

EGb761 attenuates depressive-like behaviours induced by long-term light deprivation in C57BL/6J mice through inhibition of NF- κ B-IL-6 signalling pathway

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Abstract

Our previous investigation found that Ginkgo extract EGb761 could attenuate the depressive-like behaviours induced by a single injection of lipopolysaccharide in mice. However, it has not been investigated whether EGb761 is effective on depressive-like behaviours induced by long-term light deprivation and whether its effects are associated with the inhibition of NF- κ B-IL-6 signalling pathway. In this study, three groups (vehicle group, EGb761 low-dose group, and EGb761 high-dose group) of C57BL/6J male mice were exposed to constant darkness for four weeks. The control mice remained on a 12 : 12 light-dark cycle. Depressive-like behaviours were evaluated by tail suspension test (TST), forced swim test (FST), and sucrose preference test (SPT). Spontaneous locomotor activity was evaluated by open field test (OFT). Levels of IL-6, IL-6 mRNA, NF- κ B p65, phospho-NF- κ B p65, I κ B α , and phospho-I κ B α were measured using Elisa, western blotting, or PCR assays. NF- κ B p65 DNA binding activity was evaluated using Chemi Transcription Factor Assay Kit. Results showed long-term light deprivation prolonged the immobile time in TST and FST, shortened the latency to immobility in FST, reduced spontaneous locomotor activity in OFT, decreased sucrose preference in SPT, and increased levels of IL-6, IL-6 mRNA, NF- κ B p65, phospho-NF- κ B p65, and phospho-I κ B α in hippocampal tissue. EGb761 dose-dependently reversed the changes of the above parameters induced by long-term light deprivation, without affecting spontaneous locomotor activity. We conclude that EGb761 could attenuate the depressive-like behaviours and inhibit the NF- κ B-IL-6 signalling pathway in a light-deprivation-induced mouse model of depression.

Key words: EGb761, antidepressant-like activity, light deprivation-induced depression, NF- κ B-IL-6 signalling pathway.

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Introduction

Depression was the third leading cause of global burden of disease in 2004 and will move into first place by 2030, affecting about 20% of the population worldwide [1, 2]. Efforts have been made to find novel pharmacological agents with effective antidepressant-like efficacy, which target various pathogeneses of depression. The pathogenesis of depression is complex. Besides the genetic abnormality, environmental factors are believed to contribute to depression [3, 4]. Seasonal affective disorder is a specific form of recurrent depressive disorder that can be induced

by shortened light period [5]. Recently it has been reported that long-term exposure to constant darkness could induce depressive-like behaviour and change the expression of inflammatory cytokines in rodent animals [6, 7].

Inflammatory cytokines have been well demonstrated to play a role in depression [8-10]. Increased levels of IL-1 β , TNF- α , and IL-6 have been found in patients with depression [11, 12]. Preclinical studies also showed that some depression animal models (repeated stress-induced depression, lipopolysaccharide-induced depression, and so on) had increased levels of IL-1 β , TNF- α , and IL-6 [1, 13]. Treatments targeted at controlling these cytokines have ex-

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hibited therapeutic effects on depressive behaviours [14-16]. Anti-inflammation has been regarded as a new strategy in management of depression. Interestingly, different from the other types of depression animal models, a recent study revealed that long-term light-deprivation-induced depression model had increased serum levels of IL-6, but comparable levels IL-1 β and TNF- α compared to the control animals [6]. As is known, the production of IL-6 can be regulated by various pathways. In that study the authors also found that circulating and hippocampal IL-6 levels were dependent on the nuclear factor kappaB (NF- κ B) signalling pathway [6].

NF- κ B is a transcription factor that induces expression of cytokines that regulate the inflammatory cascade. NF- κ B signalling pathways are involved in neurological and psychotic disorders that are associated with inflammation [17, 18]. Koo *et al.* reported that NF- κ B was a critical mediator of stress-impaired depressive behaviour [19]. Regulating the expression of NF- κ B and inhibitory kappaB alpha (I κ B α) by some agents could attenuate the depression-like behaviours induced by chronic unpredictable stress [20]. Monje *et al.* also found blockage of NF- κ B ameliorated constant darkness-induced depression-like behaviour in mice [6]. Inactive NF- κ B in the cytoplasm is combined with I κ B. The activation of NF- κ B is initiated by the signal-induced degradation of I κ B proteins. The activated NF- κ B is rapidly translocated to the nucleus. In the nucleus, NF- κ B activates the transcription of target genes, leading to more production of some cytokines, such as IL-6, which is involved in the pathogenesis of depression. Considering of the role of NF- κ B-IL-6 signalling pathway in depression, inhibition of the NF- κ B-IL-6 signalling pathway might be a potential strategy in depression treatment.

Ginkgo extract EGb761 has exhibited anti-inflammatory, anti-oxidative, and neuroprotective activities in a number of preclinical studies [21, 22]. Some of its effects result from the inhibition of NF- κ B activity. Moreover, our previous study revealed that EGb761 attenuated depressive-like behaviours induced by LPS injection in C57BL/6J male mice, and in parallel, inhibited the expression of TNF- α , IL-1 β , IL-6, and IL-17A in the C57BL/6J mice with lipopolysaccharide injection [1]. However, as mentioned above, the long-term light deprivation-induced depression animal model has different immunological characteristics from the other depression models. For example, it has normal levels of TNF- α and IL-1 β . To our knowledge, it is not clear whether EGb761 also has antidepressant-like activities in the long-term light deprivation-induced depression model. Furthermore, it has not been investigated whether its effects are associated with inhibition of the NF- κ B-IL-6 signalling pathway. Thus, this study aimed to investigate the effects of EGb761 on the depressive-like behaviours and NF- κ B-IL-6 signalling pathway in the long-term light deprivation-induced mouse model of depression.

Material and methods

Animals

All experiments were performed using C57BL/6J male mice, which were housed in a temperature-controlled room (22 \pm 1 $^{\circ}$ C). Mice were either maintained on a 12 : 12 light-dark cycle or in 24 hours of constant darkness with *ad libitum* access to food and water unless stated otherwise. The research protocols were performed with the approval of the Committee on the Ethics of Animal Experiments of Shandong University. Animals were cared in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

Constant darkness procedure

Mice were divided into: control group, vehicle group, EGb761 low-dose group, and EGb761 high-dose group ($n = 16$ per group). Following a week of acclimatisation to the new environment under light-dark cycle conditions, mice in the vehicle group, the low-dose group, and the high-dose group were exposed to four weeks of constant darkness, while mice in the control group remained under light-dark cycle conditions.

EGb761 administration

EGb761 (Dr. Willmar Schwabe GmbH and Co. KG) was orally administrated to the EGb761 low-dose group (100 mg/kg/day) and the EGb761 high-dose group (150 mg/kg/day) for four weeks, starting from day 1 of the constant darkness procedure. Mice in the control group and the vehicle group received equal volumes of vehicle saline only.

Behavioural testing

Forced swim test (FST)

Testing was performed as we described previously [1]. During the test, mice were put in a cylinder (20 cm in diameter \times 25 cm tall) filled with 24 \pm 1 $^{\circ}$ C water (10 cm deep) for six minutes. Each mouse's FST behaviour in the container was digitally recorded for later analysis. The container was cleaned and the water was changed after every swim session. The FST behaviour of the last four minutes was analysed by two trained investigators blinded to treatment. Immobility was defined as no movement other than that necessary to keep the animal's head above water.

Tail suspension test

In the tail suspension test, the tail of the mouse was taped to a horizontal bar 50 cm above the floor. The six-minute suspension was videotaped using a camera. Immobility time in the final five minutes was measured by two trained investigators blinded to treatment.

Sucrose preference test

To quantify constant darkness-induced anhedonia, a common symptom of major depression, we subjected mice to a two-bottle sucrose preference test, which measures the preference for a sweetened solution over tap water. Prior to the test, mice were trained to consume sweetened solution by simultaneous presentation with a bottle of 1% sucrose solution and a bottle of tap water. Following the training, sucrose preference testing was conducted for 24 hours. The mice were allowed free access to the two bottles. Preference was calculated as sucrose solution consumed compared to the total fluid intake (sucrose intake/total fluid intake \times 100).

Open field test

Open field test was performed to evaluate the spontaneous locomotor activity of the experimental mice. The open field was a square arena (100 \times 100 \times 50 cm) divided into 25 equal squares. The mice were put in the central square of the clean arena and were permitted to freely explore the novel open arena for five minutes. Locomotor activity was digitally recorded for the rearing and crossing analysis.

Elisa assays

The left hippocampus was used to make tissue homogenate using a homogeniser ($n = 8$). Levels of IL-6 (Cusabio Company, Wuhan, China), NF- κ B p65 (Cusabio Company, Wuhan, China), and I κ B α (Jining Company, Shanghai, China) were measured using commercially available ELISA kits following the manufacturer's instructions. Samples were analysed in duplicate.

Western blot

The right hippocampus was lysed in RIPA lysis buffer containing 1 mM PMSF ($n = 8$). Protein samples (40 μ g) extracted from the hippocampus were separated by 12% SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 3% bovine serum albumin at room temperature for one hour and incubated in primary antibody solutions (1 : 1000) (rabbit anti-mouse phospho-NF- κ B p65 and phospho-I κ B α : Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. Then the membranes were washed three times and incubated in HRP-conjugated secondary antibody solution (1 : 1000) (donkey anti-rabbit IgG: Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for two hours at room temperature. Enhanced chemiluminescence was used to visualise the protein bands.

NF- κ B p65 DNA Binding Activity Assay

TranAM™ NF- κ B p65 Chemi transcription factor assay kit was used (Active Motif, Carlsbad, CA, USA) to detect NF- κ B p65 DNA-binding activity, following the in-

structions of the manufacturer ($n = 8$). Briefly, nuclear protein was extracted from the hippocampus using a nuclear extraction kit (Active Motif, Carlsbad, CA, USA). Nuclear protein (10 μ g) was incubated in plates that were coated with oligonucleotides containing a consensus binding site for p65. Following incubations with primary antibody against NF- κ B p65 and horseradish peroxidase-conjugated secondary antibody, the substrate solution was added to the wells in the dark. After the chromogenic reaction with substrate solution, stop solution was added to the wells to stop the reaction. The absorbance at 450 nm was measured to determine the NF- κ B p65 DNA-binding activity.

IL-6 mRNA detection

Total RNA was isolated from the hippocampal tissue samples using a RNeasy Mini kit (Qiagen, Hilden, Germany, according to the manufacturer's instruction) ($n = 8$). cDNA was synthesised using a RevertAid™ first-strand cDNA synthesis kit (Thermo Scientific Inc, Wilmington, DE, USA). cDNA was amplified using PCR assay with the mouse primers: IL-6: forward: 5'-GCTAC-CAAACCTG-GATATAATCAGGA-3', reverse: 5'-CCAG-GTAGCTATGGTACTCCAGAA-3'; β -actin: forward: 5' ATCATGTTTGAGACCTTCAACA 3', reverse: 5' CATCTCCTGCTCGAAGTCTA 3'. The amplification was performed using the following protocol: 50°C (2 min), 95°C (5 min), followed by 50 cycles at 95°C (15 sec) and 60°C (30 sec).

Statistical analysis

All results of the investigations were presented as mean \pm SEM. Differences in mean values between groups were assessed by one-way analysis of variance (ANOVA) followed by Students-Newman-Keuls (SNK) test. $P < 0.05$ was considered to be statistically significant.

Results

Behavioural investigation

Forced swim test

Forced swim test (FST) was performed to evaluate the depressive-like behaviour of the mice. Mice from the vehicle group performed poorly in the cylinder, with more immobility time and shorter latency to immobility than the mice from the control group (both $p < 0.05$). Compared to the vehicle group, both the low-dose group and the high-dose group spent less immobility time and had longer latency to immobility in the cylinder (all $p < 0.05$), and the high-dose group showed even less immobility time and longer latency to immobility than the low-dose group (both $p < 0.05$) (Figs. 1 and 2).

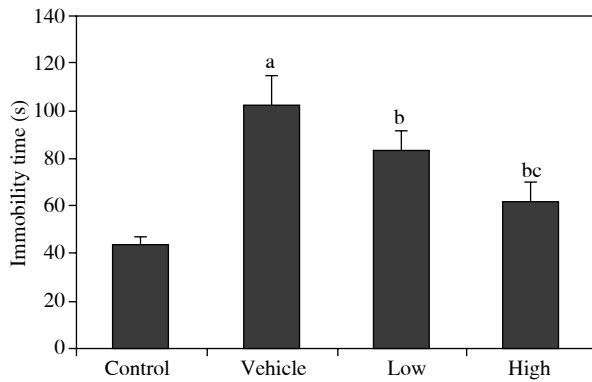


Fig. 1. Effects of EGb761 on immobility time in forced swim test (FST)

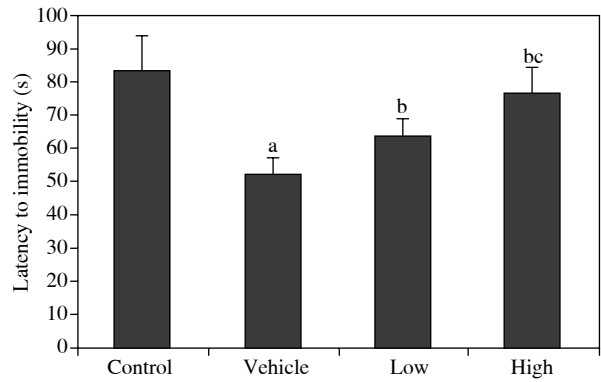


Fig. 2. Effects of EGb761 on latency to immobility in forced swim test (FST)

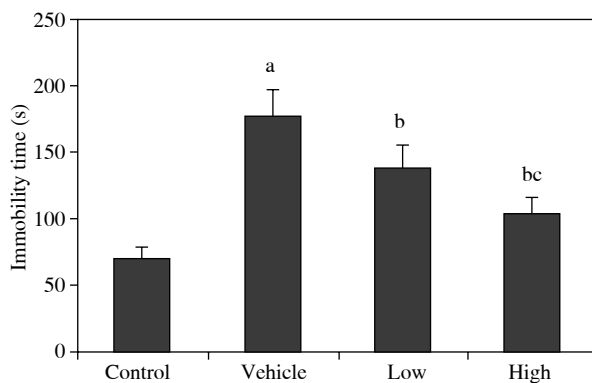


Fig. 3. Effects of EGb761 on immobility time in tail suspension test (TST)

Tail suspension test

The depressive-like behaviour of the mice was also evaluated by tail suspension test (TST). Mice from the vehicle group had longer immobility time than the mice from the control group ($p < 0.05$). Both the low-dose group and the high-dose group showed shorter immobility time than the vehicle group (both $p < 0.05$); the immobility time of the high-dose group was even shorter than that of the low-dose group ($p < 0.05$) (Fig. 3).

Locomotor activity in the open field test

Locomotor activity of the mice was evaluated by open field test (OPT). Light deprivation reduced the number of rearings and crossings of the vehicle group compared to the control group (both $p < 0.05$). The low-dose group and the high-dose group had comparable rearings and crossings with the vehicle group (both $p > 0.05$) (Fig. 4).

Sucrose preference test

The anhedonia of the mice was evaluated by sucrose preference test (SPT). During the test, mice from the vehicle group consumed much lower volume of sweetened

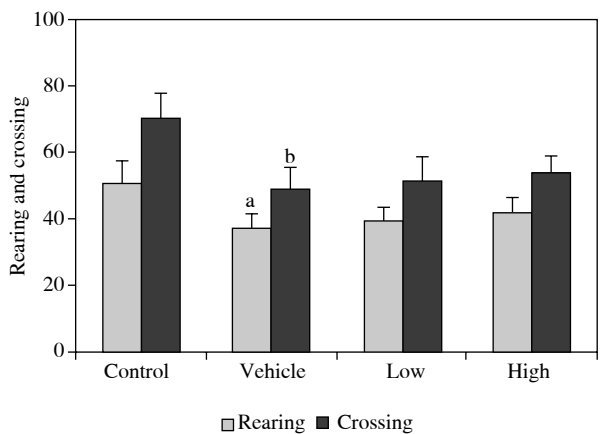


Fig. 4. Effects of EGb761 on spontaneous locomotor activity

solution than the control mice ($p < 0.05$). Mice from the low-dose group and the high-dose group consumed a higher volume of sweetened solution than mice from the vehicle group (both $p < 0.05$). The high-dose group consumed even more sweetened solution than the low-dose group ($p < 0.05$) (Fig. 5).

Levels of NF-κB p65, phospho-NF-κB p65, IκBα, and phospho-IκBα

Elisa or western assays showed that the vehicle group had higher levels of NF-κB p65, phospho-NF-κB p65, and phospho-IκBα than the control group, the low-dose group, and the high-dose group (all $p < 0.05$). The high-dose group had lower levels of those parameters than the low-dose group (all $p < 0.05$). All the groups had comparable levels of IκBα ($p > 0.05$) (Figs. 6-9).

NF-κB p65 DNA binding activity

Light deprivation increased the NF-κB p65 DNA binding activity of the vehicle group compared to the control

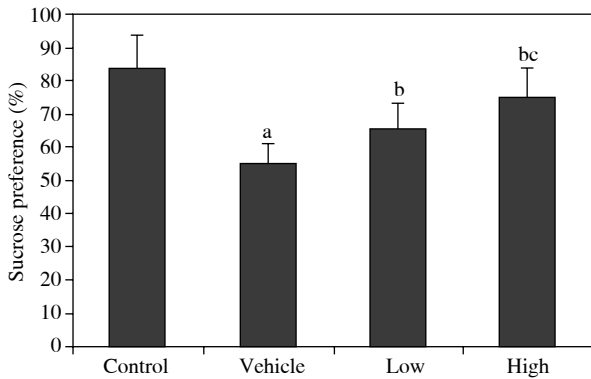


Fig. 5. Effects of EGb761 on sucrose preference in sucrose preference test (SPT)

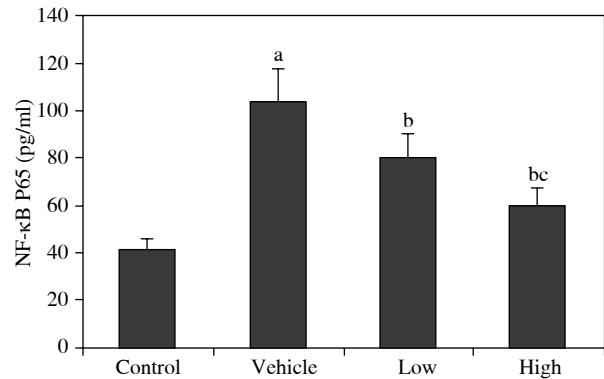


Fig. 6. Effects of EGb761 on NF-κB p65 levels in hippocampus

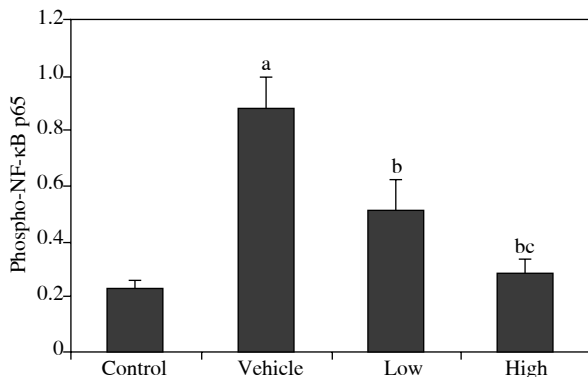
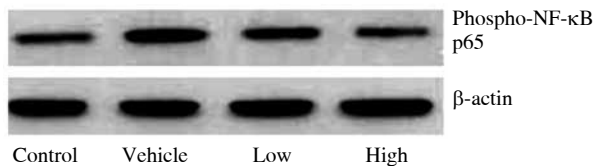


Fig. 7. Effects of EGb761 on phospho-NF-κB p65 levels in hippocampus

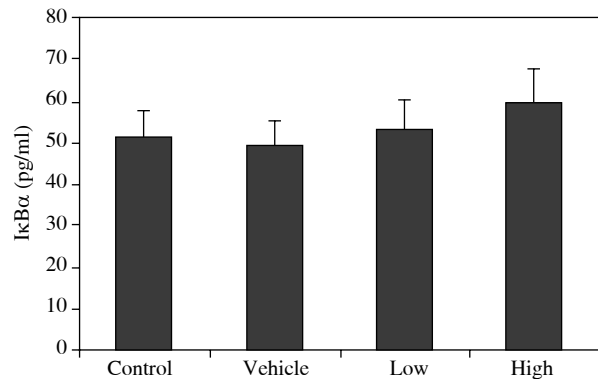


Fig. 8. Effects of EGb761 on IκBα levels in hippocampus

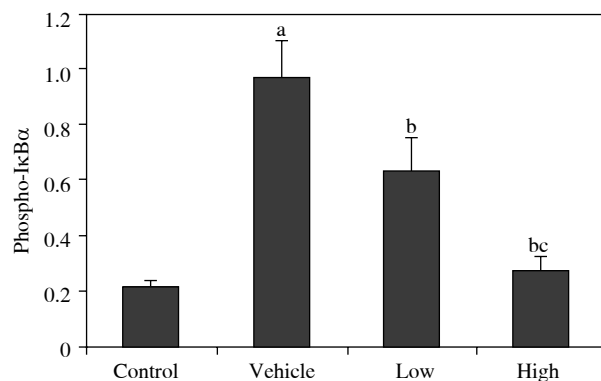
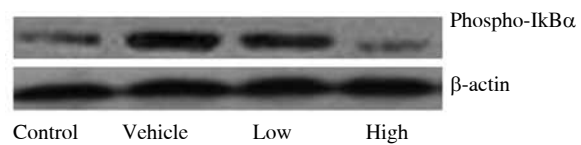


Fig. 9. Effects of EGb761 on phospho-IκBα levels in hippocampus

group ($p < 0.05$). The low-dose group and the high-dose group had lower NF-κB p65 DNA binding activity than the vehicle group (both $p < 0.05$). The activity of the high-dose group was even lower than the low-dose group ($p < 0.05$) (Fig. 10).

Levels of IL-6 and IL-6 mRNA

Levels of IL-6 and IL-6 mRNA of the vehicle group were higher than the control group, the low-dose group, and the high-dose group (all $p < 0.05$). Their levels in the high-dose group were lower than in the low-dose group (both $p < 0.05$) (Figs. 11 and 12).

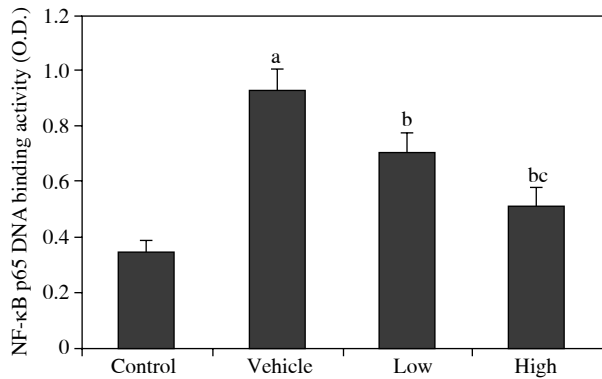


Fig. 10. Effect of EGb761 on NF- κ B p65 DNA binding activity

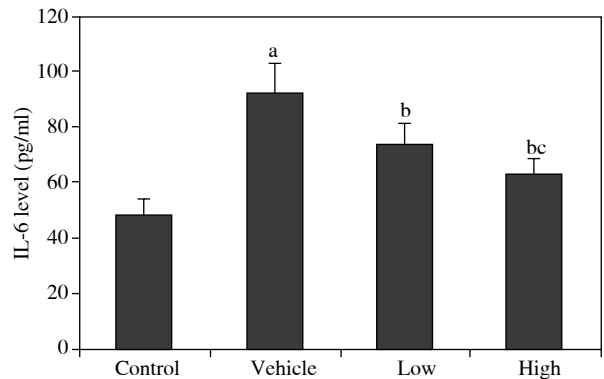


Fig. 11. Effect of EGb761 on IL-6 levels in hippocampus

Discussion

Our main findings of this work are that EGb761 can attenuate the depressive-like behaviours and inhibit the NF- κ B-IL-6 signalling pathway in C57BL/6J mice with light deprivation.

Various factors including environmental stimuli contribute to the development and progression of depression. Some species present physiological and behavioural changes in response to seasonal changes in day length [23]. Although human beings are less affected by the changes in day length than the other species, some people still have seasonal affective disorder characterised by major depression episodes in the autumn and winter [24-26]. Preclinical studies have demonstrated that long-term exposure to constant darkness could induce depression-like behaviour in rodent animals and such a depression animal model had different immunological characteristics from other stimuli-induced depression [6], which might result in different response to some specific medications.

In order to obtain optimal therapeutic effect, people are trying to find novel antidepressant agents specific to the different pathogenesis of depression. Some traditional Chinese medicine and compounds extracted from them have been used to manage depression in preclinical and clinical studies [27-29]. Ginkgo extract EGb761 has anti-inflammatory, anti-oxidative, and neuroprotective activities [21, 22]. A recent study showed that EGb761 could decrease the immobility time of BALB/c mice in the forced swimming test [30]. Our previous study and Yeh *et al.* study found that EGb761 attenuated the depressive-like behaviours induced by lipopolysaccharide injection [1, 31]. However, it has not been studied whether EGb761 has antidepressant-like activities in the light deprivation-induced animal model of depression, which has unique immunological features. In order to observe EGb761's effects on light deprivation-induced depression, we exposed mice to constant darkness conditions for four weeks and evaluated

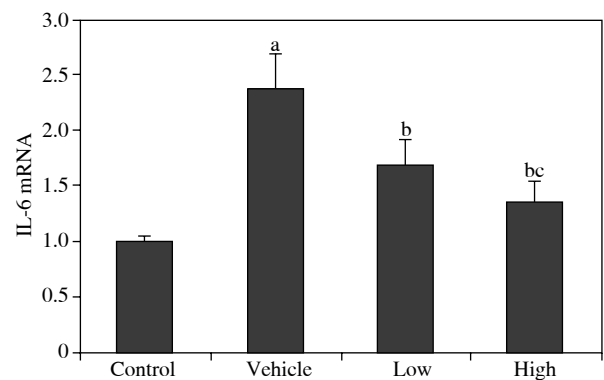


Fig. 12. Effects of EGb761 on IL-6 mRNA levels in hippocampus

the animal's behaviours. FST is widely used to screen the depressive-like behaviour and evaluate the antidepressant efficacy in animals. In our FST, mice from the vehicle group performed poorly in the cylinder, with more immobility time and shorter latency to immobility than the mice from the control group, indicating that light deprivation induced depressive-like behaviour in mice. In the EGb761 treated groups, EGb761 dose-dependently reduced the immobility time and prolonged the latency to immobility compared to the vehicle group, indicating that EGb761 could dose-dependently attenuate the depressive-like behaviour induced by light deprivation. The TST evaluation also showed that light deprivation induced depressive-like behaviour in mice, and EGb761 could dose-dependently attenuate the depressive-like behaviour. However, locomotor activity can influence the performance of the animals in behavioural tests, which may lead to false positive results in the FST and TST. We introduced OPT to evaluate the locomotor activity of the mice. The result showed that light deprivation reduced the locomotor activity of the mice,

but each of the three groups exposed to constant darkness had comparable locomotor activity. The results meant that there were no false positive results resulting from the locomotor activity in the FST and TST. Besides, we also evaluated the anhedonia of the mice by SPT. Light deprivation induced significant anhedonia in mice, and EGb761 dose-dependently attenuated the light-deprivation-induced anhedonia. All the behavioural evaluations demonstrated that light deprivation could induce depressive-like behaviours in mice and EGb761 could attenuate the depressive-like behaviours induced by light deprivation.

There is a great deal of evidence from preclinical and clinical studies demonstrating that NF- κ B signalling pathways are involved in the pathogenesis of depression [17, 19, 32, 33]. Depression animal models induced by unpredictable chronic mild stress, lipopolysaccharide injection, or other stimuli have been found to exhibit increased NF- κ B activity, and some agents that inhibit NF- κ B activity have exhibited antidepressant activity in such animal models [34-37]. A recent study found that mice exposed to constant darkness also have higher NF- κ B DNA binding activity, and pharmacological blockers of the NF- κ B pathway attenuated the depressive-like behaviours induced by light deprivation [6]. Similarly, we also found that the mice in the vehicle group had increased NF- κ B p65 DNA binding activity in hippocampal tissue in the present study. Inactive NF- κ B dimers are retained into the cytoplasm by interaction with I κ B [38]. The phosphorylation of I κ B α leads to the activation of NF- κ B. Following activation, NF- κ B p65/p50 heterodimer is translocated to the nucleus, where it promotes the transcription of target genes. Besides determining the NF- κ B p65 DNA binding activity of the mice, we also measured levels of NF- κ B p65, phospho-NF- κ B p65, I κ B α , and phospho-I κ B α in this study. Our results showed the mice in the vehicle group had increased levels of NF- κ B p65, phospho-NF- κ B p65, and phospho-I κ B α , as well as comparable level of I κ B α , if compared to the control groups. The data suggest that light deprivation promoted the production of NF- κ B p65 and induced the activation of NF- κ B p65 by accelerating the degradation of I κ B α through phosphorylation. However, EGb761 dose-dependently reversed the light deprivation-induced changes of NF- κ B p65, phospho-NF- κ B p65, phospho-I κ B α , and NF- κ B p65 DNA binding activity, suggesting that EGb761 could inhibit the NF- κ B signalling in hippocampal tissue of the mice. The inhibition of NF- κ B signalling should contribute to the attenuation of the depressive-like behaviours.

As is known, the activated NF- κ B in the nucleus can promote the transcription of genes of some cytokines including IL-6. IL-6 has been well demonstrated to have a role in depression [39], and a targeted approach to selectively inhibit IL-6 trans-signalling may offer putative antidepressant effects [40]. In the present study, we found that light deprivation induced increased production of IL-6 and

IL-6 mRNA. The result was in line with the recent study reporting that mice exposed to constant darkness had higher levels of IL-6, but comparable levels of TNF- α and IL-1 β than the control animals. However, in contrast to the light deprivation mouse model of depression, some studies, including our previous work, found some other depression animal models had increased levels of TNF- α , IL-1 β , and IL-6 [1, 13, 14]. Our present study also found that EGb761 could dose-dependently decrease IL-6 and IL-6 mRNA levels in the light-deprived mice. The reduction in production of IL-6 might contribute to the attenuation of the depressive-like behaviours.

Taken together, EGb761 could attenuate the depressive-like behaviours in mice exposed to light deprivation. The antidepressant-like activities might be associated with the inhibition of the NF- κ B-IL-6 signalling pathway.

The authors declare no conflict of interest.

References

- Zhao Y, Zhang Y, Pan F (2015): The effects of EGb761 on lipopolysaccharide-induced depressive-like behaviour in C57BL/6J mice. *Cent Eur J Immunol* 40: 11-17.
- Xue R, Jin ZL, Chen HX, et al. (2013): Antidepressant-like effects of 071031B, a novel serotonin and norepinephrine reuptake inhibitor. *Eur Neuropsychopharmacol* 23: 728-741.
- Anisman H, Matheson K (2005): Stress, depression, and anhedonia: caveats concerning animal models. *Neurosci Biobehav Rev* 29: 525-546.
- Krishnan V, Nestler EJ (2008): The molecular neurobiology of depression. *Nature* 455: 894-902.
- Jurvelin H, Takala T, Nissilä J, et al. (2014): Transcranial bright light treatment via the ear canals in seasonal affective disorder: a randomized, double-blind dose-response study. *BMC Psychiatry* 14: 288.
- Monje FJ, Cabatic M, Divisch I, et al. (2011): Constant darkness induces IL-6-dependent depression-like behavior through the NF- κ B signaling pathway. *J Neurosci* 31: 9075-9083.
- Gonzalez MM, Aston-Jones G (2008): Light deprivation damages monoamine neurons and produces a depressive behavioral phenotype in rats. *Proc Natl Acad Sci U S A* 105: 4898-4903.
- Kim JW, Szigethy EM, Melhem NM, et al. (2014): Inflammatory markers and the pathogenesis of pediatric depression and suicide: a systematic review of the literature. *J Clin Psychiatry* 75: 1242-1253.
- Slavich GM, Irwin MR (2014): From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull* 140: 774-815.
- Bai YM, Su TP, Li CT, et al. (2015): Comparison of pro-inflammatory cytokines among patients with bipolar disorder and unipolar depression and normal controls. *Bipolar Disord* 17: 269-277.
- Rethorst CD, Toups MS, Greer TL, et al. (2013): Pro-inflammatory cytokines as predictors of antidepressant effects of exercise in major depressive disorder. *Mol Psychiatry* 18: 1119-1124.

12. Liu Y, Ho RC, Mak A (2012): Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J Affect Disord* 139: 230-239.
13. You Z, Luo C, Zhang W, et al. (2011): Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: involvement in depression. *Behav Brain Res* 225: 135-141.
14. Ji WW, Li RP, Li M, et al. (2014): Antidepressant-like effect of essential oil of *Perilla frutescens* in a chronic, unpredictable, mild stress-induced depression model mice. *Chin J Nat Med* 12: 753-759.
15. Li D, Zhang N, Cao Y, et al. (2013): Emodin ameliorates lipopolysaccharide-induced mastitis in mice by inhibiting activation of NF-κB and MAPKs signal pathways. *Eur J Pharmacol* 705: 79-85.
16. Köhler O, Benros ME, Nordentoft M, et al. (2014): Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatry* 71: 1381-1391.
17. Bortolotto V, Cuccurazzu B, Canonico PL, et al. (2014): NF-κB mediated regulation of adult hippocampal neurogenesis: relevance to mood disorders and antidepressant activity. *Biomed Res Int* 2014: 612798.
18. Lanzillotta A, Porrini V, Bellucci A, et al. (2015): NF-κB in innate neuroprotection and age-related neurodegenerative diseases. *Front Neurol* 6: 98.
19. Koo JW, Russo SJ, Ferguson D, et al. (2010): Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. *Proc Natl Acad Sci U S A* 107: 2669-2674.
20. Quan W, Liu F, Zhang Y, et al. (2015): Antidepressant-like effects of magnesium lithospermate B in a rat model of chronic unpredictable stress. *Pharm Biol* 53: 1168-1175.
21. Montes P, Ruiz-Sanchez E, Rojas C, et al. (2015): Ginkgo biloba Extract 761: A Review of Basic Studies and Potential Clinical Use in Psychiatric Disorders. *CNS Neurol Disord Drug Targets* 14: 132-149.
22. Cao S, Gao M, Wang N, et al. (2014): Prevention of Selenite-Induced Cataratogenesis by Ginkgo biloba Extract (Egb761) in Wistar Rats. *Curr Eye Res* 7: 1-6.
23. Uzoma HN, Reeves GM, Langenberg P, et al. (2015): Light treatment for seasonal winter depression African-American vs Caucasian outpatients. *World J Psychiatry* 5: 138-146.
24. Danilenko KV, Ivanova IA (2015): Dawn simulation vs. bright light in seasonal affective disorder: Treatment effects and subjective preference. *J Affect Disord* 180: 87-89.
25. Rosenthal NE, Sack DA, Gillin JC, et al. (1984): Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. *Arch Gen Psychiatry* 41: 72-80.
26. Swiecicki L, Scinska A, Bzinkowska D, et al. (2015): Intensity and pleasantness of sucrose taste in patients with winter depression. *Nutr Neurosci* 18: 186-191.
27. Zhou K, Jia N, Jiang N, et al. (2015): Beneficial effect of Danggui-Shaoyao-San, a traditional Chinese medicine, on drowsiness induced by chronic restraint stress. *Neurosci Lett* 597: 26-31.
28. Yang J, Jiang L, Li Y, et al. (2014): Clinical treatment of depressive patients with anshendingzhi decoction. *J Tradit Chin Med* 34: 652-656.
29. Deng XY, Li HY, Chen JJ, et al. (2015): Thymol produces an antidepressant-like effect in a chronic unpredictable mild stress model of depression in mice. *Behav Brain Res* 291: 12-19.
30. Rojas P, Serrano-García N, Medina-Campos ON, et al. (2011): Antidepressant-like effect of a Ginkgo biloba extract (EGB761) in the mouse forced swimming test: role of oxidative stress. *Neurochem Int* 59: 628-636.
31. Yeh KY, Shou SS, Lin YX, et al. (2015): Effect of Ginkgo biloba extract on lipopolysaccharide-induced anhedonic depressive-like behavior in male rats. *Phytother Res* 29: 260-266.
32. Pace TW, Mletzko TC, Alagbe O, et al. (2006): Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am J Psychiatry* 163: 1630-1633.
33. Xu Y, Sheng H, Tang Z, et al. (2015): Inflammation and increased IDO in hippocampus contribute to depression-like behavior induced by estrogen deficiency. *Behav Brain Res* 288: 71-78.
34. Li R, Zhao D, Qu R, et al. (2015): The effects of apigenin on lipopolysaccharide-induced depressive-like behavior in mice. *Neurosci Lett* 594: 17-22.
35. Liu B, Xu C, Wu X, et al. (2015): Icaritin exerts an antidepressant effect in an unpredictable chronic mild stress model of depression in rats and is associated with the regulation of hippocampal neuroinflammation. *Neuroscience* 294: 193-205.
36. Wang Z, Zhang Q, Yuan L, et al. (2014): The effects of curcumin on depressive-like behavior in mice after lipopolysaccharide administration. *Behav Brain Res* 274: 282-290.
37. Wang H, Zhang R, Qiao Y, et al. (2014): Gastrodin ameliorates depression-like behaviors and up-regulates proliferation of hippocampal-derived neural stem cells in rats: involvement of its anti-inflammatory action. *Behav Brain Res* 266: 153-160.
38. Jacobs MD, Harrison SC (1998): Structure of an Ikappa-Balpha/NF-kappaB complex. *Cell* 95: 749-758.
39. Virtanen M, Shipley MJ, Batty GD, et al. (2015): Interleukin-6 as a predictor of symptom resolution in psychological distress: a cohort study. *Psychol Med* 20: 1-8.
40. Fonseka TM, McIntyre RS, Soczynska JK, et al. (2015): Novel investigational drugs targeting IL-6 signaling for the treatment of depression. *Expert Opin Investig Drugs* 24: 459-475.