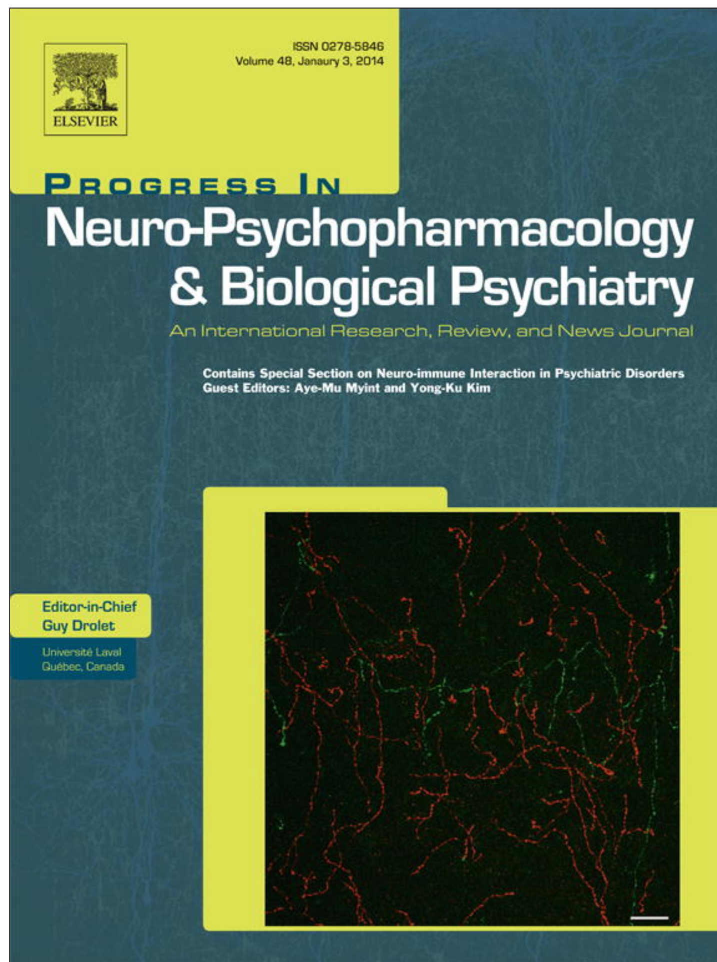
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/authorsrights

Author's personal copy

Progress in Neuro-Psychopharmacology & Biological Psychiatry 48 (2014) 135–141

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

BDNF signaling is necessary for the antidepressant-like effect of naringenin

Li-Tao Yi [a,*], Bin-Bin Liu [a], Jing Li [a], Liu Luo [a], Qing Liu [a], Di Geng [a], Yue Tang [b], Yuan Xia [b], Di Wu [b]

[a] Department of Chemical and Pharmaceutical Engineering, College of Chemical Engineering, Huaqiao University, Xiamen 361021, Fujian Province, PR China
[b] Department of Biotechnology and Bioengineering, College of Chemical Engineering, Huaqiao University, Xiamen 361021, Fujian Province, PR China

ARTICLE INFO

Article history:
Received 18 August 2013
Received in revised form 20 September 2013
Accepted 1 October 2013
Available online 10 October 2013

Keywords:
Brain-derived neurotrophic factor (BDNF)
Chronic unpredictable mild stress
Depression
Naringenin

ABSTRACT

Previous studies in our laboratory have demonstrated that naringenin produced antidepressant-like action in tail suspension test (TST). However, the underlying mechanisms involved in neurotrophin system by which naringenin works have not been investigated. The present study extends earlier works on the role of brain-derived neurotrophic factor (BDNF) in regulating the antidepressant-like actions of naringenin in chronic unpredictable mild stress (CUMS). We showed that a 21-day regimen with naringenin reversed the decreased sucrose preference in sucrose preference test (SPT) and the prolonged first feeding latency in novelty-suppressed feeding test (NSFT), without affecting home-cage feeding consumption. In addition, we also found that naringenin promoted BDNF expression in the hippocampus but not in the frontal cortex in both non-stressed and CUMS mice. Moreover, the antidepressant-like effect of naringenin in SPT and NSFT induced by naringenin administration were totally abolished by K252a, an inhibitor of BDNF receptor tropomyosin-related kinase receptor B (TrkB). In conclusion, our findings suggest that the antidepressant-like effect of naringenin may be mediated, at least in part, by the activation of BDNF signaling in the hippocampus.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Depression has become an increasingly prevalent health problem worldwide, with a high lifetime prevalence ranging from 2% to 15% (Moussavi et al., 2007). The World Health Organization predicts that depression may become the second cause of illness-induced disability by the year 2020. It is generally assumed that multiple mechanisms are responsible for the development of depression. In addition to monoaminergic deficiency and hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, neurotrophin dysfunction is also involved in the pathogenesis of depression (Angelucci et al., 2005). Brain-derived neurotrophic factor (BDNF), a member of the "neurotrophin" family of growth factors, is distributed throughout the central nervous system and the periphery. After binding with and activating tropomyosin-related kinase receptor B (TrkB), BDNF is thought to underlie the pathophysiology and treatment of depression (Lee and Kim, 2010). BDNF–TrkB signaling plays a critical role in the modulation of several functions, such as neurotransmitter release and postsynaptic responses to neurotransmitters, which are closely related to antidepressant therapy (Li and Keifer, 2012). Evidence obtained from preclinical studies indicated that BDNF levels in the brain increased compared with that of controls after antidepressant treatments (Yi et al., 2013; Zhang et al., 2010). However, in transgenic animals with decreased brain BDNF levels or inhibited BDNF–TrkB signaling, antidepressant agents fail to exert behavioral responses (Autry et al., 2011; Monteggia et al., 2007). Moreover, BDNF is now considered to be a neurophysiological biomarkers for predictors of antidepressant-like activity (Quevedo et al., 2009), as a delayed response of clinical antidepressants maybe due to the delayed elevation efficiency of BDNF (Castren and Rantamaki, 2010). These results, together with the antidepressant-like effects of BDNF in cellular and behavioral models of depression (Schmidt and Duman, 2010), suggest a central role for BDNF signaling in the molecular mechanisms of antidepressive therapy.

Although treatment using commercially available antidepressant drugs is effective, approximately 50% of individuals with depression demonstrate full remission in response to synthetic pharmaceutical treatments (Berton and Nestler, 2006). In addition, because of a prevalent belief that "natural is better," a significant amount of public interest in antidepressant development has focused on plant material or natural products extracted from plant sources (Dwyer et al., 2011). Moreover, psychiatric conditions, particularly depression, are among the most common conditions treated with complementary and alternative medicine in some national surveys (Barnes et al., 2008; Hunt et al., 2010).

Naringenin (4',5,7-trihydroxyflavanone), a dietary flavonoid abundant in the peels of citrus fruit, has been reported to have multiple

Abbreviations: BDNF, brain-derived neurotrophic factor; CUMS, chronic unpredictable mild stress; HPA, hypothalamic–pituitary–adrenal; M-MLV, Moloney Murine Leukemia Virus; NSFT, novelty-suppressed feeding test; SPT, sucrose preference test; TrkB, tropomyosin-related kinase receptor B; TST, tail suspension test.

* Corresponding author. Tel./fax: +86 592 6162302.
E-mail addresses: litao.yi@yahoo.com, litaoyi@hqu.edu.cn (L.-T. Yi).

0278-5846/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pnpbp.2013.10.002

Author's personal copy

biological effects. It prevents the cognitive deficits caused by streptozotocin (Baluchnejadmojarad and Roghani, 2006), inhibits monoamine oxidase activity (Olsen et al., 2008), exerts neuroprotective effects against carbaryl-induced neurotoxicity (Muthaiah et al., 2013) and alleviates ischemic brain injury (Raza et al., 2013). In our previous studies, naringenin has been demonstrated to exhibit an antidepressant-like effect in a mouse tail suspension test (TST), which is partly mediated by monoaminergic and HPA systems (Yi et al., 2010, 2012). However, the mechanisms underlying the regulation of the neurotrophin system by naringenin remains unknown. Considering that chronic unpredictable mild stress (CUMS) is the most valuable depressive model and is specifically used to investigate the antidepressant-like mechanisms of agents (Hill et al., 2012; Willner, 1997), our present study investigates the link between BDNF signaling and the behavioral actions of naringenin in CUMS-induced depression.

2. Methods

2.1. Animals

Male ICR mice (24 ± 2 g; 5 weeks old) were purchased from the Laboratory Animal Centre, Fujian Medical University, Fujian Province, PR China. Animals were housed eight per cage (320 × 180 × 160 cm) under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m. The animals were allowed 1 week to acclimatize themselves to the housing conditions before the start of the experiments. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, and the animals were provided standard chow and water ad libitum for the duration of the study. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on 31 October, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on 14 November, 1988).

2.2. Drugs and reagents

Naringenin (purity > 98% by HPLC) was obtained from Shanxi Huike Botanical Development Co., Ltd. (Xi'an, PR China). Fluoxetine hydrochloride was purchased from Changzhou Siyao Pharmaceuticals Co., Ltd (Changzhou, PR China). K252a was purchased from Alomone Laboratories (Jerusalem, Israel). Trizol reagent was purchased from Invitrogen (Carlsbad, USA). Reverse transcriptase Moloney Murine Leukemia Virus (M-MLV) used for cDNA synthesis was from Promega Corporation (Madison, USA). Primers and all other reagents used in RT-PCR were purchased from Sangon Biotech Co. Ltd. (Shanghai, PR China).

2.3. Drug administration

For testing of the behavioral and BDNF regulations, the mice were randomly divided into ten groups: the Control/CUMS-vehicle group (0.9 % physiological saline, p.o.), the Control/CUMS-fluoxetine group (20 mg/kg, dissolved in 0.9 % physiological saline, p.o.), and Control/CUMS groups that received 5, 10 or 20 mg/kg naringenin (suspended in 10 % Tween-80, p.o.).

To investigate whether the BDNF signaling pathway is required for the antidepressant-like effect of naringenin, the mice were randomly divided into five groups: Control-vehicle group, CUMS-vehicle group, CUMS-naringenin group (20 mg/kg, p.o.), K252a group (25 μg/kg, dissolved in 1% DMSO, i.p.) and CUMS-naringenin + K252a group (20 mg/kg, p.o. + 25 μg/kg, i.p.). For the co-injection group, the animals were first injected with K252a 30 min prior to the administration of naringenin. All these agents were administered in a volume of 10 mL/kg.

The doses were selected on the basis of the behavioral results and previous reports (Jiang et al., 2012; Yi et al., 2010, 2012). The repeated drug treatment was performed once daily for the last 3 weeks of the experiment.

2.4. CUMS

CUMS was performed as previously described (Yi et al., 2013). Briefly, the weekly stress regime consisted of food and water deprivation, exposure to an empty bottle, exposure to a soiled cage, light/dark succession every 2 h, space reduction, 45° cage tilt, overnight illumination, and predator sounds (Table 1). All stressors were applied individually and continuously, day and night. The control animals were housed in a separate room and had no contact with the stressed groups. To prevent habituation and to ensure the unpredictability of the stressors, all stressors were randomly scheduled over a 1-week period and repeated throughout the 7-week experiment. On the basis of their sucrose preference following 4 weeks of CUMS, both stressed and control mice were divided into matched subgroups (n = 8).

2.5. Sucrose preference test (SPT)

The SPT was performed at the end of 4-week and 7-week CUMS exposure. Briefly, before the test, the mice were trained to adapt to the sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, the mice were deprived of water and food for 24 h. The test was performed at 9:30 a.m. in which the mice were housed in individual cages and had free access to two bottles containing sucrose solution and water, respectively. After 24 h, the volumes of the consumed sucrose solution and water were recorded. Food was withdrawn during the SPT.

2.6. Novelty-suppressed feeding test (NSFT)

The NSFT was performed 24 h after the last SPT. The NSFT was performed during an 8-min period as previously described (Iijima et al., 2012; Santarelli et al., 2003). Briefly, the testing apparatus consisted of a plastic box (50 × 50 × 20 cm). Food was withheld from the mice for 24 h prior to the test. At the beginning of the test, a single pellet of food was placed on a white paper platform positioned at the center of the box. A mouse was placed in a corner of the maze box and a stopwatch was immediately started. Scoring to measure interest did not begin until the mouse reached for the food with its forepaws and began eating. The home-cage food consumption within 5 min was measured immediately following the test as a control value.

2.7. Tissue sample collection

Twenty-four hours after the completion of the NSFT, the mice were sacrificed by decapitation. Whole brains were rapidly removed from the mice and chilled in an ice-cold saline solution. Brain regions of the frontal cortex and hippocampus were dissected on a cold plate and immediately frozen in liquid nitrogen. The tissue samples were stored at −80 °C until assay.

2.8. Real-time RT-PCR

Total RNA was isolated from the hippocampus using Trizol reagent following the manufacturer's instructions. Reverse transcription was performed using M-MLV reverse transcriptase for cDNA synthesis. Real-time PCR reactions were performed using a SYBR Premix Ex Taq Kit in ABI-7500 system. The BDNF (forward 5′-TTATTTCATACTTCGGTTGC-3′; reverse 5′-TGTCAGCCAGTGATGTCG-3′) and the internal control GAPDH (forward 5′-GGGTGTGAACCACGAGAAAT-3′; reverse 5′-GGAAGAATGGG AGTTGCTGT-3′) primers were used. The fluorescence signal was detected

Author's personal copy

Table 1
CUMS procedure.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Food and water deprivation	09:30 → 09:30						
Exposure to an empty bottle		09:30–10:30					08:30–09:30
Soiled cage		10:30 → 10:30			20:30 → 20:30		
Light/dark succession every 2 h			10:30–20:30				09:30–21:30
Space reduction			20:30 → 08:30				21:30 →
45° cage tilt				08:30–20:30		20:30 → 08:30	
Overnight illumination				20:30 → 08:30			
Predator sounds					08:30–20:30		

at the end of each cycle. Melting curve analysis was used to confirm the specificity of the products. The results were analyzed using the $2^{-\Delta\Delta CT}$ method. The results were normalized to the mRNA expression level of GAPDH in each sample.

2.9. BDNF ELISA

Brain samples were homogenized in lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% Tergitol-type NP-40, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 0.5 mM sodium vanadate. The homogenate was centrifuged at 16,000 ×g for 30 min at 4 °C, and the supernatant was collected and stored at −80 °C until further use.

The protein levels of the samples were measured using the Lowry Method (Lowry et al., 1951). BDNF protein was measured using the BDNF ELISA kit (Boster, Wuhan, PR China) according to the manufacturer's instructions.

2.10. Statistical analyses

All data were expressed as the mean ± S.E.M. The data were analyzed using a two-way or one-way ANOVA, followed by the Dunnett or Tukey's post-hoc test. A P-value <0.05 was considered to be statistically significant for analysis.

3. Results

3.1. Chronic naringenin treatment reverses the CUMS-induced anhedonia

The effects of naringenin and fluoxetine on sucrose preference in the CUMS and control mice are shown in Fig. 1. Two-way ANOVA revealed a significant stress effect [$F(1,70) = 12.73$, $P < 0.01$] and a significant treatment effect [$F(4,70) = 2.62$, $P < 0.05$] on sucrose preference. However, there was no stress × treatment interaction [$F(4,70) = 2.17$, $P > 0.05$] concerning the sucrose preference.

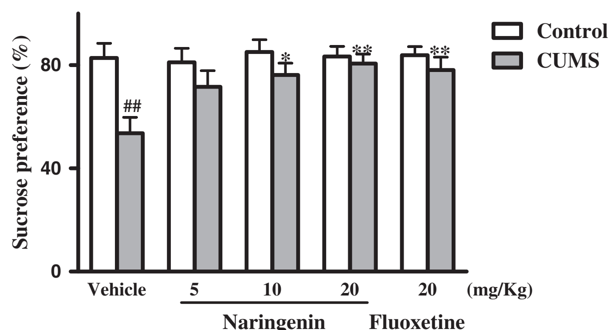


Fig. 1. Effect of naringenin on sucrose preference in non-stressed and CUMS-treated mice. The data represented the values of the mean ± S.E.M. from 8 mice/group. ##$P < 0.01$ vs. Control-vehicle group. *$P < 0.05$ and **$P < 0.01$ vs. CUMS-vehicle group. Data were analyzed using ANOVA followed by the post hoc Dunnett's test.

Separate analysis revealed that CUMS induced a significant decrease in sucrose preference [$F(1,14) = 12.20$, $P < 0.01$]. Chronic treatment with naringenin at 10 and 20 mg/kg significantly reversed the CUMS-induced reduction in sucrose preference [$P < 0.05$, $P < 0.01$, respectively]. The positive drug fluoxetine also increased sucrose preference [$P < 0.01$]. All treatments with drugs showed no significant alterations on sucrose preference in non-stressed groups.

3.2. Chronic naringenin treatment ameliorates the NSFT-induced the increase of first feeding latency

The effects of naringenin and fluoxetine on the first feeding latency and home-cage feed consumption in the CUMS and control mice are shown in Fig. 2. Two-way ANOVA revealed a significant stress effect [$F(1,70) = 4.24$, $P < 0.05$], a significant treatment effect [$F(4,70) = 2.97$, $P < 0.05$] and stress × treatment interaction [$F(4,70) = 2.83$, $P < 0.05$] on the first feeding latency.

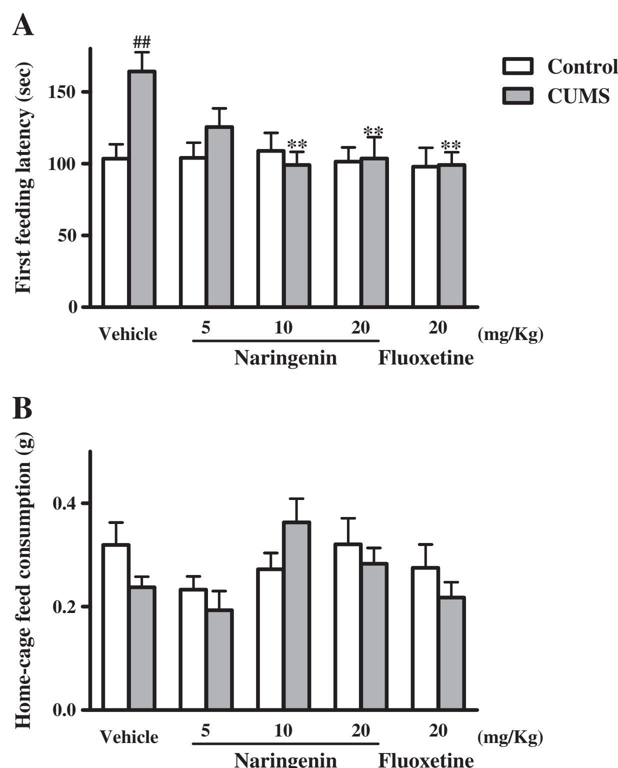


Fig. 2. Effect of naringenin on the first feeding latency (A) and home-cage feeding consumption (B) in non-stressed and CUMS-treated mice. The data represented the values of the mean ± S.E.M. from 8 mice/group. ##$P < 0.01$ vs. Control-vehicle group. **$P < 0.01$ vs. CUMS-vehicle group. Data were analyzed using ANOVA followed by the post hoc Dunnett's test.

Author's personal copy

Separate analysis revealed that CUMS induced a prolonged the first feeding latency in the NSFT [$F(1,14) = 13.14$, $P < 0.01$]. Chronic treatment with naringenin (10 and 20 mg/kg) and fluoxetine (20 mg/kg) significantly reversed the CUMS-induced elevation of the first feeding latency [$P < 0.01$, $P < 0.01$, $P < 0.01$, respectively]. All treatments with drugs showed no significant alterations on the first feeding latency in naïve mice.

However, there was no difference in the home-cage feed consumption performed immediately following the NSFT within 5 min, indicating that the effects of naringenin were not due to a general increase in feeding.

3.3. Chronic naringenin treatment attenuates the CUMS-induced BDNF down-regulation

The effects of naringenin and fluoxetine on the BDNF expression in the frontal cortex in CUMS and control mice are shown in Fig. 3. Two-way ANOVA revealed only stress effect [$F(1,40) = 11.58$, $P < 0.01$; $F(1,70) = 15.61$, $P < 0.01$] significantly affected BDNF mRNA and protein expression. However, treatment effect [$F(4,40) = 1.09$, $P > 0.05$; $F(4,70) = 1.63$, $P > 0.05$] and stress × treatment interaction [$F(4,40) = 0.37$, $P > 0.05$; $F(4,70) = 0.31$, $P > 0.05$] did not reach a statistic significance. Separate analysis revealed that CUMS induced a decrease in BNDF protein expression in the frontal cortex [$F(1,14) = 5.69$, $P < 0.05$]. Treatment with fluoxetine (20 mg/kg), but not naringenin increased the CUMS-induced reduction in BDNF protein expression [$P < 0.05$].

The effects of naringenin and fluoxetine on the hippocampal BDNF expression in the CUMS and control mice are shown in Fig. 4. Two-way ANOVA revealed a significant stress effect [$F(1,40) = 49.60$, $P < 0.01$; $F(1,70) = 68.83$, $P < 0.01$] and significant treatment effect [$F(4,40) = 8.25$, $P < 0.01$; $F(4,70) = 13.96$, $P < 0.01$] on the hippocampal BDNF mRNA and protein expression. However, there was no stress × treatment interaction [$F(4,40) = 0.83$, $P > 0.05$; $F(1,70) = 1.56$, $P > 0.05$] concerning the levels. Separate analysis revealed that CUMS induced a decrease in BNDF mRNA and protein expression in the hippocampus [$F(1,8) = 21.14$, $P < 0.01$; $F(1,14) = 43.57$, $P < 0.01$]. Chronic treatment with naringenin (10 and 20 mg/kg) and fluoxetine (20 mg/kg) significantly reversed CUMS-induced reduction of the hippocampal BDNF expression [mRNA: $P < 0.01$, $P < 0.01$, $P < 0.01$; protein: $P < 0.01$, $P < 0.01$, $P < 0.01$]. In addition, long-term treatment with fluoxetine (20 mg/kg) also significantly increased the BDNF mRNA expression in the hippocampus of non-stressed mice [$P < 0.05$] compared to the non-stressed vehicle. Furthermore, naringenin (20 mg/kg) and fluoxetine (20 mg/kg) elevated the BDNF protein levels in non-stressed mice [$P < 0.05$, $P < 0.05$].

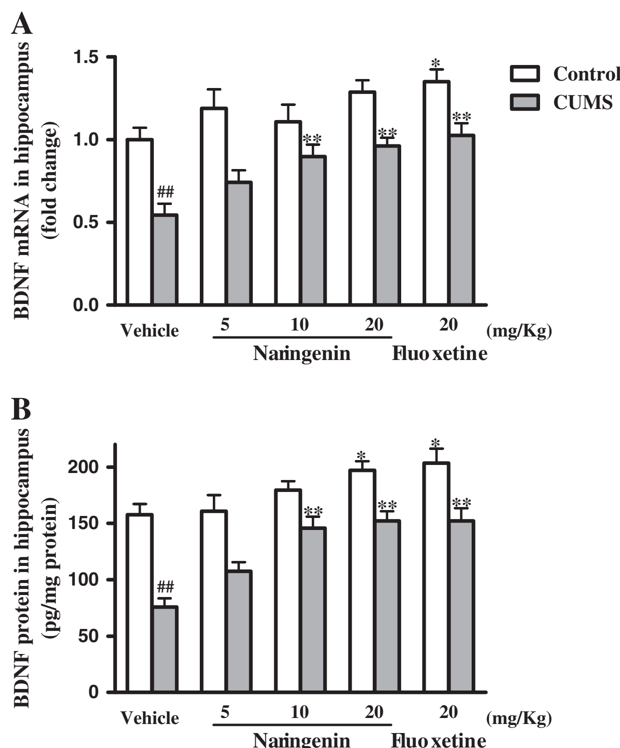


Fig. 4. Effect of naringenin on the hippocampal BDNF mRNA (A) and protein (B) expression in non-stressed and CUMS-treated mice. The data represented the values of the mean ± S.E.M. from 5 (A) or 8 (B) mice/group. ##$P < 0.01$ vs. Control-vehicle group. *$P < 0.05$ and **$P < 0.01$ vs. CUMS-vehicle group. Data were analyzed using ANOVA followed by post hoc Dunnett's test.

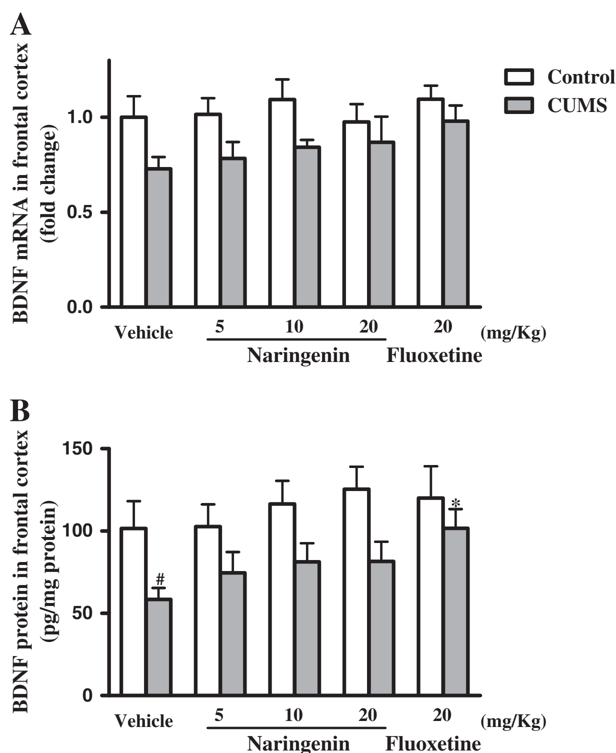


Fig. 3. Effect of naringenin on the BDNF mRNA (A) and protein (B) expression in the frontal cortex of non-stressed and CUMS-treated mice. The data represented the values of the mean ± S.E.M. from 5 (A) or 8 (B) mice/group. #$P < 0.05$ vs. Control-vehicle group. *$P < 0.05$ vs. CUMS-vehicle group. Data were analyzed using the ANOVA followed by the post hoc Dunnett's test.

3.4. BDNF is necessary for the antidepressant-like effects of naringenin

To further confirm whether BDNF signaling was necessary for the antidepressant-like effects of naringenin, CUMS mice were co-injected with naringenin (20 mg/kg) and K252a (25 μg/kg), an inhibitor of the BDNF receptor TrkB for 3 weeks. Behavioral changes were evaluated 24 h or 48 h after the last injection. For sucrose preference (Fig. 5), two-way ANOVA indicated that the effect of treatment (naringenin or vehicle) and the effect of pretreatment was significant [$F(1,28) = 9.80$, $P < 0.01$; $F(1,28) = 4.92$, $P < 0.05$, respectively]. The interaction between treatment and pretreatment was also significant [$F(1,28) = 6.90$, $P < 0.05$]. Consistent with this finding, further separate analysis indicated that naringenin [$P < 0.01$] reversed the reduction in sucrose preference induced by CUMS [$F(1,14) = 19.57$, $P < 0.01$]. This antidepressant effect was completely blocked by pretreatment of the antagonist K252a [$P < 0.01$].

For the first feeding latency in the NSFT (Fig. 6), two-way ANOVA indicated that the effect of treatment (naringenin or vehicle) and the

Author's personal copy

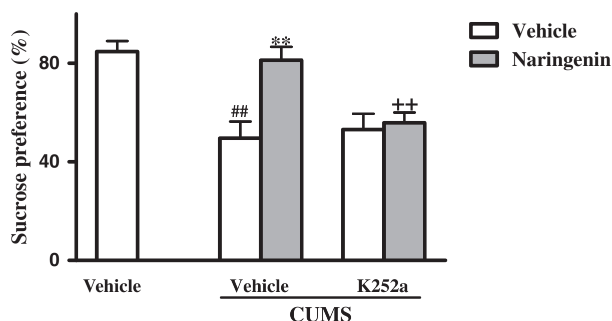


Fig. 5. Effect of pretreatment with K252a on naringenin (20 mg/kg)-induced increase in sucrose preference in CUMS mice. The data represented the values of the mean ± S.E.M. from 8 mice/group. ##$P < 0.01$ vs. Control-vehicle group. **$P < 0.01$ vs. CUMS-vehicle group. ++$P<0.01$ vs. CUMS-naringenin group. Data were analyzed using ANOVA followed by the post hoc Tukey's test.

effect of pretreatment (K252a or vehicle) was significant [$F(1,28) = 7.49$, $P<0.05$; $F(1,28) = 4.51$, $P<0.05$, respectively]. The interaction between treatment and pretreatment was also significant [$F(1,28) = 6.40$, $P<0.05$]. Consistent with this finding, further separate analysis indicated that naringenin [$P < 0.01$] reversed the increase in the first feeding latency induced by CUMS [$F(1,14) = 18.72$, $P<0.01$]. This antidepressant effect was totally blocked by pretreatment of the antagonist K252a [$P<0.05$]. In addition, the home-cage feed consumption during 5 min in each group showed no significant differences.

4. Discussion

The present study provides further evidence that naringenin exerts an antidepressant-like effect in CUMS-treated mice in the SPT and NSFT. Chronic naringenin treatment only enhanced BDNF expression in the hippocampus, but not in the frontal cortex, both in CUMS and non-stressed animals. More importantly, chronic naringenin treatment restored the stress-induced down-regulation of BDNF to the same levels as non-stressed vehicle-treated control animals. Finally, pharmacological inhibition of BDNF–TrkB signaling completely abolished the antidepressant-like effect of naringenin in the SPT and NSFT.

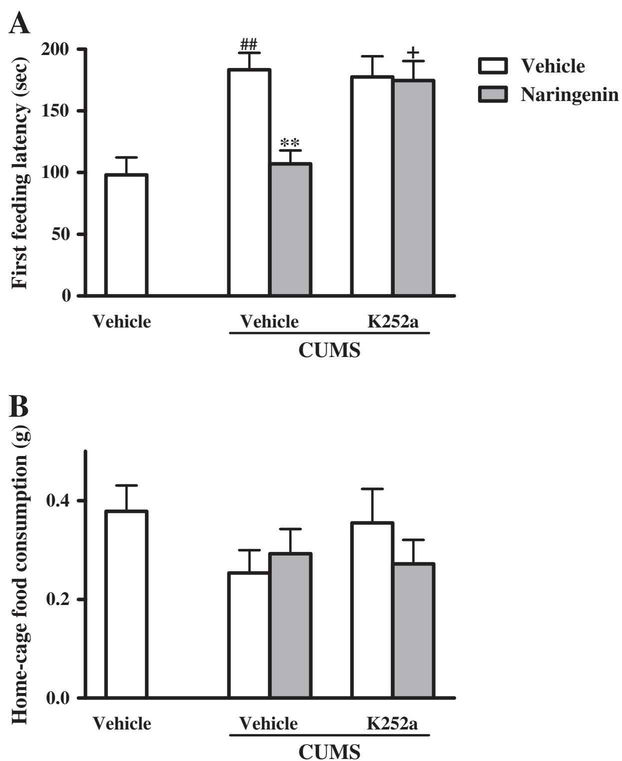


Fig. 6. Effect of pretreatment with K252a on naringenin (20 mg/kg)-induced the first feeding latency (A) and home-cage feeding consumption (B) in CUMS. The data represented the values of the mean ± S.E.M. from 8 mice/group. ##$P < 0.01$ vs. Control-vehicle group. **$P < 0.01$ vs. CUMS-vehicle group. +$P < 0.05$ vs. CUMS-naringenin group. Data were analyzed using ANOVA followed by the post hoc Tukey's test.

As a widely used model for the study and development of antidepressants (Willner, 1997), sucrose preference reduction in CUMS reflects a state of anhedonia, which produces a condition similar to human depression. This condition can be restored using therapeutically effective drugs for the treatment of depression (Zhang et al., 2010). In the present experiment, we used an efficacious mouse CUMS model to mimic stress in daily life. The result showed that a reduction in sucrose preference induced by the CUMS procedure was significantly restored with naringenin treatment.

Subsequently, we tested the first feeding latency of the mice, which is an indication of increased anxiety levels in the NSFT (Santarelli et al., 2003). The NSFT is an increasingly popular anxiety measure in depression studies, as it is a behavioral paradigm that is sensitive to chronic, but not acute antidepressants (Gordon and Hen, 2004; Li et al., 2010). In addition, considering that depression displays high comorbidity with anxiety and cognitive disorders (Bessa et al., 2009), we performed the NSFT after CUMS. We found that the prolonged first feeding latency induced by CUMS was reversed by chronic administration of naringenin, suggesting that the anxiety-related behavior was ameliorated by naringenin administration. Thus, our present study further indicates the antidepressant-like effect of naringenin, or even its potential anxiolytic-like effect. However, the home-cage food consumption among these animals showed no significant difference, indicating that the effects of naringenin were not due to a general increase in feeding.

According to the neurotrophic hypothesis of depression, stress and antidepressant treatment exert opposing effects on the regulation BDNF expression (Castren and Rantamaki, 2010). The results derived from preclinical studies demonstrate that antidepressant efficacy is mediated at least in part via an elevation of BDNF levels or BDNF signaling in the brain (O'Leary et al., 2009; Taliaz et al., 2010). Consistent with a previous study (Mao et al., 2010), the present study also found that CUMS reduced BDNF levels in both the frontal cortex and the hippocampus. The commercial antidepressant fluoxetine upregulated BDNF levels in the frontal cortex and hippocampus in mice exposed to CUMS. However, chronic administration of naringenin for 3 weeks significantly enhanced BDNF levels only in the hippocampus. Although a previous study showed that chronic administration of diverse pharmacological antidepressant treatments produced a common increase in BDNF protein levels specifically in the frontal cortex (Balu et al., 2008), several previous reports have also demonstrated that CUMS (Luo et al., 2010; Zhang et al., 2010) or antidepressant treatment (Dwivedi et al., 2006) caused a significant change in BDNF levels only in the hippocampus, but not in the frontal cortex. More importantly, regional distribution of the BDNF gene was highly variable throughout the brain, with parts of the hippocampus showing particularly high densities (De Foubert et al., 2004).

In our study, the change in BDNF mRNA expression was not always parallel to the change in BDNF protein expression after drug treatment, such as fluoxetine treatment in the frontal cortex of CUMS mice and 20 mg/kg naringenin in the hippocampus of non-stressed animals. Several reasons might explain this inconsistency. It is important to consider that total protein levels may be affected not only by new proteins derived from mRNA translation, but also by the degradation of existing proteins. On this basis, we hypothesize that the elevated BDNF protein levels observed in brain, at least in part, in response to the role of ubiquitin–proteasome degradation pathway. In addition, the inconsistent data may be due to the time points selected to examine BDNF

Author's personal copy

expression post-CUMS as mRNA levels are relatively dynamic (Hill et al., 2012). Accordingly, examination of BDNF protein levels produced much more consistent results.

According to the explanation for the delayed response of clinical antidepressants, BDNF was increased using rapid antidepressants (NMDA receptor antagonist, such as ketamine), but not traditional antidepressants (such as fluoxetine) after acute treatment (Autry et al., 2011; Castren and Rantamaki, 2010). Our preliminary experiment showed that acute administration of naringenin did not change sucrose preference and first feeding latency in mice exposed to CUMS (data not shown), which were two measurement indices used to distinguish between rapid and traditional antidepressants (Li et al., 2010). Considering that no published reports have indicated the antagonistic role of the NMDA receptor by naringenin, this observation indicated that naringenin did not produce a rapid antidepressant-like effect, indicating that acute naringenin treatment cannot increase BDNF levels.

To further determine which BDNF signaling pathway was necessary for the antidepressant-like effect of naringenin, the mice were co-injected with naringenin (20 mg/kg) and K252a (25 μg/kg), a potent pharmacological inhibitor of the BDNF receptor TrkB, for 3 weeks. Blockade of BDNF–TrkB signaling by the tyrosine kinase inhibitor K252a abolished the antidepressant-like effects of BDNF, lamotrigine, Fuzi polysaccharide-1 and ginsenoside Rg1 in animal models of depression (Jiang et al., 2012; Li et al., 2011; Shirayama et al., 2002; Yan et al., 2010). Our current results also found that K252a abolished the behavioral effects of naringenin in the SPT and NSFT exposed to CUMS. Considering that BDNF signaling play a critical role in the modulation of several functions, such as neurotransmitter release and postsynaptic responses to neurotransmitters, which are closely related to clinical antidepressant therapy (Li and Keifer, 2012), our current findings revealed that maintenance of the activated BDNF signaling pathway is required for the antidepressant-like effect of naringenin.

The relationship between monoamines and neurotrophins may be bidirectional. BDNF modulates the serotonergic system and promotes the growth and sprouting of the serotoninergic neuron (Mamounas et al., 1995; Quesseveur et al., 2013). Conversely, an increase in BDNF may result from the increased release of serotonin and/or norepinephrine, which may be a key mechanism underlying the therapeutic effects of antidepressants (Martinowich and Lu, 2008). Serotonergic and noradrenergic antidepressants can enhance BDNF expression (Masana et al., 2012; Zhang et al., 2010). In addition, antidepressants may induce the transcription of the BDNF gene via serotonergic and noradrenergic receptor-mediated signaling (Szewczyk et al., 2008; Wang et al., 2010). Moreover, we had previously demonstrated that naringenin might normalize the elevated corticosterone and decreased glucocorticoid receptor levels, thereby subsequently increase monoamine levels by stimulating monoamine synthesizing enzymes (Yi et al., 2012). Thus, altogether, these data might indicate that naringenin treatment can suppress neuroendocrine signaling and stimulate monoamines, which result in the up-regulation of BDNF. However, further studies are still required to examine the potential relationship between BDNF and the monoaminergic systems as well as their involvement in the antidepressant-like effect of naringenin.

5. Conclusion

In summary, our data provide new information with regard to the ability of naringenin to regulate BDNF expression following chronic administration. Although additional studies are required to further investigate in detail the BDNF downstream signaling pathway. In addition, the ability of naringenin to activate the BDNF signaling pathway in the hippocampus may, at least in part, explain the amelioration of behaviors associated with depression.

Contributors

Author L.-T. Yi designed the study. B.-B. Liu and J. Li performed the experiment (RT-PCR and ELISA) and article preparation. Y. Tang, Y. Xia and D. Wu participated in the research (CUMS, SPT and NSFT). B.-B. Liu and L. Luo acquired the data, which L. Liu and D. Geng analyzed. L.-T. Yi wrote the first draft of the manuscript, which all other authors reviewed. All the authors approved publication.

Acknowledgments

The project was supported by grants from the National Natural Science Foundation of China (No. 81202940).

References

Angelucci F, Brene S, Mathe AA. BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatry 2005;10:345–52.

Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, et al. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 2011;475:91–5.

Balu DT, Hoshaw BA, Malberg JE, Rosenzweig-Lipson S, Schechter LE, Lucki I. Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. Brain Res 2008;1211:37–43.

Baluchnejadmojarad T, Roghani M. Effect of naringenin on intracerebroventricular streptozotocin-induced cognitive deficits in rat: a behavioral analysis. Pharmacology 2006;78:193–7.

Barnes PM, Bloom B, Nahin RL. Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 2008;12:1–23.

Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 2006;7:137–51.

Bessa JM, Mesquita AR, Oliveira M, Pêgo JM, Cerqueira JJ, Palha JA, et al. A trans-dimensional approach to the behavioral aspects of depression. Front Behav Neurosci 2009;3:1.

Castren E, Rantamaki T. The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity. Dev Neurobiol 2010;70:289–97.

De Foubert G, Carney SL, Robinson CS, Destexhe EJ, Tomlinson R, Hicks CA, et al. Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. Neuroscience 2004;128:597–604.

Dwivedi Y, Rizavi HS, Pandey GN. Antidepressants reverse corticosterone-mediated decrease in brain-derived neurotrophic factor expression: differential regulation of specific exons by antidepressants and corticosterone. Neuroscience 2006;139:1017–29.

Dwyer AV, Whitten DL, Hawrelak JA. Herbal medicines, other than St. John's Wort, in the treatment of depression: a systematic review. Altern Med Rev 2011;16:40–9.

Gordon JA, Hen R. Genetic approaches to the study of anxiety. Annu Rev Neurosci 2004;27:193–222.

Hill MN, Hellemans KG, Verma P, Gorzalka BB, Weinberg J. Neurobiology of chronic mild stress: parallels to major depression. Neurosci Biobehav Rev 2012;36:2085–117.

Hunt KJ, Coelho HF, Wider B, Perry R, Hung SK, Terry R, et al. Complementary and alternative medicine use in England: results from a national survey. Int J Clin Pract 2010;64:1496–502.

Iijima M, Fukumoto K, Chaki S. Acute and sustained effects of a metabotropic glutamate 5 receptor antagonist in the novelty-suppressed feeding test. Behav Brain Res 2012;235:287–92.

Jiang B, Xiong Z, Yang J, Wang W, Wang Y, Hu ZL, et al. Antidepressant-like effects of ginsenoside Rg1 are due to activation of the BDNF signalling pathway and neurogenesis in the hippocampus. Br J Pharmacol 2012;166:1872–87.

Lee BH, Kim YK. The roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. Psychiatry Investig 2010;7:231–5.

Li W, Keifer J. Rapid enrichment of presynaptic protein in boutons undergoing classical conditioning is mediated by brain-derived neurotrophic factor. Neuroscience 2012;203:50–8.

Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 2010;329:959–64.

Li N, He X, Zhang Y, Qi X, Li H, Zhu X, et al. Brain-derived neurotrophic factor signalling mediates antidepressant effects of lamotrigine. Int J Neuropsychopharmacol 2011;14:1091–8.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

Luo KR, Hong CJ, Liou YJ, Hou SJ, Huang YH, Tsai SJ. Differential regulation of neurotrophin S100B and BDNF in two rat models of depression. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:1433–9.

Mamounas LA, Blue ME, Siuciak JA, Altar CA. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 1995;15:7929–39.

Mao QQ, Xian YF, Ip SP, Tsai SH, Che CT. Long-term treatment with peony glycosides reverses chronic unpredictable mild stress-induced depressive-like behavior via increasing expression of neurotrophins in rat brain. Behav Brain Res 2010;210:171–7.

Author's personal copy

Martinowich K, Lu B. Interaction between BDNF and serotonin: role in mood disorders. Neuropsychopharmacology 2008;33:73–83.
Masana M, Castane A, Santana N, Bortolozzi A, Artigas F. Noradrenergic antidepressants increase cortical dopamine: potential use in augmentation strategies. Neuropharmacology 2012;63:675–84.
Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, et al. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol Psychiatry 2007;61:187–97.
Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet 2007;370:851–8.
Muthaiah VP, Venkitasamy L, Michael FM, Chandrasekar K, Venkatachalam S. Neuroprotective role of naringenin on carbaryl induced neurotoxicity in mouse neuroblastoma cells. J Pharmacol Pharmacother 2013;4:192–7.
O'Leary OF, Wu X, Castren E. Chronic fluoxetine treatment increases expression of synaptic proteins in the hippocampus of the ovariectomized rat: role of BDNF signalling. Psychoneuroendocrinology 2009;34:367–81.
Olsen HT, Stafford GI, van Staden J, Christensen SB, Jäger AK. Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. J Ethnopharmacol 2008;117:500–2.
Quesseveur G, David DJ, Gaillard MC, Pla P, Wu MV, Nguyen HT, et al. BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities. Transl Psychiatry 2013;3:e253.
Quevedo J, Comim CM, Gavioli EC. Ketamine induces rapid onset of antidepressant action: neurophysiological biomarkers as predictors of effect. Biomark Med 2009;3:5–8.
Raza SS, Khan MM, Ahmad A, Ashafaq M, Islam F, Wagner AP, et al. Neuroprotective effect of naringenin is mediated through suppression of NF-κB signaling pathway in experimental stroke. Neuroscience 2013;230:157–71.
Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–9.
Schmidt HD, Duman RS. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 2010;35:2378–91.
Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002;22:3251–61.
Szewczyk B, Poleszak E, Sowa-Kucma M, Siwek M, Dudek D, Ryszewska-Pokrasniewicz B, et al. Antidepressant activity of zinc and magnesium in view of the current hypotheses of antidepressant action. Pharmacol Rep 2008;60:588–9.
Taliaz D, Stall N, Dar DE, Zangen A. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. Mol Psychiatry 2010;15:80–92.
Wang R, Li YH, Xu Y, Li YB, Wu HL, Guo H, et al. Curcumin produces neuroprotective effects via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3K cascades in rodent cortical neurons. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:147–53.
Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl) 1997;134:319–29.
Yan HC, Qu HD, Sun LR, Li SJ, Cao X, Fang YY, et al. Fuzi polysaccharide-1 produces antidepressant-like effects in mice. Int J Neuropsychopharmacol 2010;13:623–33.
Yi LT, Li CF, Zhan X, Cui CC, Xiao F, Zhou LP, et al. Involvement of monoaminergic system in the antidepressant-like effect of the flavonoid naringenin in mice. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:1223–8.
Yi LT, Li J, Li HC, Su DX, Quan XB, He XC, et al. Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test. Prog Neuropsychopharmacol Biol Psychiatry 2012;39:175–81.
Yi LT, Li J, Geng D, Liu BB, Fu Y, Tu JQ, et al. Essential oil of *Perilla frutescens*-induced change in hippocampal expression of brain-derived neurotrophic factor in chronic unpredictable mild stress in mice. J Ethnopharmacol 2013;147:245–53.
Zhang Y, Gu F, Chen J, Dong W. Chronic antidepressant administration alleviates frontal and hippocampal BDNF deficits in CUMS rat. Brain Res 2010;1366:141–8.