



Antidepressive effects of kaempferol mediated by reduction of oxidative stress, proinflammatory cytokines and up-regulation of AKT/ β -catenin cascade

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Abstract

Kaempferol (KFL), the major constituent of various fruits and vegetables, could attenuate oxidative stress and inflammation. The aims of the present study were to explore the ameliorative abilities of KFL on the depressive-like behaviors in a chronic social defeat stress (CSDS) mouse model, and to determine the potential mechanisms on oxidative stress, neuroinflammation, and AKT/ β -catenin signaling pathway. Three behavioral tests, sucrose preference test (SPT), social interaction test (SIT), and tail suspension test (TST), were used to evaluate the antidepressive effects of KFL in CSDS mice. Activity levels of antioxidant enzyme, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione s-transferase (GST), and concentrations of malonaldehyde (MDA) and protein carbonylation in the prefrontal cortex were assessed by commercial kits, respectively. Elisa was used to detect the levels of interleukin-1 β (IL-1 β) and tumor necrosis factor α (TNF- α). Q-PCR was used to determine the mRNA level of CD-11b. Furthermore, activity level of AKT/ β -catenin signaling in the prefrontal cortex of CSDS mice was investigated by western blot. In addition, LY294002, a PI3-K inhibitor, was used to investigate the role of AKT/ β -catenin signaling in the antidepressant effects of KFL. Social defeat stress reduced the bodyweights, sucrose consumptions, social interaction times, and the tail suspension mobility times in mice. CSDS mice were also exhibited remarkably increased levels in oxidative stress markers, inflammatory mediators, and decreased activity of AKT/ β -catenin cascade in the prefrontal cortex, which were reversed by treatment with KFL. Interestingly, LY294002 appeared to partly inhibit the overall KFL-mediated protective effects in the CSDS mice. These results confirmed that KFL exerted antidepressive effects, which might be mediated, at least in part, by enhanced antioxidant abilities and anti-inflammation effects via up-regulation AKT/ β -catenin cascade activity in the prefrontal cortex of CSDS mice. Thus, KFL might be a promising, effective, and safe food medicine for depression treatment.

Keywords Kaempferol · Major depression · Chronic social defeat stress · Oxidative stress · Inflammation · AKT/ β -catenin

Abbreviations

KFL Kaempferol
SIT Social interaction test
SPT Sucrose preference test

TST Tail suspension test
CAT Catalase
SOD Superoxide dismutase
GPx Glutathione peroxidase
GST Glutathione S-transferase
MDA Malonaldehyde
IL-1 β Interleukin-1 β
TNF- α Tumor necrosis factor α
Iba-1 ionized calcium binding adaptor molecule-1

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Introduction

Depression is the most common debilitating psychiatric disease with lifetime prevalence of more than 10%. The World Health Organization estimates that more than 350 million individuals

of all ages suffer from depression, highlighting this illness as a major contributor to the global burden of disease (Whiteford et al. 2013; Walker et al. 2015). At present, the available antidepressants are effective, but 20% of these patients do not respond to any intervention and almost 50% of patients fail to reach sustained remission against depression (Gaynes et al. 2009). Because of these drawbacks, better drugs for more effective treatment are considered and needed to explore.

Currently, it is suggested that oxidative stress and neuroinflammation play important roles in the pathogenesis of major depression (Pandya et al. 2013). For example, depressed patients have been reported to show enhanced oxidative damage and disrupted levels of antioxidant enzymes (Ng et al. 2009; O'Donnell et al. 2014). Evidences demonstrate chronic treatments with antidepressants reduce the levels of oxidative stress markers and increase several endogenous antioxidants (Maes et al. 2011a, b). Additionally, multiple investigations suggest there is close linkage between inflammation and depression. Many inflammatory mediators, such as TNF- α and IL-1 β are involved in the pathogenesis of depression (Lopresti et al. 2012; Rawdin et al. 2013). In animal models, the enhanced levels of inflammatory factors might result in depressive-like behaviors (Sukoff et al. 2012). Therefore, oxidative stress and neuroinflammation are crucial components in the biological and cellular pathogenesis of depression (Xu et al. 2014).

In past decades, more and more molecules are discovered to play critical roles in the pathogenesis of depression, especially AKT and β -catenin. A large number of studies have shown that AKT/GSK-3 β is an important neuroprotective survival kinase, especially under stress (Li et al. 2017). Atto vastatin exhibits an antidepressant effect by regulating AKT/GSK-3 β mediated neuroprotection (Ludka et al. 2016). AKT activation consequently downregulates glycogen synthase kinase (GSK-3 β) and increases β -catenin levels. A study with postmortem suggests that the protein level of β -catenin is decreased in the prefrontal cortex region. Fluoxetine (FLX) and citalopram exhibit antidepressive effects through modulation of β -catenin (Hui et al. 2015; Chen et al. 2012). What is more, recent studies show that β -catenin regulates multiple biological and pathological process, including attenuation ROS (reactive oxygen species) and inflammatory factors (Chen et al. 2015). Therefore, AKT/ β -catenin pathway has become a potential drug target and the regulation of this pathway benefits the treatment of depression as well.

There is an increasing interest in exploring new antidepressants based on antioxidation and anti-inflammation properties. Food-medicines, such as natural extracts and plant preparations, were particular considered and popularly in use. Kaempferol (KFL), a natural flavonoid, was the major constituent of various fruits and vegetables, and exerted a wide range of pharmacological actions, such as anti-inflammation and antioxidant activity (Kim et al. 2007, 2008). Recently, more

and more studies report that KFL possesses neuroprotective effects, including ameliorating cerebral ischemia/ reperfusion damages, attenuating stroke risk, and depressive-like behaviors (Yu et al. 2013; Lopez-Sanchez et al. 2007; Taiwe et al. 2016). However, the therapeutic actions of KFL on the depressive-like behaviors induced by chronic social defeat stress were unclear, and whether the antidepressive effects of KFL were involved in the inhibitions of oxidative stress and neuroinflammation remains unknown.

Here, we identified that KFL conspicuously ameliorated the depressive-like behaviors, reduced oxidative stress and neuroinflammation partly mediated by AKT/ β -catenin pathway. Therefore, KFL might be a promising food-medicine agent for depression treatment.

Method

Animals and experimental design

The male CD1 and C57 mice (8-week-old) were purchased from the Experimental Animal Center of China Three Gorges University and maintained in a temperature-controlled vivarium (22 ± 2 °C) under a 12-h light/dark cycle with lights on at 7:00 AM and housed five per cage (relative humidity $55 \pm 10\%$) for 1 week with free access to food and water. Behavioral experiments were carried out during the light phase. The experiment procedures involving animals and their care were approved by the Institutional Animal Care and Use Committee, China Three Gorges University, and conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Animal studies are reported in compliance with the ARRIVE guidelines.

Drug preparation

KFL was obtained from Yuanye Biotechnology Company (Shanghai, China). Fluoxetine and LY294002 were purchased from Sigma Chemical (St Louis, MO, USA). All drug were administered via direct intraperitoneal injection (i.p.). Animals received KFL at a dose of 10, 20 mg/kg. The doses of fluoxetine and LY294002 were 10 mg/kg and 7.5 mg/kg, respectively. The KFL, LY294002, and fluoxetine dosage was dependent on the previous publications (Suchal et al. 2016; Jiang et al. 2017; Jian-yong, et al. 2016). Fluoxetine was dissolved in 0.9% saline. KFL and LY294002 were dissolved in dimethyl sulfoxide (DMSO) to a volume of 10 μ l per gram of body weight. The final concentration of DMSO used in the solvent was less than 1% (V/V).

CSDS procedure and behavioral tasks

After 1 week for adaptation, all C57 mice were divided randomly into five groups ($n = 10/\text{group}$): control, CSDS, CSDS+10 mg/kg KFL, CSDS+20 mg/kg KFL, CSDS+10 mg/kg fluoxetine. Adult C57 mice were functioned as intruders and the aggressive CD1 were retired breeders. As shown in Fig. 1, the CSDS procedure, conducted as previously described (Jiang et al. 2017; Deng et al. 2018), lasted for 10 days (day1-day10). The stressed mice were housed individually. Briefly, during each day, the experimental C57 mice were exposed to different aggressive CD1 mice for up to 10 min. Then, we put the plastic dividers containing holes to separate them in next 24 h. During the defeat episode, in order to avoid the physical wounding, when C57 mice displayed submissive behaviors including fleeing, trembling, immobility, crouching and upright posture (8–10 min required in this study), then the plastic divider was set. The non-stressed mice were served as control, which were handled daily. On the day 11, behaviors tests (SPT, SIT and TST) were used to screen the successful models, which were delivered by drugs. Then the selected stressed mice were divided into different groups and received different drug daily, fluoxetine, LY294002 and vehicle for 28 days. The behavioral tests were preformed from day 29 to day 34. Mice were sacrificed at the day 35.

The sucrose preference test lasting for 4 days was carried out. During the first 2 days, the test mice were individually exposed to two bottles containing pure water and 1% sucrose solution, respectively. On the 3rd day, both the food and two bottles were deprived for 18 h. On the 4th days, the test lasted for 6 h, with the two bottles weighed before and after the test period. The sucrose preference was calculated as a percentage

of the consumed sucrose solution relative to the total amount of liquid intake.

The social interaction test containing two trials (“target absent” trial, “target present” trial) was performed. Each trial lasted for 5 min, and the duration time in the interaction zone spent by the test mice was individually recorded. In the “target absent” trial, each mouse was allowed to explore in an open-field apparatus with fixed plastic enclosure freely and the interaction zone was predefined. In the “target present” trial, each mouse was returned to the same open-field apparatus with an unfamiliar CD1 mouse. The apparatus was cleaned and olfactory cues was removed before each trial.

In the TST, the test C57 mice were individually suspended 60 cm above the floor. Adhesive tape was used to fasten the mice (1 cm from the tail tip). The duration of the immobility period was recorded during the last 5 min of the 6-min test by an investigator blind to the study, which reflected the depressive state of the animal (Steru et al. 1985). Mice were considered immobile only when they hung passively and were completely motionless, and any mice that did climb their tails were removed from the experimental analysis.

Real time quantitative PCR

Q-PCR was performed according to the previously described procedure (Gao et al. 2018). Total RNA was isolated from the entire prefrontal cortex tissues using Trizol. A reverse transcription kit (Takara, Japan) was used to synthesize the first strand of cDNA by the template RNA and quantitative PCR instrument (Agilent Technologies Co., Ltd) was performed for gene amplification. The relative mRNA expression was calculated

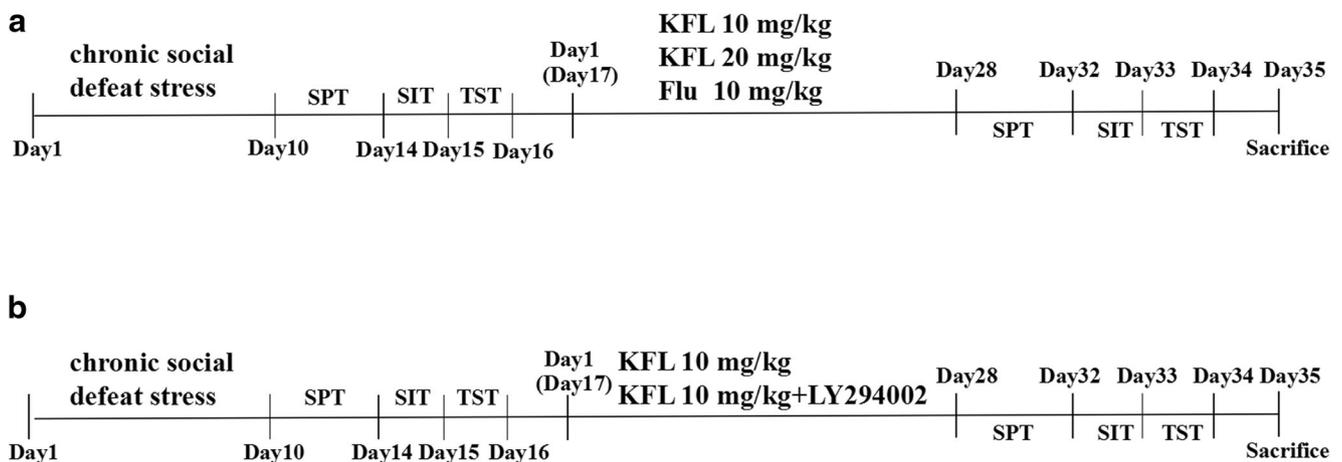


Fig. 1 Schema for experimental schedule, behavioral experiments, and sampling. SIT: social interaction test; TST: tail suspension test; SPT: sucrose preference test

by the eq. $2^{-\Delta\Delta CT}$ and normalized to β -actin. The forward and reverse primer sequences were as below:

- β -actin:
Forward: 5'-CCTCTATGCCAACACAGT-3',
reverse: 5'-AGCCACCAATCCACACAG-3',
- CD11b:
Forward: 5'-AGGAGTGTGTTTGC GTGTC-3',
Reverse: 5'-CTTGGTGTCTTTCGGACT-3'
- Iba-1:
Forward: 5'-ATTATGTCCTTGAAGCGAATGC
Reverse: 5'-TCTCAAGATGGCAGATCTCTTG
(Invitrogen, Shanghai, China).

Western blot

Western blot was performed according to the previously described procedure (Gao et al. 2018). 80 μ g protein was separated by 10% SDS-PAGE gels and subsequently transferred to PVDF membranes (0.45 μ m, Millipore, USA). 5% nonfat milk dilution in TBST was used to blocked the membranes for 2 h at room temperature, then incubated overnight at 4 °C with the primary antibody dilutions in TBST: β -actin, total AKT (1:1000), P-AKT (1: 500), β -catenin (1:1000). All primary antibodies were obtained from Cell Signaling. Then the membranes were incubated with secondary antibody for 2 h at room temperature. Immunoreactivity was detected with chemiluminescence kit (Thermo Scientific, Rockford, USA) and visualization instrument (Clinx Science Instruments Co., Ltd). The Image J analysis software was used to quantify the optical density of each band.

Measurement of cytokine release by ELISA

To detect the levels of pro-inflammatory factors in the prefrontal cortex, brain homogenates were obtained from the prefrontal cortex, and separated by centrifugation at 14,000 g for 5 min at 4 °C to remove cellular debris. The concentration of protein was measured by the ultramicro spectrophotometer (BIOCHROM, Britain). The concentrations of IL-1 β and TNF- α in the stored supernatant were measured using a commercial kit obtained from Boster (Wuhan, China).

Measurement of oxidative products and anti-oxidant enzyme activity

The content of MDA, protein carbonylation and enzyme activities of CAT, SOD, GPx, and GST were measured according to the instruction of commercial kits (Nanjing Jiancheng, China). The concentration of protein was measured by the ultramicro spectrophotometer (BIOCHROM, Britain). The activities of enzyme and contents oxidative stress markers were then normalized to the corresponding protein concentration, and finally showed as fold changes.

Statistical analysis

The data are shown as means \pm SD. The SPSS 19.0 software (IBM, Armonk, NY, USA) was used to perform the analysis. The comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by post hoc LSD test. A value of $P < 0.05$ was considered as significant.

Results

Effects of KFL on behavioral tasks of CSDS mice

Figure 1 showed the schematic timeline of the experimental procedure. At the first, the CSDS model was built, and the SPT, SIT, and TST were used to screen the CSDS mice, and the effects of KFL (10 and 20 mg/kg) were also examined. The results of the behavioral tests were shown in Fig. 2. Our data showed that KFL (10 and 20 mg/kg) and fluoxetine (10 mg/kg) significantly increased the bodyweights, sucrose consumption, social interaction time, and the mobility time of TST in the CSDS mice. The data were subjected to a one-way ANOVA with drug treatment as the factor and revealed a significant main effect of drug treatment [bodyweight: $F(4,45) = 5.167$, $p < 0.01$; SPT: $F(4, 45) = 66.285$, $p < 0.01$; SIT: $F(4,45) = 18.001$, $p < 0.01$; TST: $F(4,45) = 47.373$, $p < 0.01$]. The results suggested that KFL contributed antidepressive effects in the CSDS mice model of depression. Based on the above results, dose of 20 mg/kg KFL was used to investigate the potential mechanism.

Effects of KFL on oxidative stress markers in the prefrontal cortex

As shown in Fig. 3, the activities of SOD, CAT, GPx, and GST significantly decreased in the prefrontal cortex tissues of CSDS mice. Treatment with 20 mg/kg KFL significantly increased the enzyme activities of CSDS mice. Social defeat stress caused significant oxidative damage as evidenced by increase in MDA and protein carbonylation in the prefrontal cortex compared to the non-stressed group. KFL (20 mg/kg) treatment

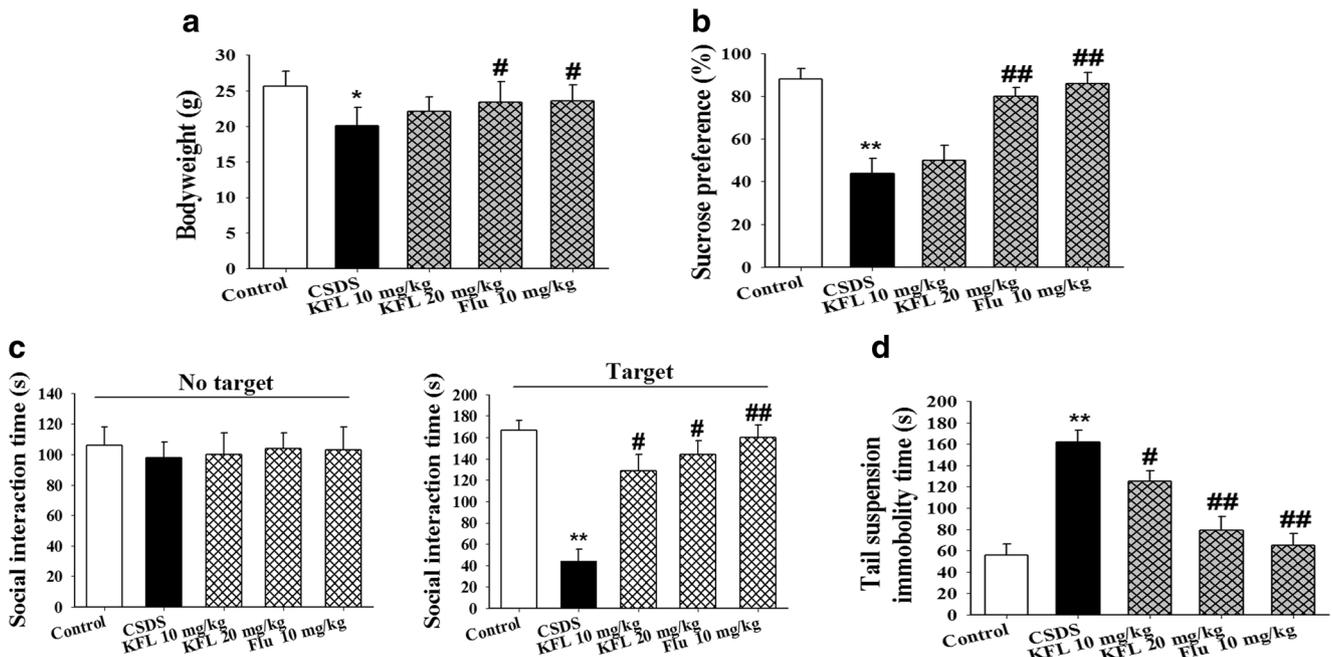


Fig. 2 Effects of social defeat stress and kaempferol (KFL) treatment on the body weight (a), sucrose preference (b), social interaction test (c), and immobility time in the tail suspension test (d). *n* = 10 mice/group. Values

represent the mean ± SD, **p* < 0.05, ***p* < 0.01 vs control group. #*p* < 0.05, ##*p* < 0.01 vs CSDS model

significantly attenuated the MDA and protein carbonylation levels in the prefrontal cortex of CSDS mice. Treatment with fluoxetine (10 mg/kg) in CSDS mice showed similar results, as shown in Fig. 3a–f. These results indicated that KFL possessed potential antioxidation effects in the CSDS model.

Effects of KFL on neuroinflammation in the prefrontal cortex

As shown in Fig. 4, CSDS mice showed a significant increase in the IL-1β and TNF-α concentrations respectively in the

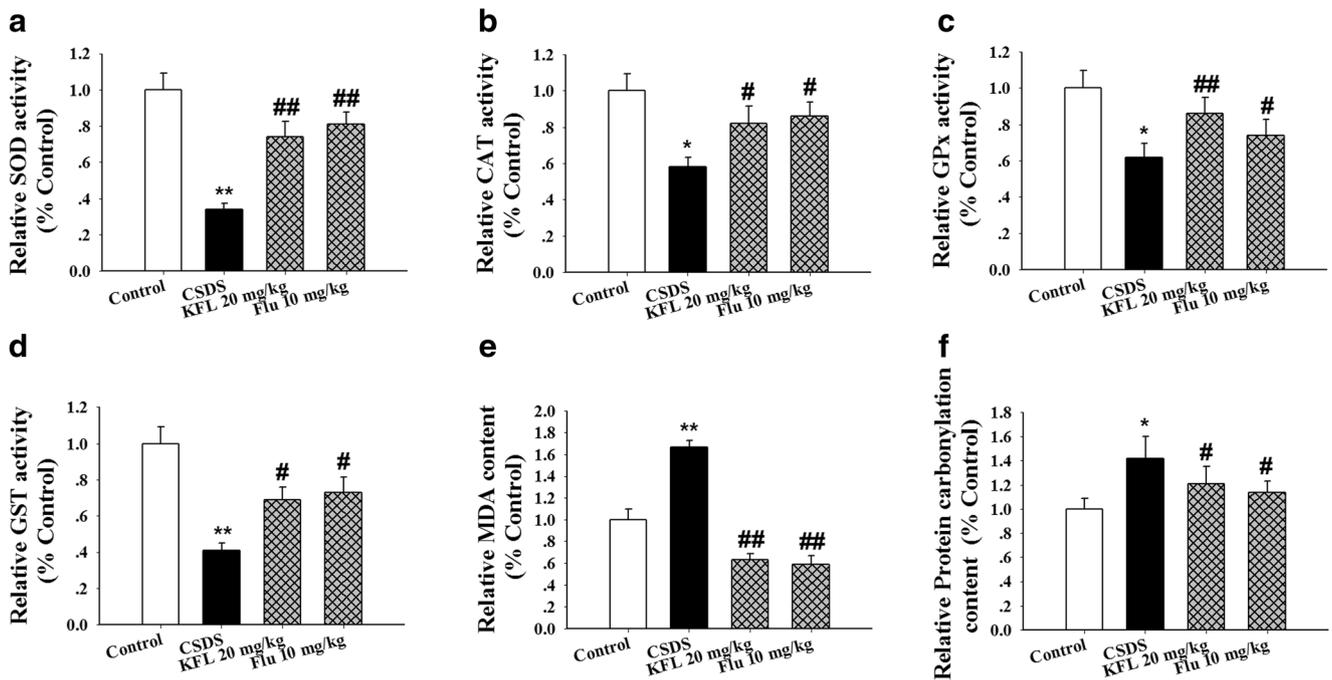
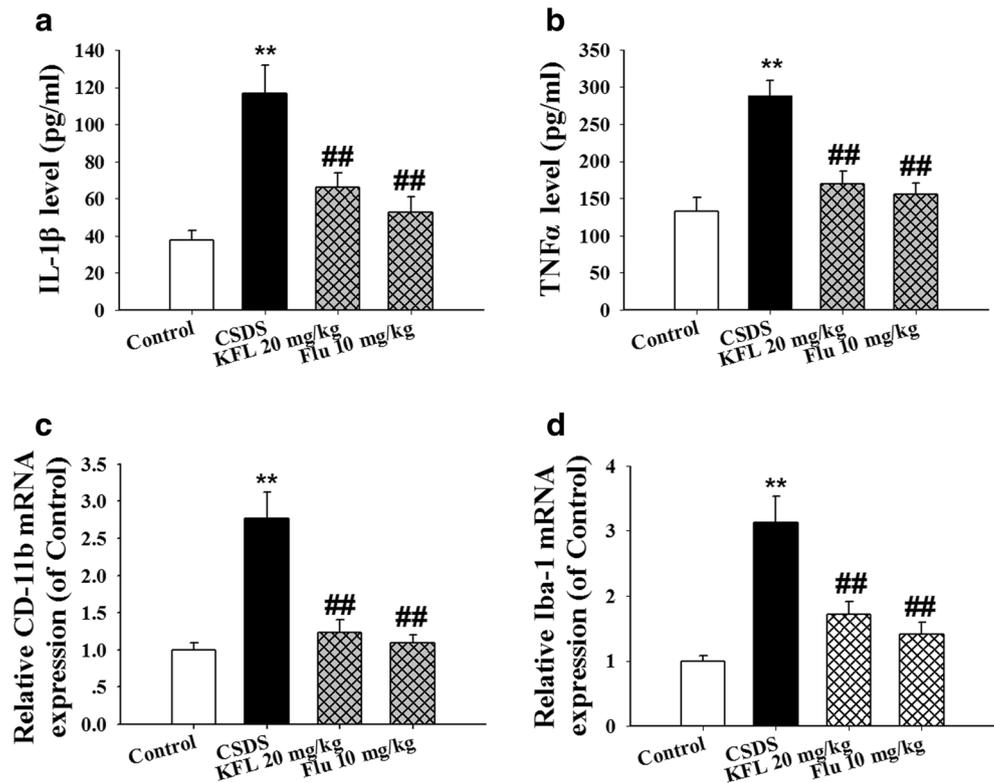


Fig. 3 Effects of social defeat stress and KFL treatment on the biochemical markers in the prefrontal cortex. a SOD, b CAT, c GPx, d GST, e MDA, f Protein carbonylation. *n* = 6 mice/group. Values represent the mean ± SD. **p* < 0.05, ***p* < 0.01 vs control group. #*p* < 0.05, ##*p* < 0.01 vs CSDS model

Fig. 4 Effects of social defeat stress and KFL administration on the levels of IL-1 β (a), TNF- α (b), and mRNA expression of CD11b (c) and Iba-1 (d) in the prefrontal cortex. $n = 6$ mice/group. Values represent the mean \pm SD. ** $p < 0.01$ vs control group. ## $p < 0.01$ vs CSDS model



prefrontal cortex compared to the non-stressed group. Treatment with KFL (20 mg/kg) and fluoxetine (10 mg/kg) markedly attenuated the concentrations of TNF- α and IL-1 β . The effects of KFL and fluoxetine on neuroinflammation were also evaluated by examining the gene expression of the microglial cell surface marker, CD11b. The results revealed that elevation expression of CD11b mRNA was exhibited in the prefrontal cortex of CSDS mice, which was obviously inhibited by KFL and fluoxetine. These results indicated that KFL decreased levels of inflammatory mediators and microglial activation in the CSDS mice.

Effects of KFL on Akt/ β -catenin cascade in the prefrontal cortex

Studies showed that increased Akt activity might reduce brain damage (Matsuda et al. 2016). Furthermore, elevation of Akt/ β -catenin pathway activity linked the alleviation of inflammation and oxidative stress (Covey et al. 2010). Therefore, we investigated the change of Akt/ β -catenin activation level in the prefrontal cortex of CSDS model. As shown in Fig. 5, social defeat stress decreased P-Akt and β -catenin protein expressions in the prefrontal cortex of mice. Treatment with 20 mg/kg KFL and 10 mg/kg fluoxetine significantly increased protein expressions of P-Akt and β -catenin. These results indicated that KFL activated Akt/ β -catenin cascade in the prefrontal cortex of CSDS mice.

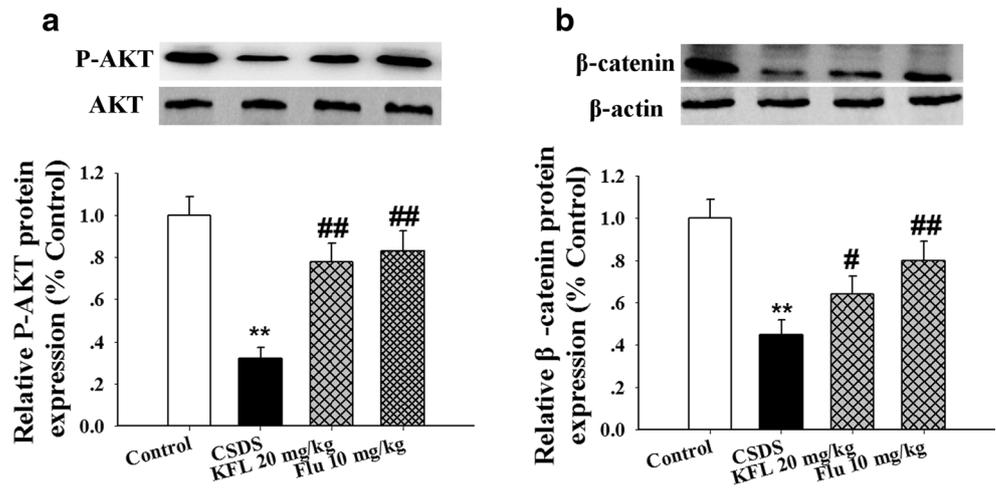
KFL reversed depression behaviors of CSDS mice involved in Akt/ β -catenin pathway

We next investigated whether Akt/ β -catenin pathway was involved in KFL protective effects. LY294002, blocking the expression of P-Akt, was used. First, we found LY294002 decreased the P-AKT protein level. Then, when CSDS mice were treated with LY294002 for 28 days, there were no significant improvements on CSDS mice behavioral deficits in KFL (20 mg/kg) + LY294002 treatment group. The data were subjected to a one-way ANOVA with social defeat stress as the factor and revealed a significant main effect of social defeat stress [SPT: $F(3,36) = 18.374$, $p < 0.01$; SIT: $F(3,36) = 79.662$, $p < 0.01$; TST: $F(3,36) = 27.705$, $p < 0.01$]. Taken together, these results suggested that KFL was able to confer antidepressive effects in CSDS mice by enhancing Akt/ β -catenin pathway activity (Fig. 6).

KFL ameliorated oxidative stress and neuroinflammation in the prefrontal cortex of CSDS mice via Akt/ β -catenin pathway

As shown in Fig. 7, the improvements of KFL on the oxidative stress markers were significantly prevented by LY294002 in the prefrontal cortex of CSDS mice. What is more, KFL (20 mg/kg) + LY294002 could not decrease the concentrations of IL-1 β and TNF- α , and the gene

Fig. 5 Effects of social defeat stress and KFL administration on the protein expressions of P-AKT (a) and β -catenin (b) in the prefrontal cortex. $n = 4$ mice/group. Values represent the mean \pm SD. ** $p < 0.01$ vs control group. # $p < 0.05$, ## $p < 0.01$ vs CSDS model



expression of CD11b in the prefrontal cortex of CSDS mice (Fig. 8). These results indicated that the effects of KFL on anti-oxidative stress and anti-inflammation through AKT/ β -catenin pathway in the prefrontal cortex.

Discussion

In the present study, we examined for the first time the antidepressive effects of KFL, which protecting mice against

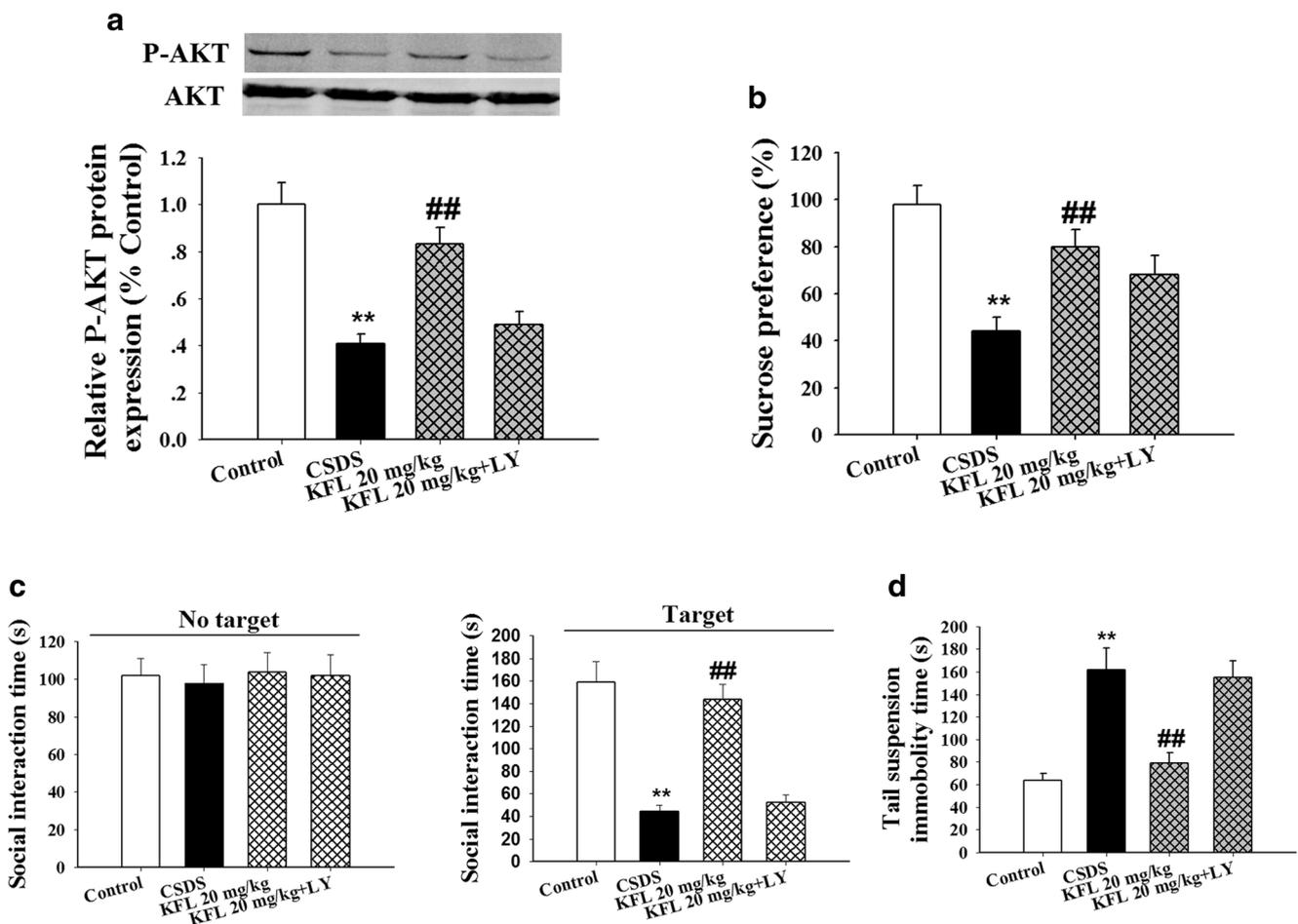
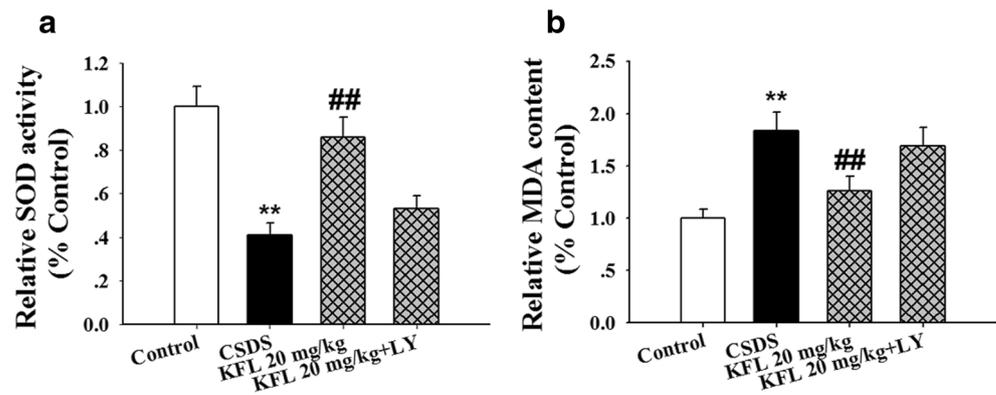


Fig. 6 Effects of LY294002 administration on the P-AKT (a), sucrose preference (b), social interaction test (c), and immobility time in the tail suspension test (d). $n = 4$ mice/group for P-AKT measurement, $n = 10$

mice/group for behavioral tasks. Values represent the mean \pm SD, ** $p < 0.01$ vs control group. ## $p < 0.01$ vs CSDS model

Fig. 7 Effects of LY294002 administration on the biochemical markers in the prefrontal cortex. **a** SOD, **b** MDA. $n = 6$ mice/group. Values represent the mean \pm SD. ** $p < 0.01$ vs control group. ## $p < 0.01$ vs CSDS model



social defeat stress mediated behavioral deficits, oxidative damages, and neuroinflammation. KFL was a major constituent isolated from many plants used in food and medicine. Researches frequently focused on its action in inhibition of inflammation and oxidative stress among its wide array of bioactivities. There have been a number of studies showed that KFL might act on multiple targets and led to a concerted positive effect, such as preventing cerebral ischemia/reperfusion injury and stroke, attenuating oxidative stress and targeting to inflammation in brain (Lagoa et al. 2010). In the present study, we demonstrated that KFL treatment ameliorated depression-like behaviors, attenuated oxidative stress damages and neuroinflammation via AKT/ β -catenin pathway.

Large number of studies reported that oxidative disturbances were remarkably exhibited in depressed patients, especially increased oxidative damage and decreased antioxidant enzyme levels (O'Donnell et al. 2014). In animal model, the brain tissue of CUMS rats showed decreased antioxidant enzyme activities and glutathione levels (Sahin and Gümüşlü 2016). Furthermore, antidepressants treatment improved oxidative stress damage, as well as increased levels of several endogenous antioxidants (Maes et al. 2011a). In addition, antioxidants were reported to possess antidepressant effects by regulation the antioxidant abundances (Pandya et al. 2013). In addition to oxidative stress, several adverse mechanisms contributed pathogenesis of depression more complex and heterogeneous. Depressive-like behaviors accompanied with neuroinflammation as well, including increased levels in

proinflammatory cytokines and microglial activation (Fan et al. 2017; Yamawaki et al. 2018). In the serum of depression patients, the levels of proinflammatory cytokines were higher than those of normal people (Azar and Mercer 2013). Microglial activation might induce the over-production of multiple cytotoxic substances, especially proteolytic enzymes, complement proteins, reactive oxygen species and nitric oxide (Noworyta-Sokołowska et al. 2013). More importantly, exaggerated oxidative stress and neuroinflammation exerted detrimental damages on membrane lipids, functional proteins, mitochondria, and DNA, ultimately resulted in cellular dysfunction and depression occur. In this study, our data were in line with the previous studies. The behavioral tasks showed that social defeat stress-challenged mice exhibited depressive-like behaviors, as evidenced by reduction in the sucrose consumption in SPT, interaction time in SIT and mobility time in TST. CSDS mice also accompanied with elevation of oxidative damage and neuroinflammation in the prefrontal cortex region.

Numerous studies indicated the key role of AKT/ β -catenin pathway in the pathophysiology of depression. Moreover, research reports suggested that therapeutic actions of antidepressants were mediated through the AKT/ β -catenin pathway (Moriguchi et al. 2013). In the prefrontal cortex of suicide victims, a decrease of phosphorylated PI3K and Akt was observed, these results suggested that the attenuation of Akt kinase activity in depressed suicide victims might be due to the combined dysregulation of PI3K (Karege et al. 2011).

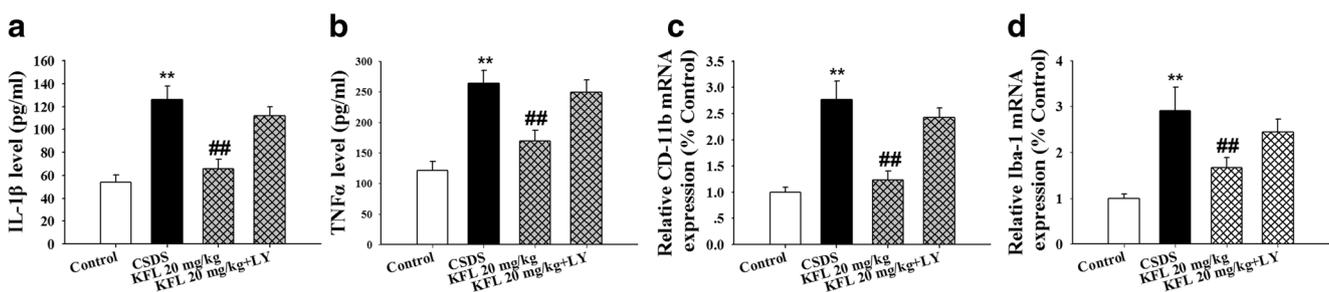


Fig. 8 Effects of LY294002 administration on the levels of IL-1 β (a), TNF- α (b), and mRNA expression of CD11b (c) and Iba-1 (d) in the prefrontal cortex. $n = 6$ mice/group. Values represent the mean \pm SD. ** $p < 0.01$ vs control group. ## $p < 0.01$ vs CSDS model

Furthermore, biological activities of up-regulation antioxidants and anti-inflammation were associated with Akt pathway activation (Chan and Chan 2015). Several studies also reported that under hypoxic conditions, cytoplasmic β -catenin reduced the gene transcription of other genes, ROS-mediate cytotoxicity inhibited activation of β -catenin-dependent transcriptional activity (Kaidi et al. 2007; Shin et al. 2006), and induced neuroinflammation, which was both a cause and consequence of chronic oxidative stress (Zhou et al. 2014). In order to understand the change of AKT/ β -catenin pathway after social defeat stress, we examined the protein expressions of P-AKT and β -catenin. We observed in the present study a significant reduction of protein expressions of P-AKT and β -catenin in the prefrontal cortex of CSDS mice. Our results provided evidences that harmful impact of chronic social defeat stress on the brain, inducing oxidative stress and neuroinflammation, decreasing AKT/ β -catenin pathway, finally impacting behaviors.

For the KFL treatment, firstly, the results showed behavioral deficits were conspicuous ameliorated by KFL therapy. Secondly, we found that KFL could attenuate the MDA and protein carbonylation contents, increase antioxidant enzyme activities, reduce IL-1 β and TNF- α concentrations, inhibit CD-11b mRNA level in the prefrontal cortex of CSDS mice, as well as enhance AKT/ β -catenin pathway. Furthermore, LY294002 appeared to partly inhibit KFL-mediated protective effects in the CSDS mice. Taken together, we speculated that the mechanisms for KFL to improve behaviors deficits might be attributed to its capacities on activating AKT/ β -catenin pathway in the prefrontal cortex of CSDS mice.

It is intriguing to compare the antidepressive effects of KFL with fluoxetine. Based on the present results, KFL and fluoxetine shared a large amount of common beneficial features, the improvements of behavioral deficits, antioxidation and anti-inflammation. These results suggested that KFL and fluoxetine might ameliorate depressive-like behavior by improving oxidative stress and inflammation in the prefrontal cortex.

In conclusion, we showed for the first time the protective effects of KFL by preventing oxidative stress and pro-inflammatory cytokine levels related to depression in the prefrontal cortex. The actions of KFL were possibly mediated by increase the AKT/ β -catenin cascade activity in the prefrontal cortex. KFL could provide a new potential option for the prevention of behavioral dysfunction in depression.

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References

- Azar R, Mercer D (2013) Mild depressive symptoms are associated with elevated C-reactive protein and proinflammatory cytokine levels during early to midgestation: a prospective pilot study. *J Womens Health* 22(4):385
- Chan JY, Chan SH (2015) Activation of endogenous antioxidants as a common therapeutic strategy against cancer, neurodegeneration and cardiovascular diseases: a lesson learnt from DJ-1. *Pharmacol Ther* 156:69–74
- Chen YC et al (2012) The effect of citalopram on chronic stress-induced depressive-like behavior in rats through GSK3 β / β -catenin activation in the medial prefrontal cortex. *Brain Res Bull* 88(4):338–344
- Chen X et al (2015) The administration of erythropoietin attenuates kidney injury induced by ischemia/reperfusion with increased activation of Wnt/ β -catenin signaling. *J Formos Med Assoc* 114(5):430–437
- Covey TM et al (2010) Alkylation of the tumor suppressor PTEN activates Akt and β -catenin signaling: a mechanism linking inflammation and oxidative stress with cancer. *Plos One* 5(10):e13545
- Deng Z-F, Zheng H-L, Chen J-G, Luo Y, Xu J-F, Zhao G, Lu J-J, Li H-H, Gao S-Q, Zhang D-Z, Zhu L-Q, Zhang Y-H, Wang F (2018) miR-214-3p targets β -catenin to regulate depressive-like behaviors induced by chronic social defeat stress in mice. *Cereb Cortex* 1–11. <https://doi.org/10.1093/cercor/bhy047>
- Fan Ni, Luo Y, Ou Y, He H (2017) Altered serum levels of TNF- α , IL-6, and IL-18 in depressive disorder patients. *Hum Psychopharmacol Clin Exp* e2588. <https://doi.org/10.1002/hup.2588>
- Gao W, Xiao C, Hu J, Chen B, Wang C, Cui B, Deng P, Yang J, Deng Z (2018) Qing brick tea (QBT) aqueous extract protects monosodium glutamate-induced obese mice against metabolic syndrome and involves up-regulation transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) antioxidant pathway. *Biomed Pharmacother* 103:637–644
- Gaynes BN et al (2009) What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatr Serv* 60(11):1439
- Hui J, Zhang J, Kim H, Tong C, Ying Q, Li Z, Mao X, Shi G, Yan J, Zhang Z, Xi G (2015) Fluoxetine regulates neurogenesis in vitro through modulation of GSK-3 β / β -catenin signaling. *Int J Neuropsychopharmacol* 1–12. <https://doi.org/10.1093/ijnp/ppy099>
- Jiang B et al (2017) Antidepressant-like effects of fenofibrate in mice via the hippocampal brain-derived neurotrophic factor signalling pathway. *Br J Pharmacol* 17(42):177–194
- Jian-yong, et al (2016) Salvianolate reduces murine myocardial ischemia and reperfusion injury via ERK1/2 signaling pathways in vivo. *Chin J Integr Med* 23(1):1–8
- Kaidi A, Williams AC, Paraskeva C (2007) Interaction between β -catenin and HIF-1 promotes cellular adaptation to hypoxia. *Nat Cell Biol* 9(2):210–217
- Karege F et al (2011) Alterations in phosphatidylinositol 3-kinase activity and PTEN phosphatase in the prefrontal cortex of depressed suicide victims. *Neuropsychobiology* 63(4):224–231
- Kim HK, Park HR, Lee JS, Chung TS, Chung HY, Chung J (2007) Down-regulation of iNOS and TNF-alpha expression by kaempferol via NF-kappaB inactivation in aged rat gingival tissues. *Biogerontology* 8(4):399–408
- Kim SK, Kim HJ, Choi SE, Park KH, Choi HK, Lee MW (2008) Antioxidative and inhibitory activities on nitric oxide (NO) and prostaglandin E2 (COX-2) production of flavonoids from seeds of *Prunus tomentosa* Thunberg. *Arch Pharm Res* 31(4):424–428
- Lagoa R et al (2010) Kaempferol protects against rat striatal degeneration induced by 3-nitropropionic acid. *J Neurochem* 111(2):473–487

- Li Z et al (2017) Valproate attenuates endoplasmic reticulum stress-induced apoptosis in SH-SY5Y cells via the AKT/GSK3 β signaling pathway. *Int J Mol Sci* 18(2):315
- Lopez-Sanchez C et al (2007) Blood micromolar concentrations of kaempferol afford protection against ischemia/reperfusion-induced damage in rat brain. *Brain Res* 1182:123–137
- Lopresti AL, Hood SD, Drummond PD (2012) Multiple antidepressant potential modes of action of curcumin: a review of its anti-inflammatory, monoaminergic, antioxidant, immune-modulating and neuroprotective effects. *J Psychopharmacol* 26(12):1512–1524
- Ludka FK, Constantino LC, Dal-Cim T, Binder LB, Zomkowski A, Rodrigues ALS, Tasca CI (2016) Involvement of PI3K/Akt/GSK-3 β and mTOR in the antidepressant-like effect of atorvastatin in mice. *J Psychiatr Res* 82:50–57
- Maes M et al (2011a) A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* 35(3):676–692
- Maes M et al (2011b) IgM-mediated autoimmune responses directed against multiple neoepitopes in depression: new pathways that underpin the inflammatory and neuroprogressive pathophysiology. *J Affect Disord* 135(1-3):414–418
- Matsuda S et al (2016) Effective PI3K modulators for improved therapy against malignant tumors and for neuroprotection of brain damage after tumor therapy (review). *Int J Oncol* 49(5):1785–1790
- Moriguchi S et al (2013) Stimulation of the Sigma-1 receptor by DHEA enhances synaptic efficacy and neurogenesis in the hippocampal dentate gyrus of olfactory bulbectomized mice. *Plos One* 8(4):e60863
- Ng F et al (2009) Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Cheminform* 40(22):851–876
- Noworyta-Sokołowska K, Górka A, Gołombiowska K (2013) LPS-induced oxidative stress and inflammatory reaction in the rat striatum. *Pharmacol Rep* 65(4):863–869
- O'Donnell P, Do KQ, Arango C (2014) Oxidative/Nitrosative stress in psychiatric disorders: are we there yet? [J]. *Schizophr Bull* 40(5):960–962
- Pandya CD, Howell KR, Anilkumar P (2013) Antioxidants as potential therapeutics for neuropsychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 46:214–223
- Rawdin BJ et al (2013) Dysregulated relationship of inflammation and oxidative stress in major depression. *Brain Behav Immun* 31(7):143–152
- Sahin E, Gümüşlü S (2016) Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. *Behav Brain Res* 87(3):421–425
- Shin S et al (2006) Involvement of glycogen synthase kinase-3 β in hydrogen peroxide-induced suppression of Tcf/Lef-dependent transcriptional activity. *Cell Signal* 18(5):601–607
- Steru L et al (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 85(3):367
- Suchal K et al (2016) Kampeferol protects against oxidative stress and apoptotic damage in experimental model of isoproterenol-induced cardiac toxicity in rats. *Phytomedicine* 23(12):1401–1408
- Sukoff Rizzo SJ et al (2012) Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes. *Transl Psychiatry* 2(12):e199
- Taiwe GS et al (2016) Anticonvulsant activity of an active fraction extracted from *Crinum jagus* L. (Amaryllidaceae), and its possible effects on fully kindled seizures, depression-like behaviour and oxidative stress in experimental rodent models. *J Ethnopharmacol* 194:421–433
- Walker ER, Mcgee RE, Druss BG (2015) Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. *Jama Psychiatr* 72(4):334
- Whiteford HA et al (2013) Global burden of disease attributable to mental and substance use disorders: findings from the global burden of disease study 2010. *Lancet* 382(9904):1575–1586
- Xu Y, Wang C, Klabnik JJ, O'Donnell JM (2014) Novel therapeutic targets in depression and anxiety: antioxidants as a candidate treatment. *Curr Neuropharmacol* 12(2):108–119
- Yamawaki Y, Yoshioka N, Nozaki K, Ito H, Oda K, Harada K, Shirawachi S, Asano S, Aizawa H, Yamawaki S, Kanematsu T, Akagi H (2018) Sodium butyrate abolishes lipopolysaccharide-induced depression-like behaviors and hippocampal microglial activation in mice. *Brain Res* 1680:13–38
- Yu L, Chen C, Wang LF, Kuang X, Liu K, Zhang H, du JR (2013) Neuroprotective effect of kaempferol glycosides against brain injury and neuroinflammation by inhibiting the activation of NF-kappaB and STAT3 in transient focal stroke. *PLoS One* 8(2):e55839
- Zhou WW, Lu S, Su Y-j, Xue D, Yu X-l, Wang S-w, Zhang H, Xu P-x, Xie X-x, Liu R-t (2014) Decreasing oxidative stress and neuroinflammation with a multifunctional peptide rescues memory deficits in mice with Alzheimer disease. *Free Radic Biol Med* 74:50–63