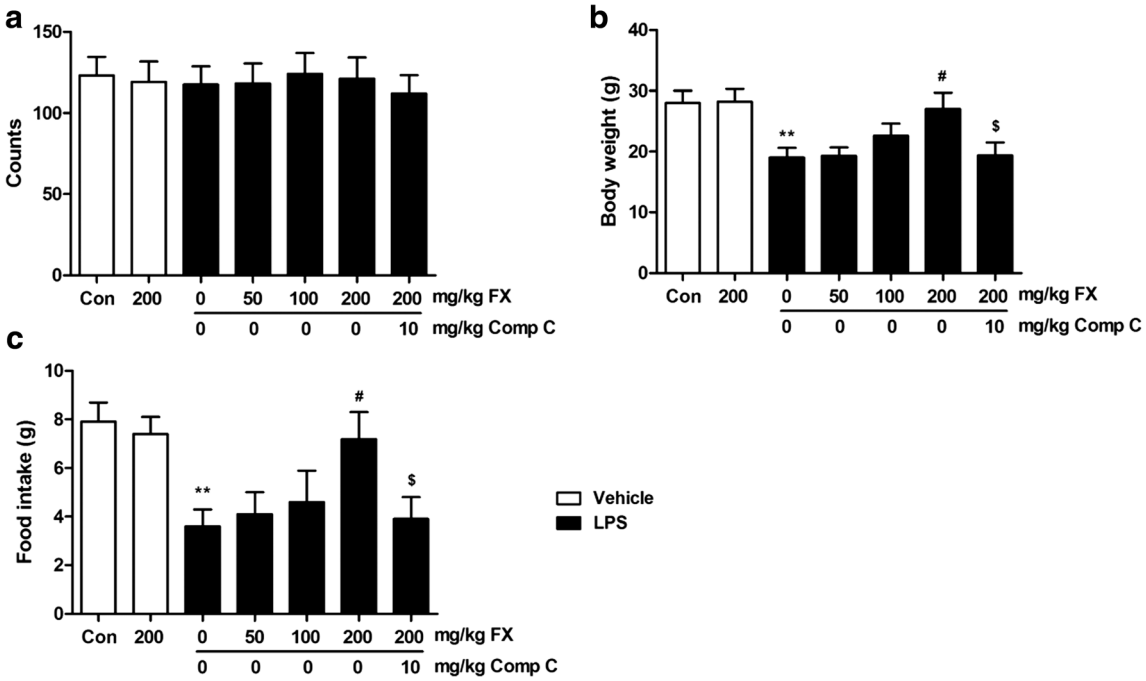

Fig. 2 Effect of FX on locomotor activity (**a**), body weight (**b**) and food intake (**c**) in mice. Mice were administered vehicle or proanthocyanidin (50, 100 and 200 mg/kg) or LPS before testing. An other 200 mg/kg FX treated group of mice co-treated compound C (10 mg/kg, i.p.) for 7 days with LPS injection. The locomotion counts were recorded for 10 min. Data of body weight and food intake on day 9 were shown in **b** and **c**. Values were the mean ± S.E.M. with 10 mice in each group. ** $P < 0.01$ vs. the vehicle-treated control group. #$P < 0.05$ vs. the vehicle-treated LPS group. $P < 0.05$ vs. the FX-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

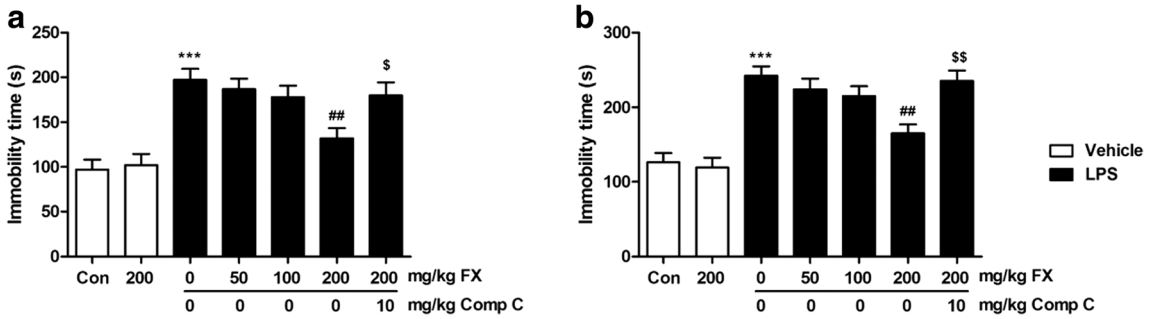

Fig. 3 Effect of FX on the immobility time in the force swimming task (**a**) and tail suspension task (**b**) in mice. Mice were administered vehicle or FX (50, 100 and 200 mg/kg) for 7 days before LPS treatment. The immobility time of force swimming task and tail suspension task were assessed 24 h after injection of saline or LPS. Values were the mean ± S.E.M. with 10 mice in each group. *** $P < 0.001$ vs. the vehicle-treated control group. ##$P < 0.01$ vs. the vehicle-treated LPS group. $$P < 0.05$ and $$$P < 0.01$ vs. the FX-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

pretreatment markedly decreased the immobility time in LPS treated mice ($P < 0.001$), which was attenuated by co-treatment with compound C ($P < 0.05$).

The protective effect of FX on LPS-induced depressive-like behaviors of mice were further in tail suspension test. Consistent with the results of force swimming test, the immobility time of LPS treated mice was significantly increased compared with control mice ($P < 0.001$), which was significant reversed by the pretreatment with FX at 200 mg/kg ($P < 0.01$, Fig. 3b). Co-treatment with compound C attenuated the protective effect of FX ($P < 0.01$).

FX reverses LPS-induced anxiety-like behaviors on mice

In marble-burying test, no significant changes of buried marbles were observed between the control mice and LPS treated mice. However, the reduction of buried marble numbers was observed in 200 mg/kg FX pretreated LPS-induced and control mice ($P < 0.05$ and $P < 0.01$, Fig. 4a).

In elevated plus maze test, time spent in the close arm, open arm and number of entries in the open arm had no significant difference between the LPS-treated mice and the control mice. Interestingly, 200 mg/kg

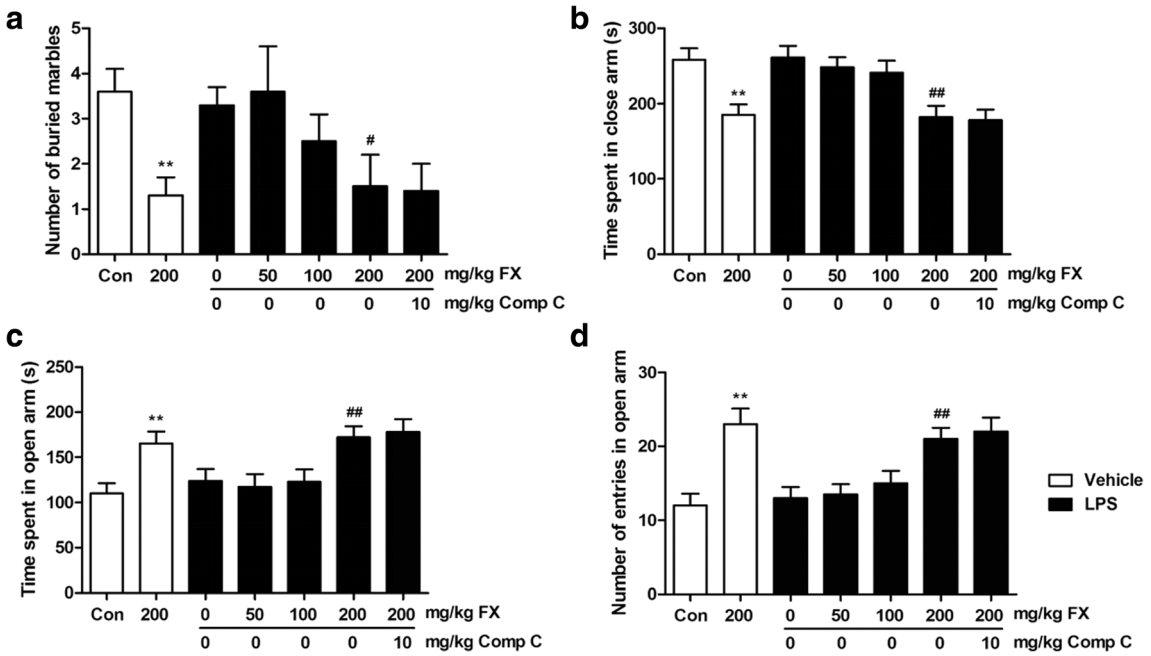

Fig. 4 Effect of FX on the anxiety behaviors in mice. Mice were administered vehicle or FX (50, 100 and 200 mg/kg) for 7 days before LPS treatment. Anxiety-like behavior test: marble-burying test (**a**) and elevated plus maze test (**b**, **c**, **d**). Values were the mean ± S.E.M. with 10 mice in each group. ** $P < 0.01$ vs. the vehicle-treated control group. #$P < 0.05$ and ##$P < 0.01$ vs. the vehicle-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

FX pretreatment significantly decreased the time spent in the close arm ($P < 0.01$, Fig. 4b), increased the time spent in the open arm ($P < 0.01$, Fig. 4c) and number of entries in the open arm in both control mice and LPS treated mice ($P < 0.01$, Fig. 4d). However, co-treatment with Comp C did not affect FX functions on LPS-induced anxiety-like behaviors in mice.

FX increases AMPK activations in the hippocampus, frontal cortex and hypothalamus of mice

As shown in Fig. 5, the phosphorylation of AMPK (pAMPK) was significantly decreased in the hippocampus, frontal cortex and hypothalamus of LPS treated mice, respectively ($P < 0.01$ for hippocampus, Fig. 5b; $P < 0.05$ for frontal cortex, Fig.5c; $P < 0.001$ for hypothalamus, Fig. 5d). Significantly, pretreatment with FX (200 mg/kg, i.g.) markedly reversed the reductions of phosphorylation of AMPK in the hippocampus ($P < 0.01$), frontal cortex ($P < 0.05$) and hypothalamus ($P < 0.01$) on LPS treated mice. Importantly, FX's activation on pAMPK/AMPK were dramatically abolished by Comp C treatment in each brain region ($P < 0.01$). Moreover, FX (200 mg/kg, i.g.) exhibited no effect on AMPK activation in control mice.

The inhibition of FX on NF-κBp65 in the hippocampus, frontal cortex and hypothalamus of mice

LPS injection induced a significantly increase of NF-κBp65 expression in the hippocampus ($P < 0.01$, Fig. 6a), frontal cortex ($P < 0.01$, Fig. 6b) and hypothalamus ($P < 0.001$, Fig. 6c), which were greatly reversed by FX pretreatment at 200 mg/kg ($P < 0.01$ in hippocampus, $P < 0.05$ in frontal cortex and $P < 0.01$ in hypothalamus). Compound C treatment attenuated the inhibiton of FX on NF-κBp65 expression in these brain regions ($P < 0.05$). Besides, FX (200 mg/kg, i.g.) did not influence NF-κBp65 expression in these three brain regions of control mice.

Effects of FX on iNOS and COX-2 expression in the hippocampus, frontal cortex and hypothalamus of mice

The significant increase of iNOS expressions was observed in the hippocampus($p < 0.001$, Fig.7b), frontal cortex ($P < 0.01$, Fig. 7c) and hypothalamus($p < 0.001$, Fig. 7d) of LPS treated mice, that were markedly reversed by FX treatment ($P < 0.05$). However, iNOS expression in each brain region in the FX co-treatment with Comp C group was still as high as that in LPS only group ($p < 0.05$), indicating Comp C

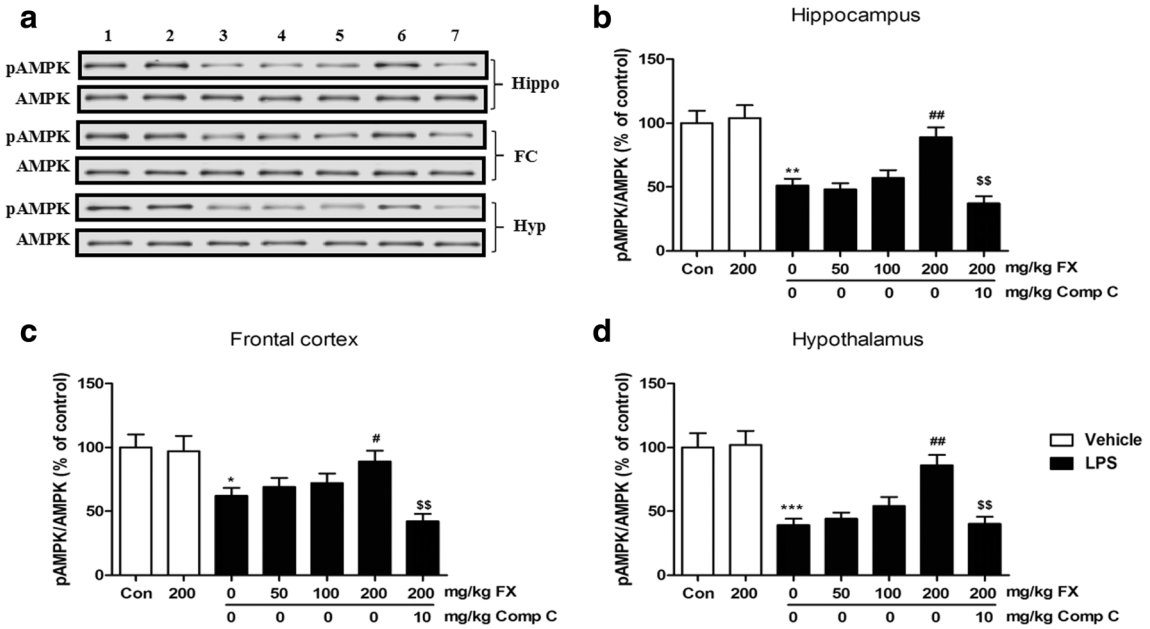

Fig. 5 Effect of FX on the pAMPK/AMPK expression in the hippocampus, frontal cortex and hypothalamus in mice. The blot of pAMPK and AMPK (**a**) expression in these brain regions shown is a representative of results obtained from six separate experiments. Lane 1: vehicle-treated group; Lane 2: 200 mg/kg FX treated control group; Lane 3: vehicle-treated LPS group; Lane 4–6: FX (50, 100, 200 mg/kg) treated LPS group; Lane 7: co-treatment with compond C group. Western blot products of pAMPK and AMPK in the hippocampus (**b**), frontal cortex (**c**) and hypothalamus (**d**) were quantified by densitometric scanning and protein expression was normalized relative to the steady-state expression of β-actin used as internal control. Values were the mean ± S.E.M. with 6 mice in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. the vehicle-treated control group. #P < 0.05 and ##P < 0.01 vs. the vehicle-treated LPS group. $$P < 0.01 vs. the FX-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

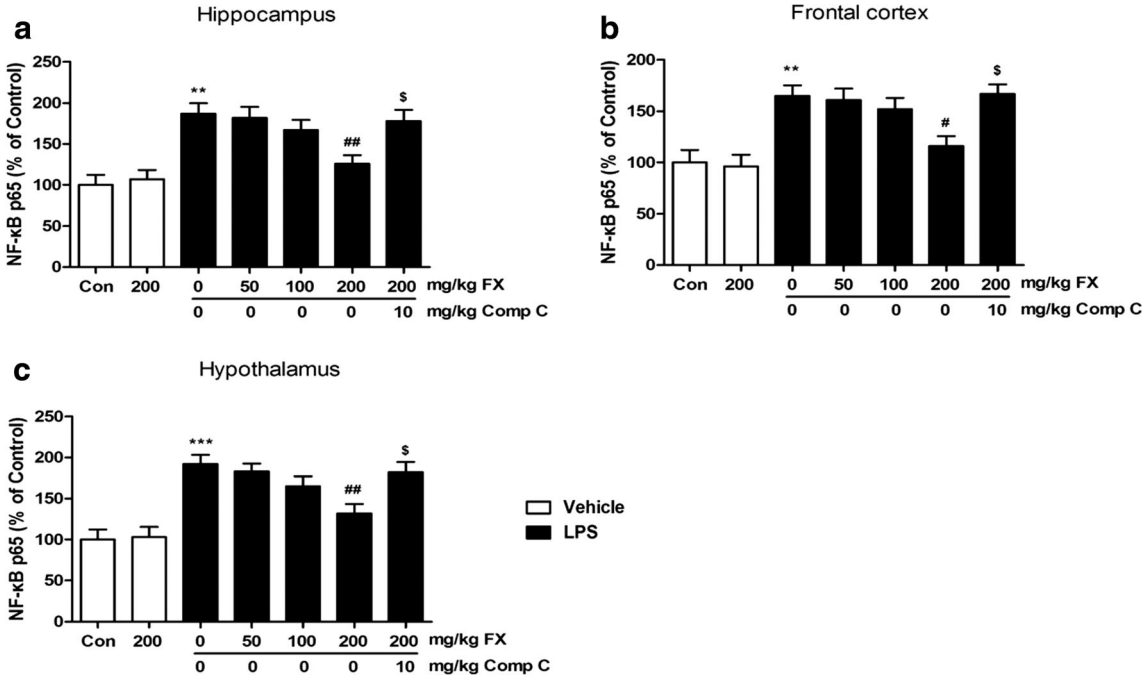

Fig. 6 Effect of FX on the NF-κB p65 expression in the hippocampus (**a**), frontal cortex (**b**) and hypothalamus (**c**) in mice. Values were the mean ± S.E.M. with 6 mice in each group. ** P < 0.01 and *** P < 0.001 vs. the vehicle-treated control group. #P < 0.05 and ##*P* < 0.01 vs. the vehicle-treated LPS group. $*P* < 0.05 vs. the FX-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

treatment abolished the inhibitive effect of FX on iNOS. The similar results had been observed in the results of COX-2 expression. COX-2 levels were increased in these three brain regions after LPS injection (*P* < 0.001, Fig. 8), and strongly decreased by the treatment of FX (*P* < 0.05). Besides, FX (200 mg/kg, i.g.) pretreatment did not influence iNOS and COX-2 expression in control mice.

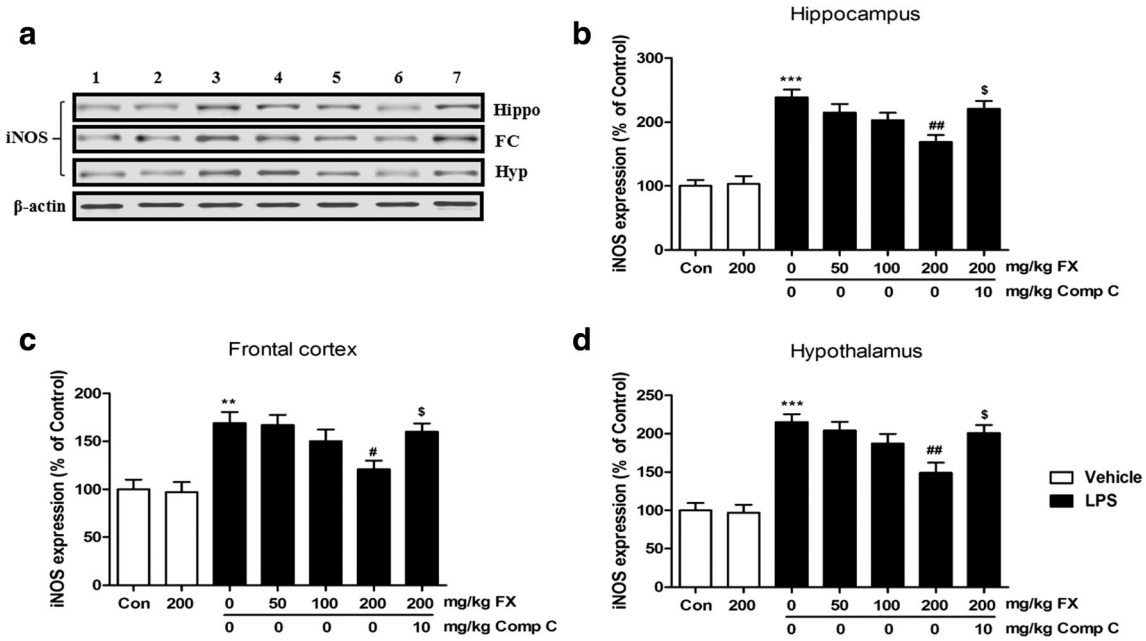

Fig. 7 Effect of FX on the iNOS expression in the hippocampus (**b**), frontal cortex (**c**) and hypothalamus (**d**) in mice. Blots of iNOS in three brain regions are summarized in (**a**). Lane 1: vehicle-treated group; Lane 2: 200 mg/kg FX treated control group; Lane 3: vehicle-treated LPS group; Lane 4–6: FX (50, 100, 200 mg/kg) treated LPS group; Lane 7: co-treatment with compond C group. Values were the mean ± S.E.M. with 6 mice in each group. ** *P* < 0.01 and *** *P* < 0.001 vs. the vehicle-treated control group. #P < 0.05 and ##P < 0.01 vs. the vehicle-treated LPS group. $P < 0.05 vs. the FX-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

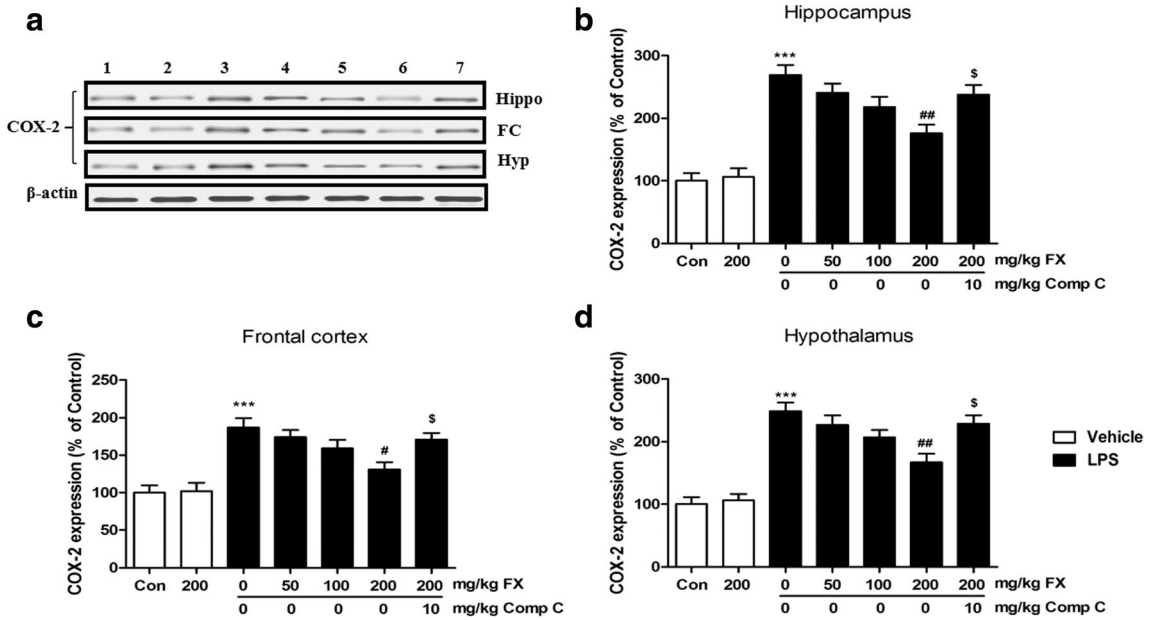

Fig. 8 Effect of FX on the COX-2 expression in the hippocampus (**b**), frontal cortex (**c**) and hypothalamus (**d**) in mice. Blots of COX-2 in three brain regions are summarized in (**a**). Lane 1: vehicle-treated group; Lane 2: 200 mg/kg FX treated control group; Lane 3: vehicle-treated LPS group; Lane 4–6: FX (50, 100, 200 mg/kg) treated LPS group; Lane 7: co-treatment with compond C group. Values were the mean ± S.E.M. with 6 mice in each group. *** $P < 0.001$ vs. the vehicle-treated control group. #$P < 0.05$ and ##$P < 0.01$ vs. the vehicle-treated LPS group. $P < 0.05$ vs. the FX-treated LPS group. LPS: lipopolysaccharide, FX: fucoxanthin, Comp C: Compound C

Effect of FX on pro-inflammatory cytokines levels in the hippocampus, frontal cortex and hypothalamus of mice

Tables 1, 2 and 3 presented the IL-1β, IL-6 and TNF-α expression followed by FX pretreatment in the hippocampus, frontal cortex and hypothalamus, respectively. The IL-1β, IL-6 and TNF-α levels in the hippocampus ($p < 0.01$ for IL-1β, $p < 0.001$ for IL-6 and TNF-α, Table 1), frontal cortex ($p < 0.01$ for IL-1β and IL-6, $p < 0.001$ for TNF-α, Table 2) and hypothalamus ($P < 0.001$ for IL-1β and IL-6, $p < 0.01$ for TNF-α, Table 3) were dramatically increased by the treatment of LPS, which were significantly reversed by 200 mg/kg FX pretreatment ($P < 0.05$ or $P < 0.01$). Co-treatment with Comp C attenuated FX's effect on IL-1β, IL-6 and TNF-α in these three brain regions ($P < 0.05$). Moreover, FX (200 mg/kg, i.g.) administration did not influence pro-inflammatory cytokines levels in the hippocampus, frontal cortex and hypothalamus of control mice.

Discussion

In recent, FX has been reported as a promising medicinal and nutritional ingredient that has multiple beneficial properties in the diseases, such as in obesity, tumor and diabetes (Zhang et al. 2015). The present study indicated that FX had the great preventive effects on LPS-induced depressive like behavior and anxiety like behavior in mice. These protective effects of FX on LPS treated mice were strongly accompanied with the inhibition on LPS-induced inflammatory mediators, iNOS and COX-2 expressions via AMPK-NF-κB pathway.

Systemic administration of LPS causes not only depressive-like behavior but also leads to sickness behavior (Dantzer et al. 2008). To minimize the experimental bias caused by sickness response, we performed all the behavioral tests after 24 h post LPS administration as previous study (Remus and Dantzer 2016). Our study confirmed that depressive like behavior was present after LPS injection at 24 h, without causing the disorder of the locomotor activity in mice. FX treatment reversed these decreased immobility time induced by LPS injection, and improved LPS induced body weight loss and food intake decreases, suggesting that FX had strong antidepressant like activity in LPS induced depression.

In our study, there were no obvious anxiety like symptoms observed after LPS injection as indicated by the no differences in anxiety behavior test between LPS and control group, which was agreed with previous study (Kirsten et al. 2015). Interestingly, FX showed an anxiolytic effect in control mice but also in LPS treated mice as indicated by the marble-burying test and elevated plus maze test. This first and novel funding of FX's anxiolytic effect in the present study need to be further demonstrated in our following study.

AMPK is a well-studied phosphorylated kinase that controls multiple metabolic processes, such as autophagy, oxidation stress, apoptosis resistance and neuroinflammation (Salminen and Kaarniranta 2012). Previous studies had

Table 1 Effect of FX on IL-1β, IL-6 and TNF-α expressions of hippocampus in LPS-treated mice

Group	Dose (mg/kg)	Hippocampus (pg/mg per tissue)		
		IL-1β	IL-6	TNF-α
Control		7.3 ± 1.1	6.8 ± 1.3	7.7 ± 1.3
FX	**200**	7.5 ± 1.0	6.9 ± 1.2	8.1 ± 1.6
LPS		15.2 ± 2.0**	14.7 ± 1.2***	17.1 ± 1.5***
FX + LPS	**50**	13.7 ± 1.4	13.1 ± 2.1	14.9 ± 1.7
	100	12.5 ± 1.6	12.0 ± 1.8	12.7 ± 1.6
	200	8.8 ± 1.2##	8.1 ± 1.4##	8.9 ± 1.2##
Comp C + FX + LPS		13.9 ± 1.3$	13.4 ± 1.5$	15.2 ± 1.6$

Mice were administered vehicle or FX (50, 100, 200 mg/kg, i.g.) for 7 days before LPS treatment. Levels of IL-1β, IL-6 and TNF-α in the hippocampus expressed as pg/mg per tissue. Values are expressed as mean ± S.E.M. for 10 mice in each group. Data analysis was performed using Dunnett's t-test

FX fucoxanthin, *LPS* lipopolysaccharide, *Comp C* Compound C

** $P < 0.01$

*** $P < 0.001$ vs. the control group

$P < 0.01$ vs. the LPS treated group

$ $P < 0.05$ vs. the 200 mg/kg FX + LPS treated group

reported LPS treatment decreased pAMPK expression in the brains of mice (Kim et al. 2015; Yu et al. 2015a, 2015b). In our results, the decreased pAMPK expressions by LPS in each brain region of mice was significant upregulated by FX treatment. Among these regions, the effect of FX on pAMPK expression in the hypothalamus was more sensitive than frontal cortex and hippocampus. In fact, AMPK expression in the hypothalamus is related to the balance of body weight and regulation of food intake (van Dam et al. 2015). The inhibition on AMPK lead to loss of body weight and food intake, conversely the activation of AMPK promotes the balance of body weight and food intake. Meanwhile, many previous studies indicated that FX had the potential effects in activating the AMPK activation in vitro and in vivo (Kang et al. 2012; Chang et al. 2018). Therefore, our results found that FX treatment significantly reversed the LPS induced weight loss and food intake decrease, which was might strongly related to AMPK activation in the hypothalamus. To test our hypothesis, we found that FX improved LPS induced decreased expressions of pAMPK in the frontal cortex and hippocampus. Thus, we assumed that the protective mechanism of FX may be attributed to its regulation of AMPK signaling pathway in

Table 2 Effects of FX on IL-1β, IL-6 and TNF-α expressions of frontal cortex in LPS-treated mice

Group	Dose (mg/kg)	Frontal cortex (pg/mg per tissue)		
		IL-1β	IL-6	TNF-α
Control		6.2 ± 1.4	7.1 ± 1.0	6.5 ± 1.1
FX	**200**	**6.5** ± 1.3	**7.3** ± 1.1	**6.8** ± 1.2
LPS		12.9 ± 1.0**	13.8 ± 1.3**	15.7 ± 1.2***
FX + LPS	**50**	10.2 ± 1.4	11.5 ± 1.2	12.5 ± 1.0
	100	9.1 ± 1.1	10.3 ± 1.2	9.1 ± 1.2#
	200	7.2 ± 0.9#	8.2 ± 0.9#	7.1 ± 1.1##
Comp C + FX + LPS		11.7 ± 1.2$	11.9 ± 1.0$	13.9 ± 1.2$

Mice were administered vehicle or FX (50, 100, 200 mg/kg, i.g.) for 7 days before LPS treatment. Levels of IL-1β, IL-6 and TNF-α in the hippocampus expressed as pg/mg per tissue. Values are expressed as mean ± S.E.M. for 10 mice in each group. Data analysis was performed using Dunnett's t-test

FX fucoxanthin, *LPS* lipopolysaccharide, *Comp C* Compound C

** $P < 0.01$

*** $P < 0.001$ vs. the control group

# $P < 0.05$

$P < 0.01$ vs. the LPS treated group

$ $P < 0.05$ vs. the 200 mg/kg FX + LPS treated group

Table 3 Effects of FX on IL-1β, IL-6 and TNF-α expressions of hypothalamus in LPS-treated mice

Group	Dose (mg/kg)	Hypothalamus (pg/mg per tissue)		
		IL-1β	IL-6	TNF-α
Control		6.1 ± 1.1	7.2 ± 1.2	5.1 ± 1.2
FX	**200**	**6.3** ± 1.2	**7.0** ± 1.4	**5.3** ± 1.4
LPS		13.9 ± 1.2***	15.2 ± 1.1***	10.1 ± 1.2**
FX + LPS	**50**	11.1 ± 1.4	13.1 ± 1.1	9.2 ± 1.3
	100	9.8 ± 1.5	10.6 ± 1.3	8.8 ± 1.5
	200	8.4 ± 0.9#	7.9 ± 1.1##	6.4 ± 1.0#
Comp C + FX + LPS		12.7 ± 1.1$	13.9 ± 1.3$	9.4 ± 1.3$

Mice were administered vehicle or FX (50, 100, 200 mg/kg, i.g.) for 7 days before LPS treatment. Levels of IL-1β, IL-6 and TNF-α in the hippocampus expressed as pg/mg per tissue. Values are expressed as mean ± S.E.M. for 10 mice in each group. Data analysis was performed using Dunnett's t-test

FX fucoxanthin, *LPS* lipopolysaccharide, *Comp C* Compound C

** $P < 0.01$

*** $P < 0.001$ vs. the control group

# $P < 0.05$

$P < 0.01$ vs. the LPS treated group

$ $P < 0.05$ vs. the 200 mg/kg FX + LPS treated group

LPS mice. To confirm whether AMPK pathway play a key role for FX antidepressant function, we examined the antidepressant effects of the AMPK inhibitor compound C and FX co-treatment group in LPS mice. Our data found that antidepressant like activities showed by FX treatment were markedly reversed by co-treatment with AMPK inhibitor compound C, demonstrating that AMPK would be a target factor for FX antidepressant function in LPS induced mice.

Recently, studies have shown that the inhibitory effects of AMPK activation on proinflammatory cytokine production via altering NF-κB activation (Russo et al. 2014). NF-κB is known as a nuclear transcription factor that can regulate various pro-inflammatory cytokines and neurotoxic mediators, such as IL-1β, IL-6, TNF-α and iNOS (Csaki et al. 2009; Koo et al. 2010; Monje et al. 2011). Malignant increase of these genes can cause behavioral abnormalities, mood and sleep fluctuation, learning and memory deterioration. The present study found FX treatment significantly inhibited the LPS induced NF-κB p65 expressions in the hippocampus, frontal cortex and hypothalamus, indicating the anti-depressant function of FX may be owing to its inhibitory effect on the NF-κB activation. Moreover, our results showed AMPK inhibitor compound C treatment enhaced the expressions of NF-κB in each brain region, suggesting AMPK-NF-κB pathway might involve in the anti-depressant effects of FX.

To investigate whether proinflammatory cytokines were involved in the antidepressant effect of FX, we measured the levels of IL-1β, IL-6 and TNF-α in the hippocampus, frontal cortex and hypothalamus. Our results showed that IL-1β, IL-6 and TNF-α expressions were greatly increased after LPS injection. Which was consistent with previous studies (Jiang et al. 2016; Jiang et al. 2017). Previous studies showed that intracerebral administration of IL-1β or TNF-α could induce the depressive-like behaviors, including increases of immobility time in tail suspension test and force swimming test (Hayley et al. 1999; Wohleb et al. 2011). In present study, FX treatment significantly inhibited these proinflammatory cytokines expressions in LPS induced mice.

In addition to the proinflammatory cytokines, many neurotoxic mediators, such as iNOS and COX-2, are also involved in the pathophysiology of depression (Tomaz et al. 2014; Zlatković and Filipović 2013). iNOS is an important biomarker of depression, can generate nitric oxide (NO), as well as a critical element of NF-kB pathway. The specific inhibition of iNOS performed the potential antidepressant-like effects in several animal depression models (Montezuma et al. 2012; Yoshino et al. 2015). Present study found that the significant increased expressions of iNOS in the hippocampus, frontal cortex and hypothalamus of LPS induced mice were greatly inhibited by FX treatment. Furthermore, COX-2, another downstream target of NF-κB pathway, is also related to development of depression and anxiety (Girotti et al. 2011). Our data suggested that FX treatment reversed LPS induced abnormal expressions of COX-2 in the hippocampus, frontal cortex and hypothalamus. The antidepressant-like effects of FX was associated with its inhibition against the NF-κB activation related genes expressions, IL-1β, IL-6, TNF-α iNOS and COX-2, in LPS induced mice, However, these effects were dramatically abolished by the co-treatment with AMPK inhibitor Compound C. Taken together, the protection of FX in LPS induced mice was largely via the upregulation of the AMPK activation resulting to the inhibition on the NF-kB signaling pathway mediated depress-like behaviors.

The current study demonstrates that FX has the great potential to reverse the depressive-like behavior in LPS-induced depression in mice. The underlying mechanisms of this effect is largely mediated by reducing the expressions of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and neurotoxic mediators (iNOS and COX-2) via AMPK-NF-κB signaling pathway.

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Author contributions Xi Jiang, Qizhi Yan and Xuefeng Yu designed the research study. Xi Jiang, Qizhi Yan and Guokang Wang conducted the experiments. Xi Jiang and Xuefeng Yu analyzed the data. Zhihua Tang, Guokang Wang, Qian Lin and Xuefeng Yu wrote the manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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