Chelidonic acid evokes antidepressant-like effect through the up-regulation of BDNF in forced swimming test

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Abstract

Depression is usually accompanied by neuro-inflammatory reactions. Chelidonic acid, in particular, has shown anti-inflammatory effects. The objective of this study was to evaluate the anti-depressant effects of chelidonic acid and to discuss the potential mechanisms of a forced swimming test. Chelidonic acid was administered orally once a day for 14 days. On the 14th day, chelidonic acid resulted in a significant decrease in immobility time during the forced swimming test without alteration of locomotor activity, in an open field test. Chelidonic acid also increased the number of nissl bodies in the hippocampus. Brain-derived neurotrophic factor expression and extracellular signal-regulated protein kinase phosphorylation in the hippocampus were up-regulated by the administration of chelidonic acid. Chelidonic acid administration significantly increased the mRNA expression of hippocampal estrogen receptor- β . The levels of hippocampal interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α were effectively attenuated by the administration of chelidonic acid. In addition, chelidonic acid significantly increased the levels of 5-hydroxytryptamine (serotonin), dopamine, and norepinephrine compared with those levels for the mice that were administered distilled water in the hippocampus. These results suggest that chelidonic acid might serve as a new therapeutic strategy for the regulation of depression associated with inflammation.

Keywords: Depression, chelidonic acid, brain-derived neurotrophic factor, estrogen receptor- β , serotonin

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Introduction

Depression is characterized by hopelessness, inappropriate guilt, thoughts of death or suicide, and low self-esteem.¹ Depression is also associated with neuroplasticity, neurotransmitter imbalance, neurodegeneration, and inflammation by a reduced brain-derived neurotrophic factor (BDNF), increased inflammatory mediators, and altered hypothalamic–pituitary–adrenal axis activity.^{2,3} Depression is a condition characterized by impairment of hippocampal neurogenesis and administration of antidepressants restores neurogenesis.⁴ Although antidepressants are generally safe and effective, patients with depression need to continue antidepressant medication for a long time to see clinical benefit and side effects are still a problem.⁵ Therefore, it is necessary to create new antidepressant agents that are faster acting, safer, and that have greater levels of efficacy.

BDNF is a growth factor of neuron cells that up-regulates neurogenesis by increasing cell survival and synaptic functions in nervous systems.⁶ The activation of extracellular signal-regulated protein kinase (ERK) induced by the interaction of BDNF and TrkB receptors conduces the neuroprotection against neuron cell death.⁷ In addition to BDNF, estradiol regulates neurotransmission in the hippocampus and induces BDNF expression through the activation of ERK.⁸ However, the blockage of the BDNF signal pathway by proinflammatory cytokines decreases neurogenesis and neurotrophin activity.⁹ Depressed patients exhibit increased interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α in the brain and blood.^{9,10} Results from previous studies have shown that the expression of BDNF was increased by antidepressant drugs in the brain.^{10,11}

Brain 5-hydroxytryptamine (5-HT, serotonin), dopamine, and norepinephrine (NE) are monoamine neurotransmitters

and have important roles in memory and learning.¹² Deficiencies or imbalances of 5-HT, dopamine, and NE can lead to depression by blocking the normal functioning of nerves.¹³

Chelidonium majus L. is a useful homeopathic stress medicine and has an anti-inflammatory effect.¹⁴ Chelidonic acid (CA) is a heterocyclic organic acid with a pyran skeleton and a component of *Chelidonium majus* L. It has neurological sedative, mild analgesic, anti-allergic, and anti-ulcerative colitis effects.^{15–17} Previously, we have reported that CA has an anti-inflammatory effect.¹⁸ However, the effect of CA and its mechanisms in depression have not yet been examined. Herein, we evaluated the anti-depressant effect of CA in a forced swimming test (FST), which is a useful screening test for antidepressant drugs.¹⁹

Materials and methods

Animals

Male ICR mice (three weeks old, 10–12 g) were purchased from the Dae-Han Experimental Animal Center (Daejeon, Korea) and subsequently maintained at the College of Korean Medicine, Kyung Hee University. Experiments were initiated after one week to allow for adaption to the laboratory environment. Six animals were per cage (294 mm × 190 mm × 125 mm) in a laminar air-flow room maintained at a temperature of 22 ± 1°C with a relative humidity of 55% ± 10% under a 12:12 L/D cycle light on at 07:00 h throughout the study. Food and water were available *ad libitum*. All manipulations were carried out between 09:00 and 16:00 h, and no animal was used more than once. All protocols were approved by the institutional animal care and use committee of Kyung Hee University (KHUASP(SE)-10-032).

Forced swimming test

After the first measurement at an immobile time, the mice were divided into a distilled water (DW, control) group, fluoxetine (20 mg/kg) group, and CA (0.02, 0.2, and 2 mg/kg) group based on the recorded swimming times. CA (purity: 98%) and fluoxetine were purchased from Sigma (St. Louis, MO, USA). We selected the concentrations of CA (0.02, 0.2, and 2 mg/kg) in accordance with previous reports¹⁸ and CA was dissolved in DW. Depression is a chronic disorder that requires chronic treatment and fluoxetine reduced the immobility time in the chronic FST (14 days).²⁰ Fluoxetine powder was dissolved in DW (20 mg/kg) and used as a positive control. CA (0.02, 0.2, and 2 mg/kg) was orally administered to mice once a day for two weeks to examine the effects of chronic CA treatment. On the 14th day of chronic CA, fluoxetine, or DW administration, the FST was performed. CA, fluoxetine and DW were administered 1 h prior to the FST. DW-administered mice were maintained under the same conditions as those used for the experimental animals. During the 6 min long FST, the duration of immobility was measured as previously described by Porsolt et al.¹⁹ The apparatus consisted of two Plexiglas cylinders (height: 25 cm, diameter: 10 cm) placed side by side in a Makrolon cage filled

with water (10 cm height) at 23–25°C. Two mice were tested simultaneously for a 6-min period inside the vertical Plexiglas cylinders; a nontransparent screen placed between the two cylinders prevented the mice from seeing each other. The total duration of immobility, after a delay of 2 min, was measured for a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The FST was recorded with a Canon Camcorder. The immobility times were measured with a stopwatch by a trained observer who was blind to the experimental treatments. There were six mice in each group.

Open field test

The open field test (OFT) was conducted in clear black Plexiglas boxes (40 cm × 40 cm × 40 cm) equipped with the video-based Ethovision System (Noldus, Wageningen, The Netherlands), as described previously.²¹ Mice were initially placed in the corner of the apparatus, and total distance moved was recorded for 10 min. The horizontal locomotor activity is expressed in terms of the total ambulatory distance (cm). Each box was cleaned with 70% ethanol between subjects. There were five mice in each group.

Nissl staining

The mice were perfused with 4% paraformaldehyde after being anesthetized. The coronal brain sections were serially cut after the brains were postfixed in the same fixative. To ensure hippocampal sections were matched between groups, we used anatomical landmarks provided by the brain atlas. The selected brain sections were stained with cresyl violet (nissl) and examined with a microscope to count nissl body numbers in the dentate gyrus (DG) regions of the hippocampus. Sections were coded and randomly analyzed by two blinded observers.

Western blot analysis

The hippocampus plays an important role in the pathophysiology of depression.⁴ To evaluate the anti-depressant effects of CA, hippocampus tissues were homogenized with homogenization buffer (20 mM HEPES pH 7.5, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.1 M NaCl, 0.2 mM DTT) in a motor-driven homogenizer. Samples were heated at 95°C for 5 min and briefly cooled on ice. After the centrifugation, 50 µg aliquots were resolved by 12% SDS-polyacrylamide gel electrophoresis. The resolved proteins were electrotransferred overnight to nitrocellulose membranes in 25 mM Tris, pH 8.5, 200 mM glycerol, and 20% methanol at 25 V. Blots were blocked for at least 2 h with 1 × PBS containing 0.05% Tween 20 containing 5% nonfat dry milk and then incubated with BDNF, actin, ERK, and phosphorylated-ERK (pERK) antibodies (Santa Cruz, CA, USA) for 1 h at room temperature. Blots were developed using peroxidase-conjugated secondary antibodies, and proteins were visualized by enhanced chemiluminescence

procedures (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions.

Immunohistochemistry

After FST, brains were removed, post-fixed in 4% paraformaldehyde overnight, placed in 30% sucrose in PBS for three days, removed from sucrose, and frozen at -70°C . Tissues were cut on a cryostat. After rinsing in PBS, sections were incubated in 1% H_2O_2 in PBS for 10 min, followed by 3×10 min PBS washes. Sections were blocked with bovine serum albumin and then incubated for 60 min with an anti-BDNF antibody (Santa Cruz, CA, USA) at a concentration of $1\text{ }\mu\text{g/mL}$. Each section was then reacted with 3, 3'-diaminobenzidine substrate solution for 10 min at room temperature. Residual DAB was washed off slices using three PBS washes. All specimens were coded, examined under a light microscope, and randomly analyzed by two blinded observers.

Reverse transcription-polymerase chain reaction

Total RNA was isolated from tissue according to the manufacturer's specifications using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Sungnam, Korea). The concentration of total RNA in the final elutes was determined by spectrophotometry. Total RNA ($2.5\text{ }\mu\text{g}$) was heated at 75°C for 5 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 60 min at 42°C using a cDNA synthesis kit (iNtRON Biotech, Sungnam, Korea). Polymerase chain reaction (PCR) was performed with the following primers for mouse estrogen receptor (ER)- β (5' ACA GTC CTG CTG TGA TGA AC 3'; 5' ACT AGT AAC AGG GCT GGC AC 3'), and mouse GAPDH (5' TTC ACC ACC ATG GAG AAG GC 3'; 5' GGC ATG GAC TGT GGT CAT GA 3') was used to verify whether equal amounts of RNA had been used for reverse transcription and PCR (RT-PCR) amplification under the different experimental conditions. The annealing temperature was 55°C for ER- β and 60°C for GAPDH. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

Quantitative real-time PCR

Quantitative real-time PCR was performed using an SYBR Green master mix, and the detection of mRNA was analyzed using an ABI StepOne real-time PCR System (Applied Biosystems, Foster City, CA, USA). We performed real-time with the following primers: IL-1 β (5' GGA CAG AAT ATC AAC CAA CAA GTG ATA 3'; 5' GTG TGC CGT CTT TCA TTA CAC AG 3'); IL-6 (5' CCA GAA ACC GCT ATG AAG TTC CT 3'; 5' CAC CAG CAT CAG TCC CAA GA 3'); TNF- α (5' CAG ACC CTC ACA CTC AGA TCA TCT 3'; 5' CCT CCA CTT GGT GGT TTG CTA 3'); GAPDH (5' GGC AAA TTC AAC GGC ACA 3'; 5' GTT AGT GGG GTC TCG CTC CTG 3'). The level of the target mRNA was normalized to the level of the GAPDH and compared with the levels of the control. All data were analyzed using the $\Delta\Delta\text{CT}$ method.

Enzyme-linked immunosorbent assay

Cytokines of hippocampus tissue were measured by enzyme-linked immunosorbent assay (ELISA). The ELISA was performed by coating 96-well plates with $1\text{ }\mu\text{g/well}$ of capture antibodies for IL-1 β , IL-6, and TNF- α (R&D Systems, Inc., Minneapolis, MN, USA). Before the subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween 20. All reagents and coated wells used in this assay were incubated for 2 h at room temperature. The standard curve was generated from known concentrations of cytokine, as provided by the manufacturer. After exposure to the medium, the assay plates were exposed sequentially to each of the biotin-conjugated secondary antibodies, and to avidin-peroxidase, and 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) substrate solution containing 30% H_2O_2 (Sigma Chemical Co., St Louis, MO, USA). The plates were read at 405 nm. Appropriate specificity controls were included, and all samples were run in duplicate. Cytokine level in the hippocampus was divided according to the total protein. The protein was estimated using the bicinchoninic acid method.

Monoamine neurotransmitters assay

Hippocampal 5-HT, dopamine, and NE levels were measured according to manufacturer specifications using the 5-HT assay Kit (MyBiosource, San Diego, CA, USA), dopamine assay Kit (Genway Biotech Inc., San Diego, CA, USA), and NE assay Kit (Labor Diagnostika Nord GmbH & Co.KG, Nordhorn, Germany), respectively.

Statistical analysis

Data were expressed as the mean $\pm$ SEM. Prior to analysis of variance (ANOVA) testing, all data were checked for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Statistical evaluation of the results was performed by one-way ANOVA, followed by Dunnett's test (or Dunnett's T3). A value of $P < 0.05$ was regarded as statistically significant.

Results

Effect of CA on immobility time and nissl bodies population in FST

The antidepressant effect of CA was investigated in the FST. On the 14th day, the immobility times of the DW, CA 0.02 mg/kg, CA 0.2 mg/kg, and CA 2 mg/kg groups in FST were 227.00 ± 5.19 , 172 ± 14.74 , 135.67 ± 26.35 , and 100.17 ± 25.03 sec, respectively. CA significantly abolished the immobility time in a dose-dependent manner (Figure 1(a), $P < 0.05$). Fluoxetine also significantly reduced the immobility time (Figure 1(a), $P < 0.05$). To determine whether CA exhibits any effect on locomotor activity, OFT was performed at the effective dose of 2 mg/kg. CA-administered mice were evaluated for total distance travelled. However, no statistically significant difference was observed between the DW and the CA-treated mice in the OFT (Figure 1(b)). We also investigated the effect of CA on

Figure 1 Effect of CA on FST immobility times and nissl body numbers in the hippocampus. (a) Immobility time at 14 days. Values are means $\pm$ SEM. (b) CA or fluoxetine were administered for two weeks and testing was conducted 1 h after final treatment. The locomotor activity of mice in the OFT were observed for 10 min. (c) Hippocampal tissues were stained with cresyl violet. Nissl bodies (arrow) in the dentate gyrus (DG) regions of the hippocampus. * $P < 0.05$ vs. the DW-administered control group. (Original magnification $\times 200$ and $\times 400$, scale bar = 50 μ m.) DW: distilled water. (A color version of this figure is available in the online journal.)

the nissl bodies of the hippocampus using the nissl staining method. Cresyl violet stained-nissl bodies were seen in the DG of the hippocampus in the DW- and CA-administered mice. However, the number of nissl bodies was higher in

the CA-administered mice than in the DW-administered mice (Figure 1(c)).

Effect of CA on hippocampal BDNF and pERK levels

To investigate the effect of CA on BDNF expression, we performed Western blot analysis for BDNF. As can be seen in Figure 2(a), compared with the administration of DW, the administration of CA increased the BDNF expression. Immunohistochemical analysis also revealed that CA increased the BDNF expression in the DG of the hippocampus (Figure 2(b)). We also analyzed the levels of pERK because BDNF has an antidepressant effect via the activation of ERK.⁷ As can be seen in Figure 2(c), CA or fluoxetine markedly increased the levels of pERK.

Effect of CA on hippocampal ER- β mRNA levels

To investigate whether the antidepressant effect of CA was induced through the activation of ER- β , we performed RT-PCR for hippocampal ER- β . We showed that CA significantly increased the levels of hippocampal ER- β (Figure 3, $P < 0.05$). The levels of hippocampal ER- β were found to increase approximately twice due to CA (2 mg/kg) administration compared with the level of DW-administered mice. Fluoxetine also showed a significant increase in the levels of hippocampal ER- β (Figure 3, $P < 0.05$).

Effect of CA on hippocampal inflammatory cytokine levels

Neuroinflammation often has a transition to chronicity and induces depressive conditions.⁹ To evaluate the regulatory effect of CA on inflammatory cytokines (IL-1 β , IL-6, and TNF- α) in the hippocampus, we analyzed the mRNA and protein levels of inflammatory cytokines using a real-time PCR and ELISA. As can be seen in Figure 4(a), CA significantly reduced the mRNA levels of IL-1 β , IL-6, and TNF- α in a dose-dependent manner ($P < 0.05$). For the levels of protein, CA significantly reduced the levels of TNF- α (Figure 4(b), $P < 0.05$) but CA did not cause any significant alteration in the protein levels of IL-1 β or IL-6 (Figure 4(b)).

Effect of CA on hippocampal monoamine neurotransmitters

Monoamine neurotransmitter has a pivotal role in the treatment of depression,^{12,13} and the levels of monoamine neurotransmitter in the hippocampus were increased by antidepressant drug treatment. To evaluate whether the antidepressant effect of CA is associated with biogenic amines, we analyzed the levels of hippocampal 5-HT, dopamine, and NE using 5-HT, dopamine, and NE assay kits. CA (0.2 mg/kg for 5-HT and 2 mg/kg for dopamine and NE) significantly increased the levels of hippocampal 5-HT, dopamine, and NE compared with those levels of the DW-administered mice (Figure 5, $P < 0.05$). Fluoxetine (20 mg/kg) significantly increased the levels of 5-HT, dopamine, and NE (Figure 5, $P < 0.05$).

Figure 2 Effect of CA on the levels of BDNF and pERK in the hippocampus. (a) The levels of BDNF were analyzed by Western blot analysis. (b) Hippocampal tissues were stained with DAB (for BDNF, arrow). (c) Phosphorylated ERK was analyzed by Western blot analysis. Protein levels were quantified by densitometry. Values are means $\pm$ SEM (A and C lower panels). * $P < 0.05$ vs. the DW-administered control group. (Original magnifications $\times 100$, 200 , 400 , scale bar = $50 \mu\text{m}$.) (A color version of this figure is available in the online journal.)

Discussion

Depression is a major disease affecting patients' quality of life.²² It is also a critical problem for postpartum women because postpartum depression increases the rate of suicide and consequent deaths.²³ For the last few decades, antidepressants such as monoamine oxidase inhibitors, tricyclic antidepressants, and selective serotonin reuptake inhibitors have been clinically used. However, most of these antidepressants have many serious adverse side-effects. Therefore, many alternative therapeutic strategies for the treatment of depression have been reported. In particular, many studies have reported that natural products with

anti-oxidant and anti-inflammatory effects improve neurodegeneration.^{24,25}

FST is an acute behavioral despair test, representing one of the symptoms of depression and just give preliminary idea for the antidepressant-like activity, whereas depression is a chronic disorder, which require chronic treatment. Although FST does not induce in mice a symptomatology similar to that of human depression, it nevertheless appears to be a suitable animal model to detect antidepressants. The majority of antidepressants reduce immobility time and their effectiveness correlates significantly with clinical potency and it is sufficiently specific, because it discriminates antidepressants from neuroleptics and anxiolytics.^{19,26}

Fluoxetine reduced the immobility time in the acute (3 days) and chronic (14 days) FST.²⁰ Neuron cell death was also induced during the FST. Thus, many effective antidepressants selectively increase hippocampal neurogenesis

to treat clinical symptoms of depression in the FST model. In this study, CA decreased the immobility times and increased the number of hippocampal nissl bodies in the 14th day FST. Therefore, FST was considered a complementary model that can provide additional information for a better understanding of the neurobiology of depression. Thus, we suggest that CA may have an antidepressant-like effect. In the OFT, CA did not alter the locomotor activity of mice. Therefore, it can also be thought that CA did not induce any anxiety or hyper activity-like effects after administration in mice.

BDNF is an important regulator of synaptogenesis, neurogenesis, synaptic plasticity, and cell survival underlying memory and learning.²⁷ The rate of hippocampal neurogenesis is increased by BDNF.²⁷ Depression impairs hippocampal BDNF expression and antidepressant drug effects correlate with increased BDNF synthesis and activity in the hippocampus.²⁸ Depression also increases the number of apoptotic neuron cells and decreases the activation of ERK in the CA1, CA3, and DG of the hippocampus.^{25,29} Estradiol also increases BDNF expression through the phosphorylation of the ERK by activating ER- β .⁸ Estradiol withdrawal has been found to increase immobility time in the FST.³⁰

Proinflammatory cytokine levels are significantly elevated in depressed patients.³¹ Proinflammatory cytokine-administered animals display typical characteristics of sickness behavior that resemble symptoms of depression.^{32–34} A high level of IL-1 β has been found to induce neurodegeneration.³⁵ IL-6 increases hippocampal indoleamine 2,3-deoxygenase levels with depressive-like behavior in the FST.³⁶ For depression, TNF- α activates the neuroinflammatory system.⁹ IL-1, IL-6, or TNF- α receptor-knockout mice show less depression-like behavior.^{37–39}

The monoamines (5-HT, dopamine, and NE) are broadly distributed in the brain.^{12,40} 5-HT increases the expression

Figure 3 Effect of CA on the levels of hippocampal ER- β mRNA. (a) Hippocampal ER- β mRNA was measured by RT-PCR. (b) ER- β levels were quantified by densitometry. Values are means $\pm$ SEM. * $P < 0.05$ vs. the DW-administered control group. DW: distilled water

Figure 4 Effect of CA on the mRNA and protein levels of inflammatory cytokine in the hippocampus. (a) Hippocampal IL-1 β , IL-6, and TNF- α mRNA levels in the hippocampal tissues were analyzed by real time-PCR. (b) IL-1 β , IL-6, and TNF- α protein levels were measured by ELISA. Values are means $\pm$ SEM. * $P < 0.05$ vs. the DW-administered control group. DW: distilled water

Figure 5 Effect of CA on the levels of hippocampal 5-HT, dopamine, and NE. After the last FST, hippocampal 5-HT, dopamine, and NE levels were measured using an assay kit. Values are means $\pm$ SEM. * $P < 0.05$ vs. the DW-administered control group. DW: distilled water

of BDNF in hippocampus. Some antidepressant drugs (fluoxetine and tranylcypromine) increase the dopaminergic function by enhancing both the release of dopamine and the expression of dopamine receptors.^{41,42} These increases of dopamine receptors have been found to be controlled by BDNF.⁴³ Fluoxetine also increases the levels of brain NE and its action was mediated by the central noradrenergic systems.⁴⁴ Proinflammatory cytokines play an important role in monoamine metabolism in mice and rats.^{45,46} Therefore, we suggest that monoamines, BDNF, and proinflammatory cytokines regulations become

potential targets of therapy for depression. In this study, we have shown that CA increased the levels of hippocampal monoamines and of BDNF, and decreased the levels of proinflammatory cytokines. Therefore, our results indicate that CA exerts antidepressant-like effects through the up-regulation of monoamine neurotransmitters and BDNF and through the down-regulation of proinflammatory cytokines.

Fluoxetine generally leads to a dose dependent increase in the 5-HT levels.⁴⁷ However, CA showed dose independence in the 5-HT level. A dose of CA 2 mg/kg seems to be

less effective than a dose of CA 0.2 mg/kg. Similar to CA, *Apocynum venetum* leaves have an antidepressant-like effect; they increase the dopamine and NE levels in a dose-dependent manner but showed dose independence in the 5-HT level.⁴⁸ Therefore, we suggest that the beneficial effect in the 5-HT levels was produced by a CA 0.2 mg/kg dose. We also found that levels of dopamine and NE were significantly increased by CA in a dose-dependent manner. Our results suggest that CA at 2 mg/kg exerts antidepressant-like effects through the up-regulation of dopaminergic and norepinephrine systems, although the precise action of CA is not known at this time. Further study will be needed to elucidate the detailed dose and related mechanism of CA in neurotransmitter systems.

CA is an active compound of *Chelidonium majus* L. and an inhibitor of rat brain glutamate decarboxylase.^{16,18} Kim et al.¹⁵ reported that CA reduced the dextran sulfate sodium-induced cyclooxygenase-2 and hypoxia-induced factor-1 α levels. CA inhibited IL-6 production through a blockade of NF- κ B and caspase-1 activation in mast cells.¹⁷ In addition, CA attenuated allergic inflammatory reactions in animal models.¹⁸ Therefore, we suggest that CA may achieve an antidepressant-like effect by blocking inflammatory reactions.

Conclusion

We observed for the first time that CA increased the levels of hippocampal 5-HT, dopamine, NE, and BDNF. CA also increased the levels of ER- β and pERK, whereas levels of inflammatory cytokine were reduced by CA. Therefore, these findings suggest that CA could be useful for treating depression. However, further study is needed to characterize the mechanisms and effects of CA prior to its clinical use in humans.

Author contributions: HJJ and HMK designed the research scheme. HYK performed the FST. NRK performed the OFT. HJJ, SYJ, and JBJ analyzed the data and wrote the manuscript. HJJ and SYJ contributed equally to this work.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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