Antidepressant-like and Hypnotic Effects of the Herbal Extract Combination of *Stauntonia hexaphylla* and *Vaccinium bracteatum* Fruit in Mice

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Stauntonia hexaphylla (SH) and *Vaccinium bracteatum* (VB) are herbal extracts widely used in food and traditional herbal medicine, and have the ability to perform a wide range of biological activities. We aimed to investigate the effects of the SH and VB combination (SHVB) on mice models of chronic restraint stress (CRS) and pentobarbital-induced sleeping behaviors to elucidate its possible mechanisms of action. CRS-exposed mice treated with SHVB showed significantly decreased immobility time, increased swimming and climbing times in the forced swim test (FST), and increased locomotor activity in the open field test (OFT). SHVB decreased serum CORT levels, but enhanced brain monoamine neurotransmitters. SHVB significantly decreased the sleep latency and increased total sleep duration in pentobarbital-induced sleeping behavior in mice. SHVB showed inhibitory effect on 5-HT_{2A} receptor-mediated ERK1/2 phosphorylation. These results suggest that SHVB has antidepressant and hypnotic effects by regulating the 5-HT_{2A} receptor.

keywords : *Stauntonia hexaphylla*, *Vaccinium bracteatum*, Chronic restraint stress, Antidepressants, Serotonin 2A receptor

Introduction

Stress is a risk factor for depression and insomnia (sleep problems), with insomnia being one of the key symptoms of common depressive disorder[1]. Depressive disorders are characterized by changes in the mental status such as a marked loss of interest, a low mood, fatigue, and worthlessness[2]. Several types of sedative antidepressant drugs are often used in the treatment of insomnia[3]. Chronic restraint stress (CRS) is commonly induced in animal studies to mimic the development of clinical depression. CRS exerts common depressive-like symptoms, such as changes to corticosterone (CORT) and monoamine neurotransmitter (e.g. serotonin; 5-HT, dopamine; DA, and norepinephrine; NE) levels, and locomotor activity deficit[4,5]. Serotonin and serotonin receptors (5-HT receptors) play a key role in depression. Activation of the 5-HT_{2A} receptor, a Gq-coupled receptor, leads to an accumulation of IP_3, diacylglycerol (DAG), and activation of protein kinase C (PKC), which causes the release of Ca^{2+}, thereby activating the extracellular signal-regulated kinases 1/2 (ERK1/2) pathway. 5-HT_{2A} receptors are expressed in a majority of neocortical cells (mainly prefrontal cortex; PFC), and are involved in the regulation of sleep and mood[6].

Stauntonia hexaphylla (SH) belongs to the genus Lardizabalaceae, and is widely distributed in Korea, Japan, and China. It has been commonly used as a herbal medicine in China, especially for its analgesic, sedative, and diuretic properties[7-9]. Previous studies have demonstrated that the SH leaf exerts pharmacological effects, including antidiabetic[10] and anti-osteoporosis[11] effects. We have previously reported that the SH fruit extract has anti-inflammatory effects in lipopolysaccharide (LPS)-activated RAW 264.7 cells and in carrageenan-induced paw edema rats[12]. We also reported that the SH fruit extract has antioxidant and hepatoprotective effects on hydrogen peroxide-induced cytotoxicity in HepG2 cells[13]. In addition, previous studies have shown that the constituents of SH extract includes triterpenoids, glucosides, flavonoids, and phenylpropanoids[14]. *Vaccinium bracteatum* (VB) belongs to the genus Ericaceae, and its fruits are commonly known as the "oriental blueberry" in Korea. Previous studies have demonstrated that VB exert pharmacological effects, including anti-fatigue[15], antimicrobial[16], anti-diabetes[17,18],

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anti-oxidant[16,19], retinal protection[20], anti-proliferative and anti-inflammatory effects[21,22]. We have previously reported that the VB fruit has antidepressant properties, protective effects on oxidative stress-induced apoptosis, sedative, and hypnotic effects[23-25]. However, no studies have reported the antidepressant-like effects of the combination of SH and VB extracts (SHVB), especially in models of CRS and related depression disorders.

In the present study, we investigated the effects of the SHVB on pentobarbital-induced sleeping behavior in CRS-exposed mice, through a forced swimming test (FST) and open field test (OFT). We also evaluated the levels of CORT and monoamine neurotransmitters (5-HT, DA, and NE) in the CRS-exposed mice. In addition, the effects of SHVB on sleep latency and total sleep duration associated with sleep behavior in pentobarbital-induced mice were studied. Moreover, we investigated the inhibitory effects of SHVB on 5-HT_{2A}-associated ERK1/2 phosphorylation in Chinese hamster ovary (CHO)-K1 cells transfected with the human 5-HT_{2A} receptor.

Materials and Methods

1. Preparation of plant extract

The *Stauntonia hexaphylla* (Thunb.) Decne. fruits were collected from the Jangheung-gun County (Jeollanamdo, Republic of Korea). The *Vaccinium bracteatum* Thunb. fruits used in this study were collected from the Goheung County (Jeollanamdo, Republic of Korea). The herbs were extracted with water at 100 ℃ for 4 h. The extraction yield of *Stauntonia hexaphylla* (Thunb.) Decne. fruits and *Vaccinium bracteatum* Thunb. fruits was about 8.0 % and 12.5 %, respectively. The extracts were stored at 4 ℃ for further use. The SHVB (NET-1601, SH: VB = 1:1, w/w) used in the present study was the same sample used in the clinical trial, which was approved by Institutional Review Board (IRB) at Konkuk University Medical Center (clinical trials registration number KUMC 2019-07-033-001).

2. Animals

Male ICR mice (five-week-old, weight 24-27 g) were purchased from Samtako Bio Korea (Osan, Republic of Korea). The animals were maintained at a constant room temperature of 22 ± 3 ℃, with a humidity of 50 ± 15 %, and were kept at a 12/12 h light/dark cycle. Mice were given free access to water and food. The all mice were acclimatized for 7 days prior to the experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Jeollanamdo Institute of Natural Resources Research (approval no. JINR-1806-2018 and JINR-1808-2018).

3. Drug administration and chronic restraint stress (CRS) procedure

The SHVB (100 and 200 mg/kg/day), escitalopram oxalate (EO, 10 mg/kg/day) and vehicles (saline) were administered orally for 3 consecutive weeks. Thirty minutes after drug administration, CRS was induced for a period of 6 h by clear plastic tubes, under a 60 W light for 3 consecutive weeks (11:00 and 5:00 p.m.) in accordance with a previously described method[24]. Mice were evaluated OFT and FST behavioral tests, consecutively.

4. Open field test (OFT)

General locomotor activity was evaluated using the OFT. Thirty minutes after the final drug administration, the mice were placed into a 60 cm × 60 cm wooden box with 20 cm boundary walls, divided into 25 equal squares. Each mouse was gently placed in a corner of the apparatus and counted in a 5 min session, accordance to the previously described method[23]. After each trial, the wooden box apparatus was cleaned with ethanol solution (70 % v/v).

5. Forced swim test (FST)

The FST was conducted in mice similarly to previous reports, with slight modifications[26]. The mice were individually placed in a plexiglass cylinder (diameter 15 cm) filled with 20 cm of water at 23 ± 2 ℃. Mice was exposed to 15 min of FST (pre-test session). After 24 h, the animals were forced to swim for a 6 min post-test session. The time of immobility, swimming, and climbing behavior during the last 5 min of the test were recorded, in accordance to the previously described method[23].

6. Serum and brain sampling

The mice were sacrificed immediately after the FST evaluation. The blood samples were collected during decapitation. The serum was separated by centrifuging the blood at 3,000 rpm for 20 min, and stored at -80 ℃ until further analysis. Their brains were quickly removed, and the PFC were rapidly isolated, immediately frozen in liquid nitrogen, and stored at -80 ℃ until further analysis.

7. Measurement of serum CORT levels

The serum CORT were determined by ELISA in accordance with the manufacturer's instructions (Abnova).

Briefly, the serum was diluted to 1:100 with the diluted assay buffer that was provided. The 50 μL sample or standard were added to the pre-coated antibody plate that was provided with the kit and then 25 μL of CORT conjugate and 25 μL of CORT antibody were immediately added to each well and incubated at room temperature for 1 h. After incubation, the plates were washed four times and 100 μL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added to each well for 30 min and stopped with stop solution (50 μL). The absorbance was read at 450 nm. The results were expressed in pg/mL.

8. Measurement of 5-HT, NE, and DA levels

For the quantification of monoamine levels, the PFC was lysed in 10 volumes per tissue weight of PRO-PREPTM protein extraction solution (iNtRON Biotechnology, Sungnam, Korea) on ice and incubated for 2 h at 4 ℃ with shaking. The lysate were divided centrifugation at 13,000 rpm for 20 min at 4 ℃. Protein concentration were determined using the bicinchoninic acid assay (BCA) protein assay reagent (Thermo Scientific, Rockford, IL, USA) with bovine serum albumin (BSA) used as the standard. The levels of 5-HT, NE, and DA were determined in the PFC lysate as per the procedure described by the manufacturer (Abnova Corp., Taipei City, Taiwan).

9. Pentobarbital-induced sleeping test and drug administration

The pentobarbital sodium-induced sleeping behavior of the mice were evaluated according to the method described previously[27]. For the evaluation of the SHVB activity on the combined administration with pentobarbital sodium at a hypnotic dosage, the mice were divided into four groups (n = 5 per group). Group I was treated with saline for 28 days (control group); Group II was treated with diazepam for 45 min before the experiment (DZP; 2 mg/kg) which served as a positive control; Group III and Group IV was treated with SHVB 100 and 200 mg/kg/day for 28 days (SHVB 100 and SHVB 200). After administration of pentobarbital sodium (45 mg/kg, i.p.; Sam Eung Ind. Co. Ltd, Seoul, Korea), the mice were observed for the sleep latency (loss of righting reflex), and duration of sleep (time between loss and regain of the righting reflex).

10. CHO-K1 cells cultures and human 5-HT_{2A} receptor gene transfection

CHO-K1 cells (American Type Culture Collection; ATCC, Manassas, VA, USA) were maintained in Roswell Park Memorial Institute 1640 (RPMI 1640; Invitrogen Inc., Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (FBS; Invitrogen Inc.) at 37 ℃ in a humidified atmosphere containing 5 % CO_2. For western blot analysis, CHO-K1 cells (approximately 1 × 10^6 cells/mL) were seeded in 6-well plates and incubated overnight. Cells were transiently transfected with the human 5-HT_{2A} receptor plasmid DNA (NM 000621) using the transfection reagent Lipofectamine™ 2000 (Invitrogen Inc.) for 48 h following the manufacturer's instructions.

11. Cell viability assay

Cell viability was assessed by the MTT assay. CHO-K1 cells were seeded at a density of 1.5 × 10^4 cells/well in a 96-well plate for 24 h, and exposed to various concentrations of SHVB for 24 h. At the end of the treatment, MTT solution (5 mg/mL; 20 μL/well) was added to each well and incubated for 4 h. Subsequently, the supernatants were removed and the formazan crystals were solubilized in 150 μL dimethyl sulfoxide. The optical density (OD) was determined at 540 nm using a spectrophotometer.

12. Immunoblot analysis

For the evaluation of the 5-HT_{2A} receptor-related activity, CHO-K1 cells transfected with the 5-HT_{2A} receptor were incubated with serum-free RPMI1640, and treated with SHVB (50 or 100 μg/mL) or risperidone (10 μM, 5-HT_{2A} receptor antagonist) alone for 30 min. The antagonistic effects were evaluated by exposing cells to SHVB or risperidone for 15 min followed by treatment with 5-HT (100 μM) for another 15 min. Cells were washed with cold phosphate buffered saline (PBS) and lysed with PRO-PREP™ Protein Extraction Solution on ice for 20 min, and centrifuged separately at 13,000 rpm for 5 min at 4 ℃. The supernatants were collected and used immediately or stored at −80 ℃ until assayed.

Protein concentration was determined by the BCA protein assay reagent using BSA (Sigma Chemical Co., St. Louis, MO, USA) as a standard. Protein lysates were separated on 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis using the Power Pac Basic electrophoresis apparatus (Bio-Rad, Hercules, CA, USA). The protein were transferred to a polyvinylidene difluoride membrane (0.45 mm pore size, Merck Millipore, Darmstadt, Germany). The membranes were blocked with 1 × TBS/0.2 % Tween-20 supplemented with 5 % skim milk for 1 h. Immunoblotting was performed overnight at 4 ℃ with β-actin, phospho and total ERK1/2 antibodies (Cell Signaling Technology, Beverly, MA, USA). The membranes were

incubated with diluted horseradish peroxidase (HRP)-conjugated anti-rabbit IgG secondary antibodies (Cell Signaling Technology) for 1 h. The proteins were detected using a chemiluminescent substrates kit (Merck Millipore) in accordance with the instructions provided the manufacturer.

13. High-performance liquid chromatography (HPLC) Analysis

The analysis was performed using the SHIMADZU series Ultra-fast liquid chromatography system (LC-20AD, shimadzu, Kyoto, Japan) comprised of a diode array detector (SPD-M20A). The column was a Carotenoid-C30 (250 mm x 4.6 mm, 5 μm, YMC, Japan) and the detection wavelength was set at 340 nm for SHVB extract. The column temperature was set to 35 ℃. Mobile phase A was water and mobile phase B was acetonitrile with the elution profile as follows: 86 – 84 % A, 0 – 40 min, 84 – 100 % A; 40 – 42 min, 100 % A; 42 – 52 min, 100 – 86 % A; 52 – 53 min, 86 % A; 53 – 60 min. The flow rate was 1 mL/min, and the injection volume was 10 μL. These analysis were approved by Korea Health Supplement Institute (approval no. D2019072484).

14. Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM). Data were statistically evaluated by a Student's t-test or one-way analysis of variance (ANOVA) using the GraphPad Prism version 5.00 for Windows (GraphPad software, San Diego, CA, USA) software program. The differences between the groups were assessed by using Duncan's multiple range tests. A value of $P < 0.05$ was considered statistically significant.

Results

1. Effects of SHVB on behavioral immobility in FST in CRS-exposed mice

In order to investigate the effects of SHVB on the duration of immobility, swimming, and climbing behavior, we performed an FST. As shown in Fig. 1A, CRS-exposed mice showed a significant increase in the immobility time compared with the control group ($P < 0.01$), while treatment with EO (10 mg/kg, positive control; $P < 0.05$) and SHVB (200 mg/kg, $P < 0.01$) significantly led to a decrease in the immobility time than the CRS group. In contrast, as shown in Fig. 1B and C, CRS-exposed mice showed a significant decrease in swimming ($P < 0.05$) and climbing (not significant) behavior compared with the control group. However, SHVB treatment (200 mg/kg) significantly induced an increase in the climbing and swimming behavior ($P < 0.05$ and $P < 0.001$, respectively) compared with the CRS group.

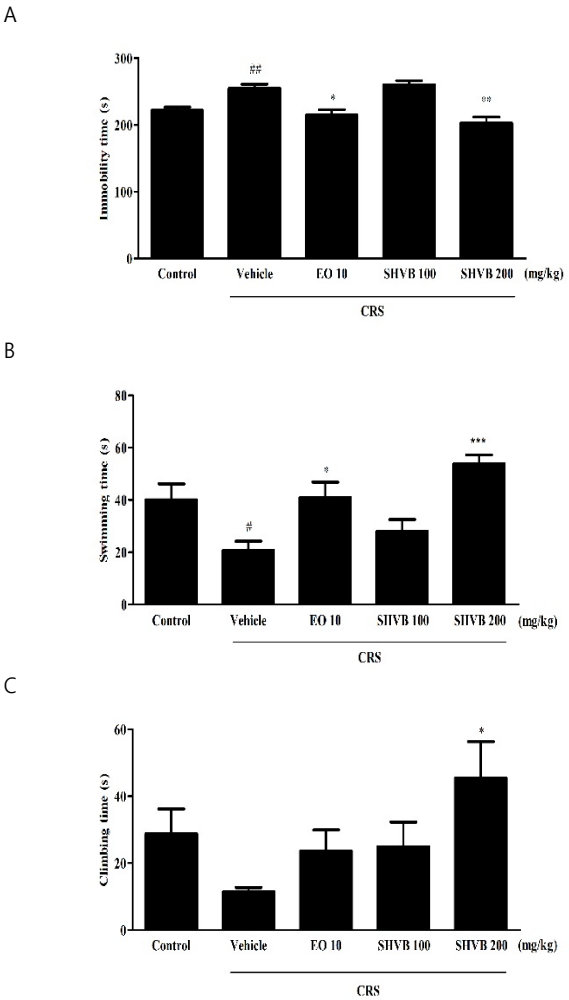

Fig. 1. Effects of SHVB treatment in the forced swim test (FST) in CRS-exposed mice. The effects of SHVB (100, and 200 mg/kg/day) on the duration of immobility (A), swimming (B), and climbing (C) behavior in the FST in CRS-induced mice. CRS, chronic restraint stress; EO 10, escitalopram oxalate 10 mg/kg; SHVB 100, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 100 mg/kg; SHVB 200, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 200 mg/kg. The values are expressed as the mean ± standard error of the mean (n = 5). $^{\#}$ $P < 0.05$ and $^{\#\#}$ $P < 0.01$ compared with the control group; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with the CRS group.

2. Effects of SHVB on the movement activity in CRS-exposed mice

The effects of SHVB (100 and 200 mg/kg/day) on the movement activity are shown in Fig. 2. CRS-exposed mice showed a significant decrease in the number of crossings compared with the control group ($P < 0.01$). However,

treatment with SHVB (100 and 200 mg/kg, $P < 0.05$, respectively) and EO (10 mg/kg, positive control, $P < 0.01$) showed higher locomotor activity compared with the CRS group.

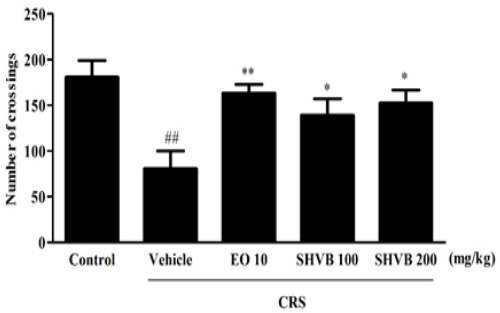

Fig. 2. Effects of SHVB on locomotor activity in CRS-exposed mice. CRS, chronic restraint stress; EO 10, escitalopram oxalate 10 mg/kg; SHVB 100, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 100 mg/kg; SHVB 200, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 200 mg/kg. The values are expressed as the mean ± standard error of the mean (n = 5). $^{\#\#}$ $P < 0.01$ compared with the control group; * $P < 0.05$, and ** $P < 0.01$ compared with the CRS group.

3. Effects of SHVB on serum CORT levels in CRS-exposed mice

As shown in Fig. 3, the levels of serum CORT significantly increased in CRS-exposed mice ($P < 0.05$) compared with the control group. However, SHVB (100 and 200 mg/kg) treatment decreased levels of CORT ($P < 0.05$, respectively) levels than the CRS group.

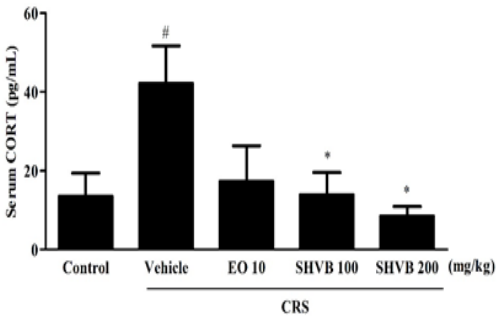

Fig. 3. Effects of SHVB on serum CORT levels in CRS-exposed mice. The levels of CORT were determined by ELISA kit (Abnova Corp.). CORT, corticosterone; CRS, chronic restraint stress; EO 10, escitalopram oxalate 10 mg/kg; SHVB 100, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 100 mg/kg; SHVB 200, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 200 mg/kg. The values are expressed as the mean ± standard error of the mean (n = 5). $^{\#}$ $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the CRS group.

4. Effects of SHVB on monoamine neurotransmitter levels in CRS-exposed mice

The levels of 5-HT, DA, and NE in the PFC when mice were exposed to CRS for 21 days are shown in Fig. 4. The 5-HT ($P < 0.05$), DA (not significant), and NE (not significant) levels in the PFC were decreased compared with those in the control group. In contrast, daily administration of SHVB (100 and 200 mg/kg) significantly increased the 5-HT ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 4A), DA ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 4B), and NE ($P < 0.05$, respectively) (Fig. 4C) levels in the PFC compared with those in the CRS group. Moreover, EO (10 mg/kg) also increased the 5-HT ($P < 0.05$), DA ($P < 0.05$), and NE ($P < 0.05$) levels in the PFC compared with those in the CRS group.

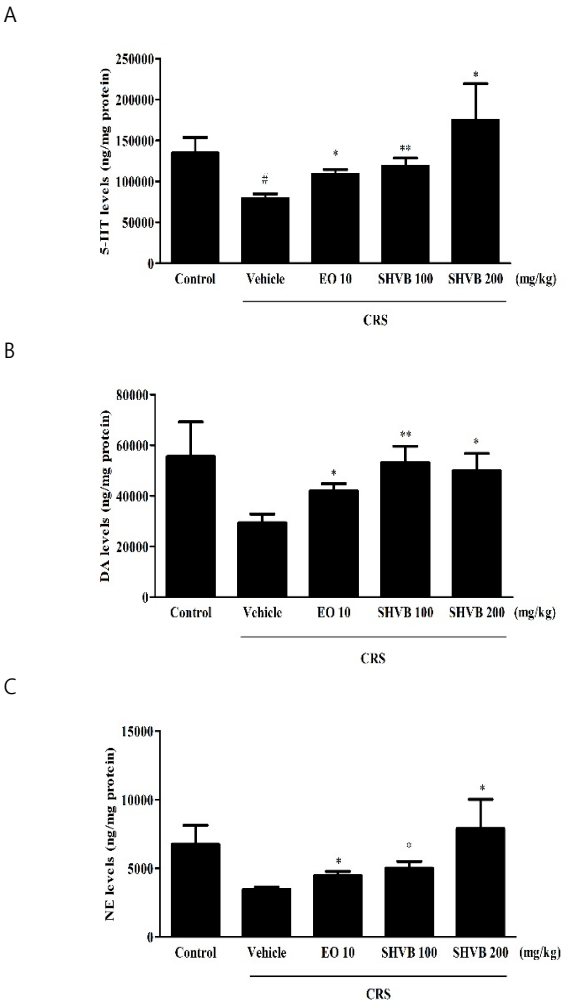

Fig. 4. Effects of SHVB on monoamine neurotransmitters levels in CRS-exposed mice: (A) 5-HT, (B) DA, and (C) NE levels in the PFC. CRS, chronic restraint stress; DA, dopamine; EO 10, escitalopram oxalate 10 mg/kg; 5-HT, serotonin; NE, norepinephrine; PFC, prefrontal cortex; SHVB 100, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 100 mg/kg; SHVB 200, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 200 mg/kg. The values are expressed as the mean ± standard error of the mean (n = 5). $^{\#}$ $P < 0.05$ compared with the control group; * $P < 0.05$, and ** $P < 0.01$ compared with the CRS group.

5. Effects of SHVB on pentobarbital sodium-induced sleep in mice

After hypnotic pentobarbital (45 mg/kg, i.p.) injection,

the latency of sleep and total sleep time were observed, and the results are shown in Fig. 5. Treatment with SHVB (200 mg/kg/day, 163.14 ± 4.17 s, $P < 0.05$) led to significantly lower sleep latency compared with the control group (326.08 ± 26.12 s) (Fig. 5A). In addition, treatment with SHVB (100 and 200 mg/kg/day, 6760.12 ± 299.90 s and 7890.08 ± 911.73 s, $P < 0.05$, respectively) led to a significantly higher total sleep duration compared with the control group (3188.51 ± 301.79 s) (Fig. 5B). Similarly, the DZP (diazepam, 2 mg/kg) treated mice also showed a decrease in the sleep latency and an increase in the total sleep duration compared with the control group (147.66 ± 3.13 s and 13770.33 ± 2897.43 s, $P < 0.01$ and $P < 0.05$, respectively).

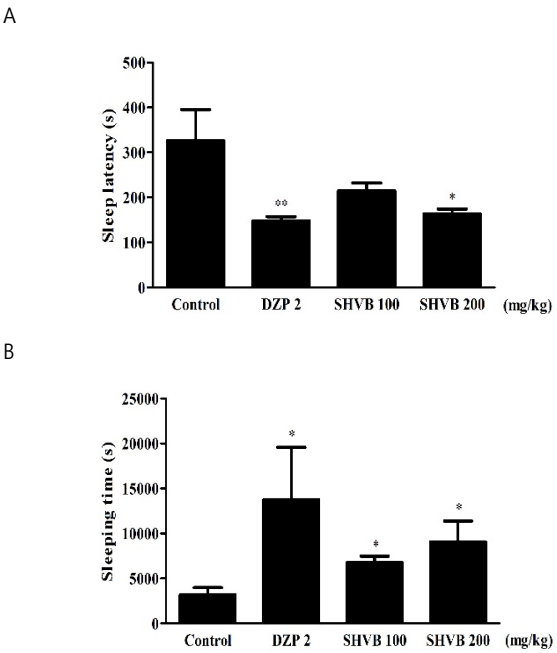

Fig. 5. Effects of SHVB on the onset and duration of sleep in pentobarbital-treated mice. The sleep latency (A) and total sleeping time (B) were measured. DZP 2, diazepam 2 mg/kg; SHVB 100, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 100 mg/kg; SHVB 200, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract 200 mg/kg. The values are expressed as the mean ± standard error of the mean (n = 5). * $P < 0.05$ and ** $P < 0.01$ compared with the control group.

6. Effects of SHVB on ERK1/2 pathways in 5-HT_{2A} receptor expressed in CHO-K1 cells

We next investigated whether the ERK1/2 phosphorylation pathway in CHO-K1 cells transfected with the human 5-HT_{2A} receptor was involved in the antidepressant-like effects caused by SHVB. We measured the cytotoxicity of SHVB using the MTT assay. The results indicated that SHVB at concentrations of 25, 50, and 100 μg/mL did not alter the viability of CHO-K1 cells (Fig. 6A). As shown in Fig. 6B, treatment with either the 5-HT_{2A} receptor antagonist, risperidone, (10 μM) or SHVB (50 and 100 μg/mL) did not affect ERK1/2 phosphorylation at 30 min. As shown in Fig. 6C and D, treatment with 5-HT (100 μM) for 15 min increased ERK1/2 phosphorylation, while pre-treatment with SHVB (50 and 100 μg/mL) or risperidone (10 μM) decreased 5-HT-mediated ERK1/2 phosphorylation.

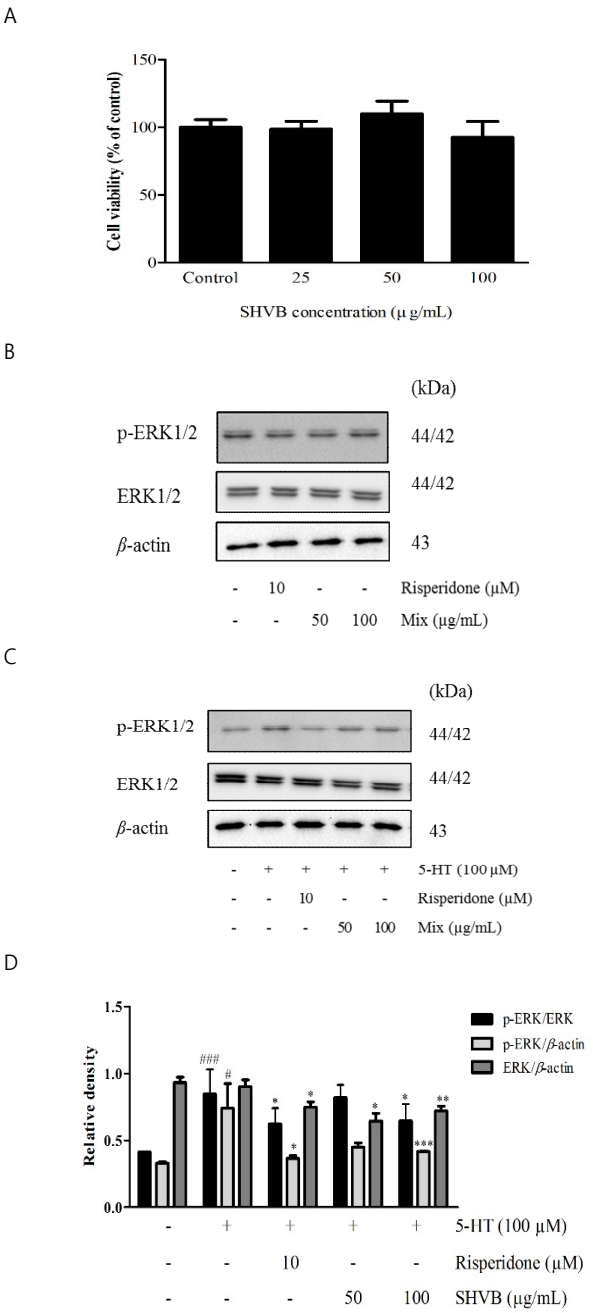

Fig. 6. Effects of SHVB on ERK1/2 phosphorylation in CHO-K1 cells transfected with the human 5-HT_{2A} receptor. (A) CHO-K1 cells were treated with SHVB for 24 h and cell viability was determined by MTT assay. (B) CHO-K1 cells transfected with the human 5-HT_{2A} receptor were treated with SHVB (50 or 100 μg/mL) or risperidone (5-HT_{2A} receptor antagonist, 10 μM) for 30 min. (C) For the evaluation of antagonistic effects, CHO-K1 cells transfected with the human 5-HT_{2A} receptor were treated with 5-HT (100 μM) for 15 min after the pre-treatment with SHVB

(50 or 100 μg/mL) or risperidone (10 μM) for 15 min. (D) Quantitative analysis of relative ERK1/2 phosphorylation. 5-HT_{2A}, serotonin receptor subunit 2A; CHO-K1, Chinese Hamster Ovary K1 cells; ERK, extracellular signal-regulated kinases; SHVB, *Stauntonia hexaphylla* and *Vaccinium bracteatum* water extract. The values are expressed as the mean ± standard error of the mean (n = 3). $^{\#}$ $P < 0.05$ and $^{\#\#\#}$ $P < 0.001$ compared with the control group; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the 5-HT group.

7. HPLC Analysis of the SHVB

We identified the SHVB by HPLC analysis. An illustration of the retention times is shown in Fig. 7.

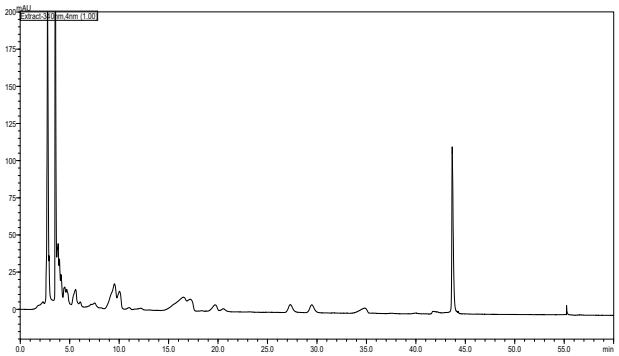

Fig. 7. HPLC chromatogram of SHVB with detection at 340 nm.

Discussion

In this study, we showed that SHVB treatment in CRS-exposed mice significantly decreased the immobility time ($P < 0.01$) and increased swimming ($P < 0.001$) and climbing ($P < 0.05$) time in the FST (Fig. 1). SHVB treatment also increased locomotor activity ($P < 0.05$) in the OFT (Fig. 2). Chronic stress models are commonly used in animal studies to mimic the pathology of human depressive-like disorders[27]. Chronic stress exposure can cause structural degeneration and the impaired functionality of the PFC[28]. Chronic stress also activates the hypothalamus-pituitary-adrenal (HPA) axis, which is accompanied by oxidative stress and neuro-inflammatory damage in the PFC. In addition, chronic stress associated with depressive disorders are accompanied by increased CORT levels and decreased monoamine neurotransmitter levels in the PFC[29]. We identified that CRS-exposed mice showed increased serum CORT levels and decreased monoamine neurotransmitter (such as 5-HT, DA, and NE) levels in the PFC, while administration of SHVB showed a significant decrease in serum CORT levels (Fig. 3), and increase in 5-HT, DA, and NE levels in the PFC (Fig. 4).

Depression is one of the main clinical manifestations of menopause, which occurs in women. Some studies suggest that menopausal depression may be associated with dysfunction of the GABA receptor and the HPA axis in response to stress, due to altered ovarian hormones and serotonin-norepinephrine levels[30,31]. In addition, sleep problems are common during the menopause, influenced by the decline of estrogen. Insomnia is very common in patients with various depressive disorders. It has been reported that chronic stress induced sleep problems are characterized by active awakening (NREM and REM sleep); in particular, sleep problems are common in stress-related disorders through regulation of CORT levels[32]. Evaluation of pentobarbital-induced sleeping behavior was used in an in vivo animal model. In the present study, we confirmed that SHVB treatment significantly decreased the sleep latency and increased the total sleep duration (100 and 200 mg/kg, $P < 0.05$, respectively) in a hypnotic dosage of pentobarbital (45 mg/kg, i.p.)-treated mice (Fig. 5). We previously reported that VB fruit extract (100 mg/kg) alone showed significantly lower sleep latency and higher total sleep duration than the control group[25]. These results suggest that the SHVB combination may have higher synergistic effects on sleep behavior than the VB fruit alone. Therefore, SHVB could be used as a potential functional therapeutic for the treatment of menopausal symptoms due to of its antidepressant effects.

Many preclinical studies suggested that targeting various 5-HT receptors (e.g. 5-HT_{1A}, 5-HT_{2A}, 5-HT_3, 5-HT_6, and 5-HT_7receptors) contribute to antidepressant effects[6]. Of these, the 5-HT_{2A} receptor, has been linked to depression by stress, which is associated with the activation of glucocorticoid receptors[33]. 5-HT_{2A} receptor antagonists, such as mirtazapine and nefazodone, may have a beneficial effects in the treatment of depression and primary insomnia[34]. In this study, we demonstrated an interaction between 5-HT_{2A} receptor and SHVB, and its related signaling pathway involving ERK1/2 phosphorylation (Fig. 6).

Recent studies have found herbal medicines to be effective complementary therapies for depression, were predicted to induce less severe side effects. St. John's wort (*Hypericum perforatum* L.) is a commonly used herbal medicine for the treatment of depression and insomnia. These herbs have been found to work through the regulation of monoamine neurotransmitters (e.g. 5-HT, DA, and NE) and gamma amino butyric acid ($GABA_A$ and $GABA_B$) receptors, as well as through the modulation of 5-HT_2 receptors in the frontal cortex[35]. Therefore, this studies demonstrated that SHVB has a potent antidepressant effect through the regulation of serum CORT and monoamine neurotransmitters, with an important role in the 5-HT_2

receptors signaling pathways. We evaluated the acute toxicity of SHVB (100 and 200 mg/kg) in normal mice and confirmed to be safe herb via liver, kidney, and spleen in individual histopathologic evaluations (data not shown). Traditional herbal medicines contain multiple compounds which act on multiple targets, and can treat complex symptoms. We previously reported that VB fruits contain active constituents such as orientin, hyperin, chlorogenic acid, neochlorogenic acid, crytochlorogenic acid, isoorientin, and rutin[23]. Moreover, we reported that SH fruits include some of these active constituents such as neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid[12]. Therefore, further studies are required to define the active constituents of SHVB that contributes to its antidepressant effects and sleep improvement.

In Conclusion, the present study demonstrates that the antidepressant effects of SHVB are mediated by levels of serum CORT and the monoamine neurotransmitters (5-HT, DA, and NE) in the PFC in CRS-induced mice. In addition, SHVB exerts hypnotic effects in pentobarbital-induced sleep behavior. Moreover, the results of the present study suggest that SHVB exert antidepressant effects possibly related to the regulation of the 5-HT_{2A} receptor systems.

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