Effect of Korean music therapy on depression: Daegeum playing of Arirang

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Abstract: - Recently, many studies have described the effectiveness of music therapy on depression. Arirang is a Korean music, often considered as the unofficial national anthem of Korea and has been listed on the UNESCO in 2012. Arirang has been sung for more than 600 years in Korea. It includes all of our feelings of joys and sorrows. Daegeum is a natural Korean transverse bamboo flute. However, the effects of the Daegeum playing of Arirang (DPA) on depression have not been investigated. Here, we evaluated the antidepressant-like effects of DPA. Mice were exposed to DPA once a day for 21 days in a sound isolation booth. Immobility times in the forced swimming test and distance moved in open field test were measured. DPA significantly decreased immobility times as compared with non-treated control without affecting locomotor activity. DPA increased levels of 5-hydroxytryptamine, brain-derived neurotrophic factor, phosphorylated-extracellular signal-regulated kinase, and estrogen receptor-β in brains. In addition, protein and mRNA levels of tumor necrosis factor-α in brains were significantly reduced in animals exposed to DPA. These findings indicate that DPA has positive effects on depression, and suggests that Korean music therapy be considered a potential therapeutic intervention for the treatment of depression.

Key-Words: - Depression, Daegeum, Arirang, Forced swimming test, 5-hydroxytryptamine, Korean music therapy

1 Introduction

Depression is a multifaceted disease with various causes and has been involved to cardiovascular disease, obesity, neurodegenerative disorder, and inflammatory diseases [1]. Conventional treatment methods of depression were both using the antidepressant and cognitive behavioral therapy [1]. However, using the antidepressant has a diverse of undesirable side effects including fatigue, insomnia, increased appetite, weight gain, and decrease of blood pressure [1]. This results in patients' poor compliance resulting in a break-up of medication with recurrence of depressive symptoms and increased suicidal risk [2]. Therefore, alternative treatment should be considered.

Music therapy is one of the complementary treatments or alternative treatments and has been used in the treatment of diverse disorders because it has multifaceted effects on human being [3]. Music eliminates unhappiness feeling and negative thinking and also causes the mental and physical stabilities in patients with depression [3]. Listening to music relieves symptoms of psychological or physical diseases and levels of depression according to its psychological influence [4]. In addition, listening to music influences the brain and thus causes the secretion of neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NE), and dopamine [5]. 5-HT and NE regulate numerous emotional and physical processes such as pain response, mental performance, and emotional states [5] and have a pivotal role in the treatment of depression [6].

Stress reduces levels of brain-derived neurotrophic factor (BDNF) and activation of extracellular signal-regulated kinase (ERK) and reduction of these induces the impaired neurogenesis and depressive symptoms [7]. The useful effect of BDNF associated with the activation of ERK [8]. Antidepressant estradiol increases the BDNF levels through the activation of estrogen receptor-β (ER-β) [9]. Proinflammatory cytokines, such as, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 are down-regulates by BDNF [10,11].

Arirang is a Korean folk song, often considered as the unofficial national anthem of Korea and has been listed on the UNESCO in 2012. Arirang has been sung for more than 600 years in Korea. Arirang is also good to remember and easy to tinge of improvisation. It has provided a sense of positive emotional support for Koreans throughout history, both in times of sorrow and times of happiness [12].

Daegeum is a natural Korean transverse bamboo flute, it has one blowing hole, six finger holes, and an extra hole covered with a thin membrane called 'Cheong' (located between the blowing hole and finger holes). Cheong is a white resonant membrane cut from a reed and makes this instrument produce a distinctive refined, calm sound. The pure and clean voice of Daegeum sounds like sigh. Also, it's like the sound of the wind through bamboo.

Many studies reported the positive effects of music therapy on depression. Thus, we hypothesized that the Daegeum playing of Arirang (DPA) would have beneficial effects on depression. Therefore, the aim of this study was to investigate the effect of antidepressant effect of DPA on mice subjected to the forced swimming test (FST).

2 Materials and Methods

2.1 Materials

Fluoxetine, avidin peroxidase, bicinchoninic acid, and other reagents were purchased from Sigma (St. Louis, MO, USA). Anti-mouse TNF-α purified antibody (Ab), anti-mouse TNF-α biotin-conjugated Ab, and recombinant mouse TNF-α were purchased from BD Biosciences Pharmingen (Sandiego, CA, USA). Abs for BDNF, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ERK, and phosphorylated-ERK (p-ERK) obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2 Animals

Male ICR mice (3 weeks old) weighing 10 - 12 g were purchased from the Dae-Han Experimental Animal Center (Daejeon, Republic of Korea), and subsequently maintained at the College of Korean Medicine, Kyung Hee University. Animals were housed 5-10% in as laminar air-flow room maintained at a temperature of 22 ± 1°C and a relative humidity of 55 ± 10% under a 12:12 L/D cycle light (on at 7:00 h) throughout the study. Food and water were provided ad libitum. All manipulations were carried out between 9:00 and 16:00 h and no animal was used more than once. The study protocol was approved beforehand by the institutional animal care use committee of Kyung Hee University (KHUASP(SE)-10-032*).*

2.3 Music treatment

ICR mice were divided in to five groups according to FST results: the untreated control group, the fluoxetine group (positive control), the white noise group, the Buk (Korean traditional drum) group, and DPA groups. Buk music was exposed to compare DPA effect against other Korean musical styles. Background sound levels in the special isolation booth constructed on the non-treated control and fluoxetine groups were 10 ~ 40 dB and in the white noise, Buk, and DPA groups was 70 dB. Mice were exposed to white noise, Buk, or DPA daily for 3 weeks. Fluoxetine was dissolved in distilled water. Fluoxetine (10 mg/kg, a classical antidepressant) was orally administered to mice once daily for 3 weeks using an atraumatic feeding needle. All experiments were carried out in a quite environment other sounds. In this study, DPA was played by Prof. Hyung-Min Kim. The music was recorded and copied using a MP3 player to ensure precisely the same music was played for mice in all treatment groups.

2.4 FST

After measuring immobility times, 5 mice were allocated to each of the three study groups in an immobility time-matched manner. FST was performed as the end of the 3 weeks administration period. During the 6 min FST, immobility times were measured as described by Porsolt et al. [13]. The apparatus consisted of two Plexiglas cylinders (height: 25 cm, diameter: 10 cm) placed side by side in a Makrolon cage filled with water at 23-25°C. Two mice were tested simultaneously for 6 min period inside the vertical Plexiglas cylinders; a nontransparent screen was placed between the two cylinders to prevent mice from seeing each other during the test. Total duration of immobility, during the 4 min period from 2 to 6 min was recorded. A mouse was considered immobile when it ceased struggling and remained floating motionless on the water, making only movements necessary to keep its head above water.

2.5 Open Field Test (OFT)

OFT was conducted in clear black Plexiglas boxes (40 × 40 × 40 cm) equipped with the video-based Ethovision System (Nol-dus, Wageningen, The Netherlands), as described previously [14]. Mice were initially placed in the corner of the apparatus, and total distance moved was recorded for 30 min. The horizontal locomotor activity is expressed in

terms of the total ambulatory distance (cm). Each box was cleaned with 70% ethanol between subjects.

2.6 5-HT assay

5-HT levels in brain were measured a 5-hydroxytryptamine assay kit (MyBiosource, San Diego, CA, USA).

2.7 Isolation of brain protein

Immediately after the FST, mice were sacrificed, brains were rapidly removed, and frozen in liquid nitrogen. Samples were dissected and homogenized in ice-cold buffer supplemented with 0.2 mM DTT, 0.5 mM sodium vanadate, and protease inhibitors. NaCl was then added to a final concentration of 0.45 M, and homogenates were centrifuged at 15,000 × g for 30 min. Supernatants were collected and stored at -70°C. Samples were subjected to Western blot analysis for BDNF, ERK, p-ERK, and GAPDH.

2.8 Western blot analysis

Brain extract samples obtained as described above were heated at 95°C for 5min and briefly cooled on ice. After centrifugation, 50 μg aliquots were resolved by 12% SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose membranes, which were blocked for 2 h with phosphate-buffered saline (PBS) containing Tween-20 (PBST) containing 6% bovine serum albumin. Membranes were incubated overnight at 4°C with primary antibodies diluted 1:500 with PBST, and washed nine times for 10 min with PBST. For protein detection, blots were incubated with secondary antibodies conjugated peroxidase (1:5000) for 2 h. Finally, protein bands were visualized using an enhanced chemiluminescence kit (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions.

2.9 Quantitative real time-PCR analysis

Quantitative real time-PCR was performed using a SYBR Green master mix and mRNA levels were analyzed using an ABI StepOne RT-PCR System (Applied Biosystems, Foster City, CA, USA). Primer sequences for the reference gene (GAPDH) and genes of interest were as follows: GAPDH (5' GGC AAA TTC AAC GGC ACA 3'; 5' GTT AGT GGG GTC TCG CTC CTG 3'); and ER-β (5' GAC TGT AGA ACG GTG TCA TCA A 3'; 5' CCT GTG AGG TAG GAA TGC GAA C 3'); TNF-α (5` AGG ACG AAC ATC CAA CCT TCC CAA 3`; 5` TTT GAG CCA GAA GAG GTT GAG GGT 3`). The quantitative PCR amplification protocol was as follows: 2 min at 50°C and 10 min 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C, with data collection during the last 30 s.

2.10 Enzyme-linked immunosorbent assay (ELISA)

TNF-α levels in serum and brain were measured by ELISA. The ELISA was performed by coating 96-well plates with 1 μg/well of capture antibodies for TNF-α. Before the subsequent steps in the assay, the coated plates were washed twice with PBST. 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 TNF-α, as provided by the manufacturer. The assay plates were exposed sequentially to each of the biotin-conjugated secondary antibodies, and avidin-peroxidase, and 2´-azino-bis (3-ethylbenzithiazoline-6-sulfonic acid) substrate solution containing 30% H_2O_2. The plates were read 405 nm. TNF-α level in brain was divided according to the total protein. The protein was estimated using the bicinchoninic acid method.

2.11 Statistical analysis

Data were expressed as the mean ± standard error mean (SEM). Prior to ANOVA testing, all data were checked for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. The independent *t*-test and one-way ANOVA with Tukey's post hoc test were used to determine statistical significance. SPSS statistical software (SPSS Inc., Chicago, IL, USA) was used throughout, and statistical significance was accepted for p values < 0.05.

3 Results

3.1 Effect of DPA on immobility time and locomotor activity

Initially, we analyzed the regulatory effect of DPA in immobility time of FST. The immobility time in DPA group was significantly decreased compared with the control groups (Fig. 1A, $p < 0.05$). Fluoxetine group also significantly decreased the immobility time compared with the control groups. However, white noise and Buk groups did not affect the immobility times (Fig. 1A, $p < 0.05$). DPA also significantly reduced the immobility time compared with the white noise group (Fig. 1A, $p < 0.05$). To determine whether DPA could affect locomotor activity in mice, DPA-exposed mice were evaluated for total distance travelled in OFT. However, no statistically significant difference was observed

between the control mice and the DPA-exposed mice in the OFT (Fig. 1B). Fluoxetine and white noise group did not change the total distance moved (Fig. 1B). Therefore, we observed only the effect of DPA and fluoxetine from the next experiments.

3.2 Effect of DPA on antidepressant-related biomarkers in brain.

5-HT and NE are neurotransmitters that are associated with mood and feeling and they are important factors in antidepressant effect [6]. So, we analyzed the levels of brain 5-HT and NE after FST. 5-HT levels in the DPA group were significantly increased compared with the control group (Fig. 2A, $p < 0.05$). But, DPA did not affect the NE levels (data not shown). Fluoxetine also increased the 5-HT levels compared with the control group (Fig. 2A, $p < 0.05$). BDNF and ERK play important roles in depression [15]. Therefore, we examined whether the BDNF and ERK pathways in effect of DPA was involved. Protein levels of BDNF and p-ERK in the DPA group were significantly increased compared with the control group (Figs. 2B and 2C, $p < 0.05$). Similarly, fluoxetine also significantly elevated the protein levels of BDNF and p-ERK (Figs. 2B and 2C, $p < 0.05$).

To investigate whether the antidepressant effect of DPA was induced through the activation of ER-β, we performed a quantitative real time-PCR for brain ER-β. DPA and fluoxetine significantly increased the levels of ER-β compared with the control group (Fig. 2D, $p < 0.05$).

3.3 Effect of DPA on levels of TNF-α

Depression was aggravated by proinflammatory cytokine [16]. Therefore, we analyzed the protein levels of TNF-α by ELISA in the brain. Protein levels of TNF-α in the DPA group were significantly decreased compared with the control group (Fig. 3A, $p < 0.05$). Furthermore, we sought to estimate the influence of DPA in the TNF-α mRNA expression using a quantitative real time-PCR. As a result, DPA significantly decreased the brain TNF-α mRNA expression compared with the control group (Fig. 3B, $p < 0.05$). However, DPA did not affect the serum TNF-α protein levels (Fig. 3C).

4 Discussion

Music therapy becomes more and more important because of increased beneficial effect and functions on people. Listening to music increases the positive feeling in people and it plays an important role in the increase confidence. Also, it induces relaxation of brain and leads to decrease the depressive feelings in brain. According to recent studies, role of music therapy may be a useful alternative therapy facilitating better emotional control and experience without medicine, reducing anxiety, stress, and depression in patients [3,17]. In this study, we showed that DPA has an antidepressant effect by increasing the levels of 5-HT and BDNF and decreasing the levels of TNF-α.

FST is often used as indices of a depressive-like state and also an important tool for studying the neurobiological mechanisms involved in antidepressant responses [18,19]. In this study, we showed that DPA significantly decreased the immobility times of FST without change of locomotor activity in OFT. Therefore, these results suggest that DPA has an antidepressant effect.

BDNF signaling was down-regulated in depression [20]. BDNF promotes the activation of ERK and differentiation of 5-HT neurons [21] and is generally known to be modulated by antidepressant drug as a down-stream target of 5-HT [22]. 5-HT is also well-known for being a target of antidepressant drugs [21]. Antidepressant estradiol increases BDNF expression through the phosphorylation of the ERK by activating ER-β [23] and estradiol withdrawal increased immobility time in the FST [24]. In this study, we showed that DPA increased the levels of 5-HT, BDNF, p-ERK, and ER- β in the brain, suggesting the antidepressant effect of DPA is associated with activation of BDNF pathways.

Patients with depression show increased levels of proinflammatory cytokines, including TNF-α, IL-1β, and IL-6 [25]. Also, the proinflammatory cytokine genes are increased in the frontal cortex of subjects with a history of depression compared with healthy controls [26]. Antidepressant treatment including the imipramine, clomipramine, venlafaxine, and fluoxetine can attenuate the expression of inflammatory biomarkers in depression [27]. In this study, we showed that DPA decreased the protein and mRNA levels of TNF-α in the brain. Therefore, this result suggests the antidepressant effect of DPA is associated with regulation of inflammatory cytokines.

Koreans often have expressed all aspects of human lives like love, anger, happy, agony, grudge, fear, envy, sorrow, life and death through Arirang. Rhythm of Arirang is Jungmori-jangdan of twelve beats. A Korean word Jangdan (rhythmic pattern) expresses the rhythm of the music. The rhythm of jungmori-jangdan is similar to normal human heart rate. Thus, Arirang is good to remember and easy to

tinge of improvisation. Therefore, we can presume that listening to DPA has healing powers because it makes our more peaceful.

5 Conclusion

In conclusion, we suggest that DPA exhibits an antidepressant-like effect and these effects are related to modulation of serotonergic system and BDNF signaling pathway. Therefore, we suggest that Korean music therapy by using DPA be considered a potential method for the regulation of depression.

References:

[1] Hesdorffer DC, Hauser WA, Annegers JF, Cascino G. Major depression is a risk factor for seizures in older adults. *Annals of Neurology*, Vol.47, No.2, 2000, pp. 246-249.

[2] Keller MB, Hirschfeld RM, Demyttenaere K, Baldwin DS. Optimizing outcomes in depression: focus on antidepressant compliance. *International Clinical Psychopharmacology*. Vol.17, No.6, 2002, pp. 256-271.

[3] Baker FA, Gleadhill LM, Dingle GA. Music therapy and emotional exploration: Exposing substance abuse clients to the experiences of non-drug-induced emotions. *The Arts in Psychotherapy*. Vol.34, No.4, 2007, pp. 321-330.

[4] Bernatzky G, Presch M, Anderson M, Panksepp J. Emotional foundations of music as a non-pharmacological pain management tool in modern medicine. *Neuroscience & Biobehavioral Reviews*. Vol.35, No.9, 2011, pp. 1989-1999.

[5] Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron*. Vol.34, No.1, 2002, pp. 13-25.

[6] Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. *Nature Reviews Neuroscience*. Vol.4, No.12, 2003, pp. 1002-1012.

[7] Masi G, Brovedani P. The hippocampus, neurotrophic factors and depression: possible implications for the pharmacotherapy of depression. *CNS Drugs*. Vol.25, No.11, 2011, pp. 913-931.

[8] Massa SM, Yang T, Xie Y, Shi J, Bilgen M, Joyce JN Nehama D, Rajadas J, Longo FM. Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *Journal of Clinical Investigation*. Vol.120, No.5, 2010, pp. 1774-1785.

[9] Firozan B, Goudarzi I, Elahdadi Salmani M, Lashkarbolouki T, Rezaei A, Abrari K. Estradiol increases expression of the brain-derived neurotrophic factor after acute administration of ethanol in the neonatal rat cerebellum. *European Journal of Pharmacology*. Vol.5, 2014, pp. 1-11.

[10] Numakawa T, Richards M, Nakajima S, Adachi N, Furuta M, Odaka H, Kunugi H. The role of brain-derived neurotrophic factor in comorbid depression: possible linkage with steroid hormones, cytokines, and nutrition. *Frontiers in Psychiatry*. Vol.5, 2014, pp. 136.

[11] Xu D, Lian D, Wu J, Liu Y, Zhu M, Sun J, He D, Li L. Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental Streptococcus pneumoniae meningitis. *Neuroinflammation*. Vol.14, No.1, 2017, pp. 156.

[12] Lee CM. Arirang: Song of Korea. 1st ed. (Korea; Seoul, Easy Publishing Co.), 2009, pp. 1-365,

[13] Porsolt RD, Lepichon M, Jalfre M. Depression, a new animal model sensitive to antidepressant treatments. *Nature*. Vol.266, No.5604, 1977, pp. 730-732.

[14] Lee B, Sur B, Park J, Kim SH, Kwon S, Yeom M, Shim I, Lee H, Hahm DH. Chronic administration of baicalein decreases depression-like behavior induced by repeated restraint stress in rats. The *Korean Journal of Physiology and Pharmacology*. Vol.17, No.5, 2013, pp. 393-403.

[15] Kim JE, Ji ES, Seo JH, Lee MH, Cho SH, Kim Park YM, Seo TB, Kim CJ. Alcohol exposure induces depression-like behavior by decreasing hippocampal neuronal proliferation through inhibition of the BDNF-ERK pathway in gerbils. *Neurobiology & Physiology*. Vol.16, No.3, 2012, pp. 393-403.

[16] Makar TK, Trisler D, Sura KT, Sultana S, Patel N, Bever CT. Brain derived neurotrophic factor treatment reduces inflammation and apoptosis in experimental allergic encephalomyelitis *Journal of the Neurological Sciences*. Vol.270, No.1-2, 2008, pp. 70-76.

[17] Cevasco AM, Kennedy R, Generally NR. Comparison of movement to music, rhythmic activities, and competitive games on depression, stress, anxiety, and anger of females in substance abuse rehabilitation.

Journal of Music Therapy. Vol.42, No.1, 2005, pp. 70-76.

[18] Izumi J, Washizuka M, Hayashi-Kuwabara Y, Yoshinaga K, Tanaka Y, Ikeda Y, Kiuchi Y, Oguchi K. Evidence for a depressive-like state induced by repeated saline injections in Fischer 344 rats. *Pharmacology Biochemistry and Behavior*. Vol.57, No.4, 1997, pp. 883-888.

[19] Lee B, Sur B, Kwon S, Yeom M, Shim I, Lee H, Hahm DH. Chronic administration of catechin decreases depression and anxiety-like behaviors in a rat model using chronic corticosterone injections. *Biomolecules & Therapeutics*. Vol.21, No.4, 2013, pp. 313-322.

[20] Duman RS. Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular Medicine*. Vol.5, No.1, 2004, pp. 11-25.

[21] Homberg JR, Molteni R, Calabrese F, Riva MA. The serotonin-BDNF duo: developmental implications for the vulnerability to psychopathology. *Neuroscience & Biobehavioral Reviews*. Vol.43, 2014, pp. 35-47.

[22] Martinowich K, Lu B. Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology*. Vol.33, No.1, 2008, pp. 73-83.

[23] Yang LC, Zhang QG, Zhou CF, Yang F, Zhang YD, Wang RM, Brann DW. Extranuclear estrogen receptors mediate the neuroprotective effects of estrogen in the rat hippocampus. *PLoS One*. Vol.5, No.5, 2010, pp. e9851.

[24] Schiller CE, O'Hara MW, Rubinow DR, Johnson AK. Estradiol modulates anhedonia and behavioral despair in rats and negative affect in a subgroup of women at high risk for postpartum depression. *Physiology & Behavior*. Vol119, 2013, pp. 137-141.

[25] Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic Medicine*. Vol.71, No.2, 2009, pp. 171-186.

[26] Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirnics K. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Molecular Psychiatry*. Vol.16, No.7, 2011, pp. 751-762.

[27] Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G, Kubera M, Bob P, Lerer B, Maj M. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metabolic Brain Disease*. Vol.24, No.1, 2009, pp. 27-53.

Appendix

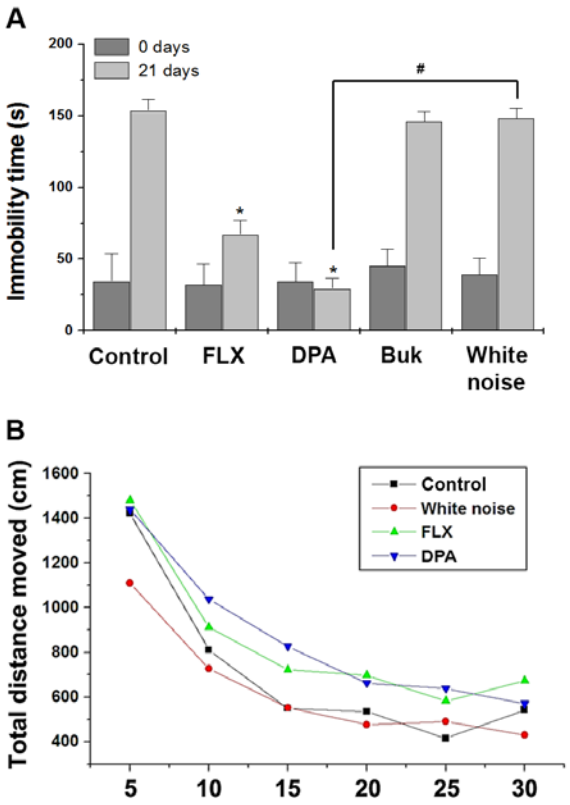

Fig. 1: Effect of DPA on immobility time and locomotor activity. DPA, white noise, or Buk was exposure for 3 weeks and fluoxetine were administered for 3 weeks. (A) Immobility times. (B) Locomotor activity of mice in the OFT were observed for 30 min. Total distance moved. Values are means ± SEMs. *$p < 0.05$ versus control group; #p < 0.05 versus white noise group. Control, untreated mice; FLX, Fluoxetine; DPA, Daegeum playing of Arirang; Buk, Buk music.

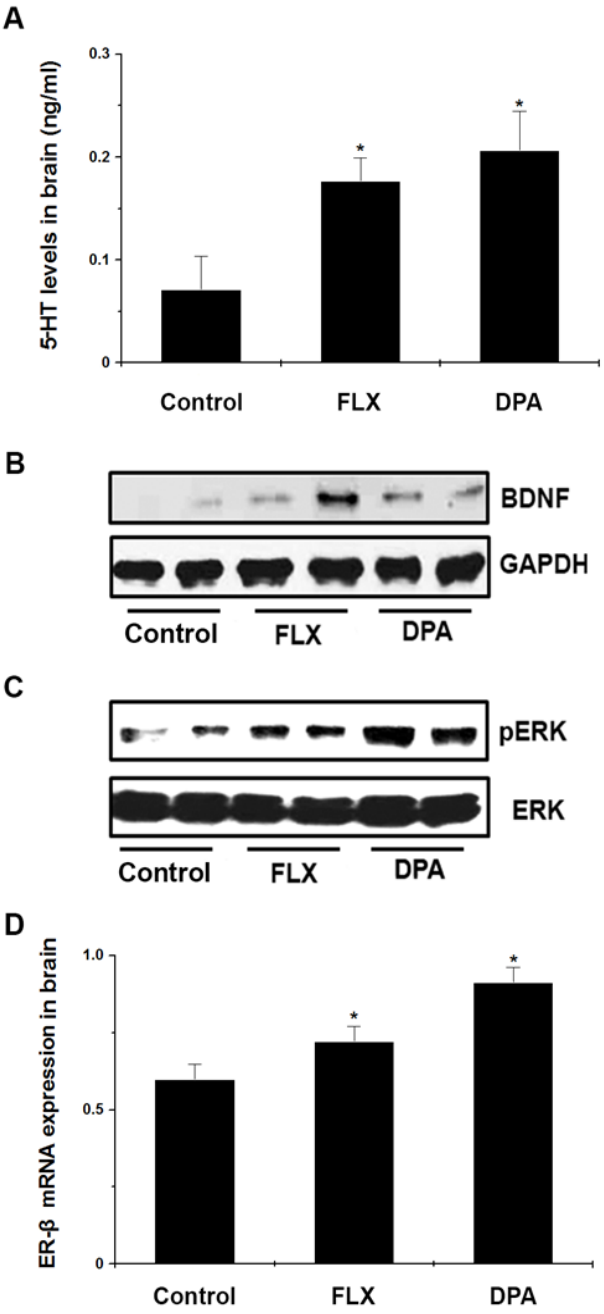

Fig. 2: Effect of DPA on antidepressant-related biomarkers. After FST, (A) 5-HT levels in brain were measured using assay kits. Protein levels of (B) BDNF and (C) p-ERK in brain were analyzed by Western blotting. (D) mRNA levels of ER-β in brain were measured using a quantitative real time-PCR. Values are means ± SEMs. *$p < 0.05$ versus control group. Control, untreated mice; FLX, Fluoxetine; DPA, Daegeum playing of Arirang.

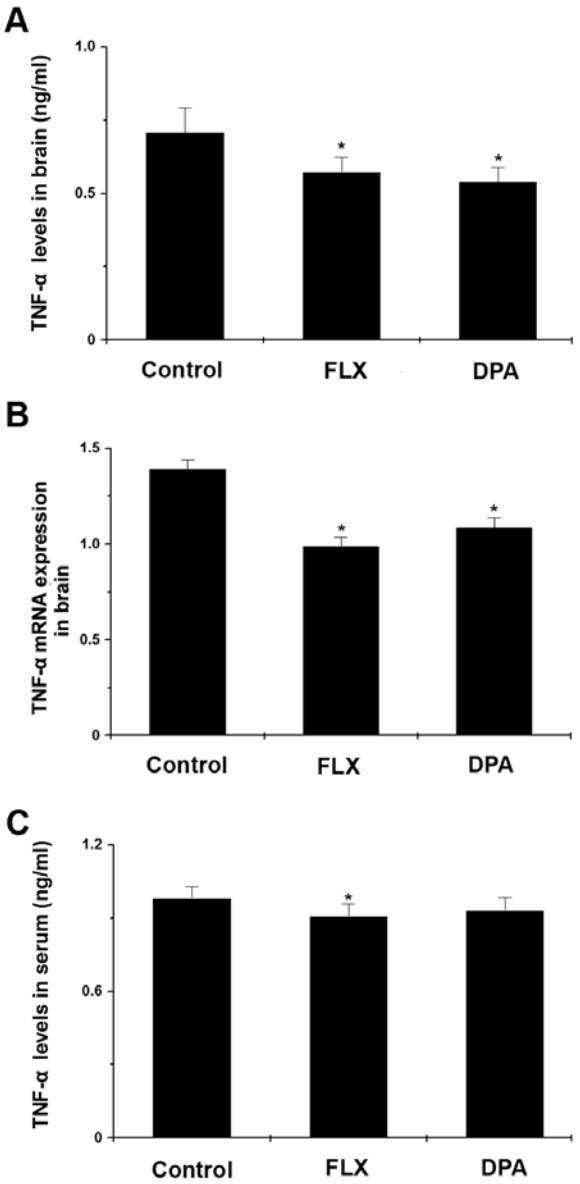

Fig. 3: Effect of DPA on levels of TNF-α. After FST, (A) Protein levels of TNF-α in brain were measured using an ELISA. (B) mRNA levels of TNF-α in brain were measured using a quantitative real time-PCR. (C) Protein levels of TNF-α in serum were measured using an ELISA. Values are means ± SEMs. *$p < 0.05$ versus control group. Control, untreated mice; FLX, Fluoxetine; DPA, Daegeum playing of Arirang.