Antinociceptive effect of *Valeriana fauriei* regulates BDNF signaling in an animal model of fibromyalgia

HWAYOUNG LEE[1], JIYUN IM[1], HANSOL WON[1], JUN YOUNG KIM[1], HYUNG-KI KIM[1], JUN-TACK KWON[1], YOUNG OCK KIM[2], SANGHYUN LEE[3], IK-HYUN CHO[4], SANG WON LEE[2] and HAK-JAE KIM[1,5]

[1]Department of Clinical Pharmacology, College of Medicine, Soonchunhyang University, Cheonan, Chungnam 31151; [2]Development of Ginseng and Medical Plants Research Institute, Rural Administration, Eumseong, Chungbuk 27709; [3]Department of Integrative Plant Science, Chung-Ang University, Anseong, Gyeonggi 17546; [4]Department of Convergence Medical Science, Brain Korea 21 Plus Program, and Institute of Korean Medicine, College of Oriental Medicine, Kyung Hee University, Seoul 02447; [5]Soonchunhyang Medical Research Institute, College of Medicine, Soonchunhyang University, Cheonan, Chungnam 31151, Republic of Korea

Received November 22, 2016; Accepted September 22, 2017

DOI: 10.3892/ijmm.2017.3203

Abstract. The genus *Valeriana* has been widely used in popular medicine for centuries, to treat sleep disorders, anxiety, epilepsy and insomnia. Recent studies have focused on the novel pharmacological effects of *Valeriana fauriei* Briq. (VF) species. Previous studies have attempted to determine the pharmacological functions of *Valeriana* in various human diseases, particularly with regards to its neuroprotective effects, and its ability to reduce pain and stress. The present study constructed an animal model of fibromyalgia (FM), which was induced by intermittent cold stress with slight modification. Subsequently, the study aimed to determine whether VF exerts antinociceptive effects on the FM-like model following oral administration of VF extracts. The effects of VF extracts on the FM model were investigated by analyzing behavioral activity, including pain, and detecting protein expression. In the behavioral analysis, the results of a nociception assay indicated that the pain threshold was significantly decreased in the FM group. Subsequently, western blotting and immunohistochemical analyses of the hippocampus demonstrated that the protein expression levels of brain-derived neurotrophic factor (BDNF) and phosphorylated-cAMP response element-binding protein were downregulated in the FM group. Conversely, VF restored these levels. These results suggested that the effects of VF extract on a model of FM may be associated with its modulatory effects on the BDNF signaling pathway in the hippocampus and medial prefrontal cortex. In conclusion, the mechanism underlying the protective effects of VF as a therapeutic agent against FM may involve the BDNF signaling pathway.

Correspondence to: Professor Hak-Jae Kim, Department of Clinical Pharmacology, College of Medicine, Soonchunhyang University, 25 Bongjeong-ro, Cheonan, South Chungcheong 31151, Republic of Korea
E-mail: hak3962@sch.ac.kr

Key words: *Valeriana fauriei* Briq., fibromyalgia, brain-derived neurotrophic factor-cAMP response element-binding protein pathway, pain, animal model

Introduction

Fibromyalgia (FM) is characterized by generalized tenderness in ≥11 of 18 tender points (1) and chronic widespread pain that lasts >3 months (2). Mechanical hyperalgesia is a common symptom of FM (3), which is a painful syndrome of largely unknown etiology and pathology that is often accompanied by various phenomena. In addition, emerging evidence has indicated that pain amplification within the central nervous system serves an important role in the pathology of FM-associated pain (4), which is associated with numerous other symptoms (5). The symptoms of this painful syndrome include fatigue (6), anxiety (7), sleep disturbance (8), temporomandibular disease and depression (9). An animal model of FM must include widespread pain and associated symptoms of fatigue and psychological disturbance (3).

There are numerous animal models of FM pain, which are induced by either intramuscular injection of acidic saline (10), vagotomy (11), sound stress (12) or depletion of biogenic amines (13). A previous study described a novel generalized chronic pain or FM-like mouse model as part of a pharmacological study. This FM model was induced by intermittent cold stress (ICS), which is useful for inducing abnormal pain (14,15). Xu *et al* previously revealed that complex interactions exist between pain and depression (16). Previous studies have suggested that FM is associated with emotional disorders, including depression (17); ≥30% of patients with chronic pain have major depression, and 30% are diagnosed with panic and diffuse anxiety disorder (18).

Brain-derived neurotrophic factor (BDNF) is an upstream activator and a downstream target of cAMP response element-binding protein (CREB)-mediated signaling (19) CREB activity is tightly regulated by its phosphorylation at serine 133 (20); therefore, it would be useful to analyze BDNF and phosphorylated (p)-CREB levels following identical treatments (21). Furthermore, the

BDNF/tropomyosin receptor kinase B-mediated signaling pathway within the spinal cord may be involved in the induction of neuropathic pain. In a previous study, treatment with a selective N-methyl-D-aspartate (NMDA)-2B receptor antagonist completely blocked BDNF-induced mechanical allodynia in animals (22). BDNF may play a protective role in FM, including pain modulation and mental disorders, such as depression. In addition, it has been suggested that the expression of BDNF is a downstream target of various antidepressants (23) and BDNF is an important candidate gene in antidepressant medication (24). In addition, the principal treatment for depression consists of pharmacotherapy with selective serotonin reuptake inhibitors (25), including Cymbalta (duloxetine), which is a Food and Drug Administration-approved drug for the treatment of FM (26).

Valeriana fauriei Briq. (VF) is a perennial herb found in all of North America, most parts of Europe, and Northern Asia (27). The genus *Valeriana* contains >250 species with many subspecies containing medicinal plants (28). VF has been used for many years in China and Korea (29), and contains 150-200 chemical constituents of biologically active components that can be divided into volatile oils, epoxy iridoid esters and alkaloids (30). These components have been reported to inhibit γ-aminobutyric acid (GABA) re-uptake (31) The genus *Valeriana* has been widely used in popular medicine for centuries to treat sleep disorders, anxiety and epilepsy. It can also modulate insomnia by interacting with various neurotransmitter systems (32). Previous studies have focused on the novel pharmacological effects of VF species in various human diseases. Some studies have attempted to explain the pharmacological functions of VF, particularly with regards to its neuroprotective effects in neurodegenerative diseases (33-35), and its ability to reduce pain, cyclic cramps and stress (36,37).

These previous findings have resulted in the hypothesis that VF may exert beneficial effects on FM with depression. Therefore, in the present study, the behavior of mice and the protein levels in the medial prefrontal cortex and hippocampus of a mouse model of FM were examined, in order to determine whether treatment with VF reverses any changes.

Materials and methods

Animals. Male adult C57BL/6J mice were obtained from Daehan Biolink, Co., Ltd. (Eumseong, South Korea). All groups contained between 12 and 14 mice with similar numbers of males: the 'control' (n=12 males) group; the drug non-treated model group 'FMS' (n=14 males); and the VF administration model group (n=14 males). Mice were housed in clear cages with access to free food and water, and were maintained in an environment with the temperature-controlled to 23±2˚C, humidity-controlled to 50-60% and under a 12-h light-dark cycle (lights were turned on at 6:30 a.m.). All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the US National Institutes of Health (38). The present study was approved by the Animal Care and Use Committee of Soonchunhyang University (Cheonan, South Korea; SCH16-0062).

Drugs and treatments. VF extract was purchased from Yunpung (Eumsung, Chungbuk, South Korea), and the specimens were identified taxonomically by an Oriental medicine physician at the National Institute of Horticultural and Herbal Science, Rural Development Administration (Eumsung, South Korea). The voucher specimen (HPR-207) was deposited in the herbarium of the Herbal Crop Research Institute (Eumsung, South Korea). VF was dissolved in tap water to a concentration of 100 mg/kg/day. The animals were orally administered VF solution during stress for 24 days (39).

Experimental model of FM. An animal model of FM was constructed as previously described, with slight modification (40,41). For the chronic restraint stress (CRS) and intermittent cold stress (ICS) paradigm, the mice were restrained for 6 h (between 12 a.m. and 6 p.m.) daily in well-ventilated 50-ml conical tubes and were deprived of food and water. Control mice remained undisturbed in their cages. The protocol was scheduled for 21 days. For the ICS paradigm, mice were placed on a stainless steel mesh plate in a cold room at 4˚C overnight (between 4:30 p.m. and 10:00 a.m.), followed by ICS with experimental temperatures alternating between 24 and 4˚C every 30 min, between 10:00 a.m. and 4:30 p.m. These procedures were repeated for 2 days. On day 3, the mice were adapted to 24˚C for 1 h prior to behavioral analysis. Control mice were maintained at 24˚C for the 3 days (from 4:30 p.m. on day 1 to 10:00 a.m. on day 3) (15).

Behavioral analysis. Behavioral assessments were conducted 1 day after the final day of ICS (n=10 mice/group). Mice were allowed to acclimate to the testing room for ≥1 h prior to the assessments. Tests for nociception and depression were performed. A tail flick test (TFT), a plantar test (PTL), and the von Frey test paw withdrawal threshold (PWT) test were used to assess nociception. Subsequently, a tail suspension test (TST) was used to assess depression. For these thermal and mechanical tests, thresholds were determined from three repeated challenges at 15 min intervals, and the averages were used for statistical analysis.

TFT. A TFT is used as a test of pain response, which can be used to assess the antinociceptive effects of drugs by measuring the latency time from the onset of radiant heat exposure to withdrawal of the tail (42,43). The thermal pain threshold of the tail was measured using tail flick apparatus (IITC Life Science Inc., Woodland Hills, CA, USA). Each animal was gently restrained under a 50-ml conical tube with light manual pressure so as to minimize stress. Radiant heat was applied to the tail (2 cm distal part) and latency (seconds) was determined as the time taken for the tail to flick away from the radiant heat source. A cutoff time of 20 sec of radiant heat application was applied to avoid tissue damage to the tail. Each mouse was tested three times at each time-point, with an interval of 15 min between replicates. The average of three replicates was calculated to obtain tail withdrawal latency.

PTL. In the thermal paw withdrawal test, nociceptive threshold is assessed by determining the latency of paw withdrawal upon thermal stimulus (44,45). Mice were placed in individual clear plastic chambers on top of a glass sheet and were acclimated for 1 h. Radiant heat (IITC Life Science Inc.) was positioned under the glass sheet, and the focus of the projection bulb was aimed exactly at the plantar surface of the hind paw of the

animal. Paw withdrawal latency (PWL), defined as the first occurrence of licking the hind paw, was scored. Each mouse was tested three times at each time-point, with an interval of 15 min between replicates. The average of three replicates was calculated to obtain PWL.

Von Frey PWT test. The pressure required to induce a flexor response was defined as the pain threshold. The Von Frey PWT test was conducted using digital von Frey apparatus (Aesthesiometer; IITC Life Sciences Inc.), as previously reported (46,47). Mice were placed in a plastic chamber on a wire mesh grid floor and were allowed to acclimate for 1 h. In this experiment, the threshold of a given pressure with a rigid tip used to induce paw withdrawal behavior was evaluated. To prevent tissue damage, the interval time was set at at least 5 min for each paw.

TST. The TST is a widely used model for the assessment of depression-like behaviors in mice. In this test, mice are subjected to short-term, inescapable stress by being suspended by the tail resulting in the development of immobility. In the present study, the total duration of tail suspension-induced immobility was measured according to Steru *et al* (44). Mice were suspended 50 cm above the floor using adhesive tape placed ~1 cm from the tip of the tail and the total duration of immobility during a 6-min period was measured.

Measurement of corticosterone. Following completion of the behavioral analyses, the mice were placed in 50-ml conical tubes for 60 min (n=6 mice/group). The animals for analysis were sacrificed by decapitation using a guillotine using a decapicone (48). Trunk blood was immediately collected in plastic tubes and was centrifuged at 15,814 x g at room temperature; the serum was placed in a fresh tube. Serum corticosterone levels were determined by immunoassay using the Cortisol ELISA kit (cat no. 500360; Cayman Chemical, Ann Arbor, MI, USA) . Assays were conducted according to the manufacturer's protocol.

Western blot analysis. mPFC and hippocampal tissues were lysed in mixture solution as radioimmunoprecipitation assay buffer [RIPA and EBA-1149 (Elpis Biotech, Inc., Daejeon, South Korea) and leupeptin and sodium fluoride (cat. nos. 103476-89-7 and 7681-49-4, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany], and were centrifuged at 18,341 x g for 10 min at 4˚C (n=6-7 mice/group). Protein concentration was calculated using a standard Bradford protein assay. These samples (100 μg) were separated by 15% SDS-PAGE and were transferred to a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). After blocking with 5% skim milk for 1h at room temperature, the membranes were probed with the following antibodies overnight at 4˚C: Anti-p-CREB (1:1,000; #9198), anti-CREB (1:1,000; #9197) (both from Cell Signaling Technology, Inc., Danvers, MA, USA), anti-BDNF (1:3,000; ab108319; Abcam, Cambridge, UK) and anti-β-tubulin (1:3,000; MA5-16308; Thermo Fisher Scientific, Inc., Rockford, IL, USA). Subsequently, the membrane was incubated with peroxidase-conjugated anti-mouse secondary antibody (1:10,000; A9044; Sigma-Aldrich; Merck KGaA) and goat anti-rabbit IgG-HRP antibody (1:5,000; LF-SA8002; Abfrontier, Seoul, Korea) for 1 h at room temperature. Immunoreactive bands were detected using an enhanced chemiluminescence kit (Elpis Biotech, Inc.). Semi-quantitative analysis of p-CREB, CREB, BDNF and β-tubulin protein expression was conducted using ImageJ software version 1.51k (National Institutes of Health, Bethesda, MD, USA).

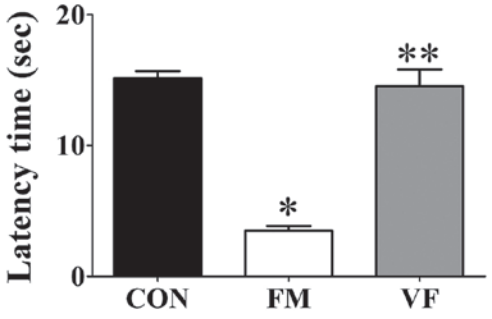

Figure 1. Differences in reaction time in the tail-flick test caused by VF administration. Effects of VF on nociceptive responses in the tail flick test. Data are presented as the mean ± standard error of the mean. *P<0.001 vs. Con group; **P<0.001 vs. FM group. P-values represent the significance of difference among the groups using two-way analysis of variance. Mean values followed within three different groups are significantly different at P<0.05 by Tukey's test. Con, control mice; FM, fibromyalgia mouse model; VF, *Valeriana fauriei* extract-administered FM mouse model.

Immunohistochemistry. The mice for immunohistochemical analysis had been anaesthetized with diethyl ether and were perfused through the left cardiac ventricle with 4% paraformaldehyde (n=4-5 mice/group). The fixed brains were removed, frozen and cut into 30-μm sections (n=4 mice/group). Frozen sections from the hippocampus were treated with 0.3% hydrogen peroxide for 5 min, blocked with normal horse serum (S-2000; Vector Laboratories, Inc., Burlingame, CA, USA), and were incubated with anti-p-CREB (1:800; #9198), anti-CREB (1:800; #9197) (both Cell Signaling Technology, Inc.). Subsequently, sections were incubated with Cy3-conjugated anti-rabbit (111-165-003) and anti-mouse (715-545-151) secondary antibodies (1:500; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). After washing in PBS, sections were stained with DAPI to identify nuclei. Fluorescent images were captured using a confocal laser-scanning microscope (FV10-ASW; Olympus Corporation, Tokyo, Japan), and images were semi-quantified with ImageJ software version 1.51k using a protocol described previously with slight modifications (49).

Statistical analysis. Data are expressed as the mean ± standard error of the mean, and assessed using one-way analysis of variance (ANOVA) with subsequent Tukey's tests. All statistical analyses were performed using IBM SPSS Statistics 19 software (IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Tail-flick latency. Tail-flick latency was significantly different among the control, FM and VF-administered FM groups (Fig. 1). The FM group exhibited decreased tail-flick latency compared with the control group (Fig. 1). Conversely, the FM-induced decrease in tail-flick latency was attenuated following VF administration (Fig. 1).

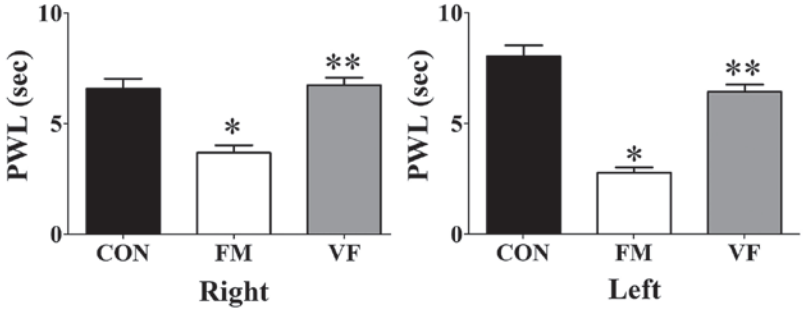

Figure 2. Differences in reaction time in the plantar test caused by VF administration. Effects of VF on nociceptive responses in the plantar test. Data are presented the mean ± standard error of the mean. *P<0.001 vs. Con group; **P<0.001 vs. FM group. P-values represent the significance of difference among the groups using two-way analysis of variance. Mean values followed within three different groups are significantly different at P<0.05 by Tukey's test. Con, control mice; FM, fibromyalgia mouse model; left, hind left paw; PWL, paw withdrawal latency; right, hind right paw; VF, *Valeriana fauriei* extract-administered FM mouse model.

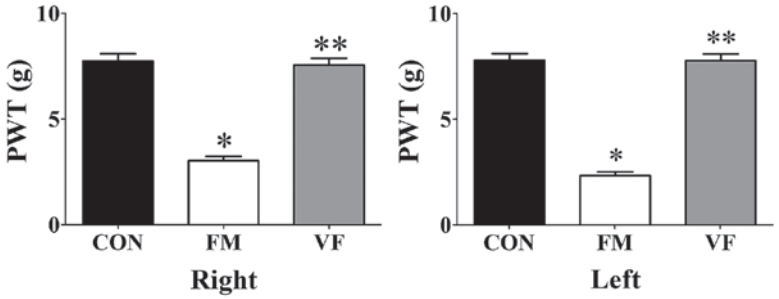

Figure 3. Differences in reaction to stimulation in the Von Frey test caused by VF administration. Withdrawal responses to the von Frey filaments from both hind paws were counted and expressed as the average in grams. Data are presented the mean ± standard error of the mean. *P<0.001 vs. Con group; **P<0.001 vs. FM group. P-values represent the significance of difference among the groups using two-way analysis of variance. Mean values followed within three different groups are significantly different at P<0.05 by Tukey's test. Con, control mice; FM, fibromyalgia mouse model; left, hind left paw; PWT, paw withdrawal threshold; right, hind right paw; VF, *Valeriana fauriei* extract-administered FM mouse model.

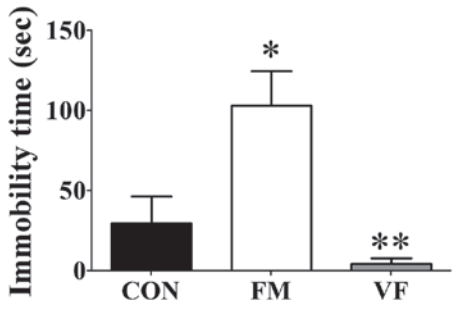

Figure 4. Effects of VF treatment on depression-like behavior in a mouse model of FM, as determined using a tail suspension test. Data are presented the mean ± standard error of the mean. *P<0.05 vs. Con group; **P<0.001 vs. FM group. P-values represent the significance of difference among the groups using two-way analysis of variance. Mean values followed within three different groups are significantly different at P<0.05 by Tukey's test. Con, control mice; FM, fibromyalgia mouse model; VF, *Valeriana fauriei* extract-administered FM mouse model.

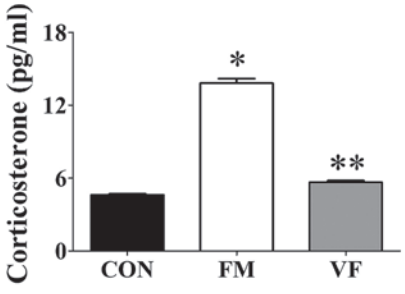

Figure 5. Effects of VF extract on serum corticosterone levels. Corticosterone levels in the VF-administered FM group returned to the control levels. Corticosterone levels were expressed as concentration in pg/ml. Data are presented the mean ± standard error of the mean. *P<0.05 vs. Con group; **P<0.001 vs. FM group. P-values represent the significance of difference among the groups using two-way analysis of variance. Mean values followed within three different groups are significantly different at P<0.05 by Tukey's test. Con, control mice; FM, fibromyalgia mouse model; VF, *Valeriana fauriei* extract-administered FM mouse model.

PWL. Significant differences among the control, FM and VF-administered FM groups were detected with regards to the PWL of both paws (Fig. 2). The FM group exhibited decreased PWL compared with the control group (Fig. 2). Conversely, this FM-induced decrease in PWL in the plantar test was attenuated following VF administration (Fig. 2).

PWT. VF exerted beneficial effects on the PWT of both paws in the VF-administered FM group (Fig. 3). The FM group exhibited a decreased PWT compared with the control group (Fig. 3). Conversely, this FM-induced decrease in PWT was attenuated following VF administration (P<0.01; Fig. 3).

Tail suspension. The duration of immobility was measured in the TST, in order to evaluate stress-associated depression in mice. The duration of immobility in the FM group was significantly increased compared with the control group (P<0.05; Fig. 4). Following VF administration, the duration of immobility was

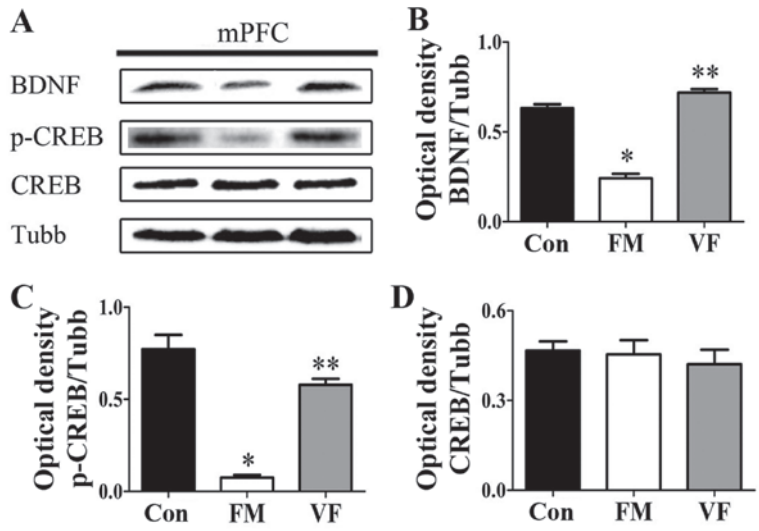

Figure 6. Effects of VF treatment on the protein expression levels of BDNF and p-CREB in FM mice (n=5 mice/group). (A) BDNF, p-CREB and CREB levels in the mPFC were determined by western blot analysis. (B-D) Semi-quantitative analysis of western blotting data for BDNF, p-CREB, CREB expression was conducted using ImageJ. There was a significant difference in BDNF and p-CREB expression between the FM and VF-administered FM groups. *P<0.05 vs. Con group; **P<0.05 vs. FM group. BDNF, brain-derived neurotrophic factor; Con, control mice; CREB, cAMP response element-binding protein; FM, fibromyalgia mouse model; mPFC, medial prefrontal cortex; p-CREB, phosphorylated-CREB; Tubb, β-tubulin; VF, *Valeriana fauriei* extract-administered FM mouse model.

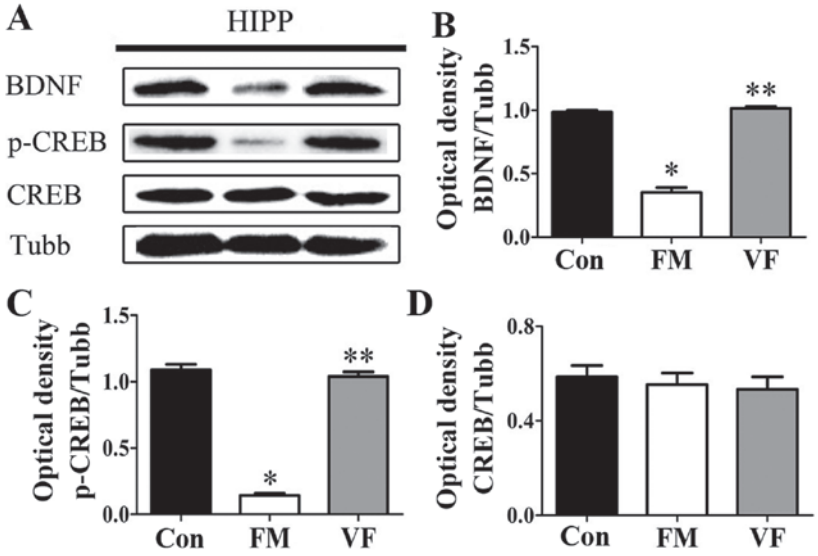

Figure 7. Effects of VF treatment on the protein expression levels of BDNF and p-CREB in FM mice (n=5 mice/group). (A) p-CREB, CREB and BDNF levels in the HIPP were determined by western blot analysis. (B-D) Semi-quantitative analysis of western blotting data for BDNF, p-CREB, CREB expression was conducted using ImageJ. There was a significant difference in BDNF and p-CREB expression between the FM and VF-administered FM groups. *P<0.05 vs. Con group; **P<0.05 vs. FM group. BDNF, brain-derived neurotrophic factor; Con, control mice; CREB, cAMP response element-binding protein; FM, fibromyalgia mouse model; HIPP, hippocampus; p-CREB, phosphorylated-CREB; Tubb, β-tubulin; VF, *Valeriana fauriei* extract-administered FM mouse model.

significantly decreased compared with the FM group (Fig. 4), thus suggesting that VF may reverse depression in the FM group.

Corticosterone levels. Corticosterone levels were increased in the FM group (Fig. 5) compared with those in the control group. However, the corticosterone levels in the VF-administered FM group returned to the control levels (P<0.001; Fig. 5).

Western blot analysis and immunohistochemistry. In order to investigate whether the BDNF-CREB pathway, which is known to be associated with depression and pain, is involved in behavioral abnormalities in the FM group, western blotting (Figs. 6 and 7) was performed on samples from the medial prefrontal cortex and hippocampus. In addition, immunohistochemical analyses (Fig. 8) were performed on samples from the hippocampus of the control, FM and VF-administered FM groups. Western blot analysis revealed that the protein expression levels of BDNF and p-CREB in the medial prefrontal cortex and hippocampus were significantly reduced in the FM group compared with in the control group (P<0.05). However, these alterations were reversed by VF administration (P<0.05; Figs. 6 and 7). In addition, p-CREB was differentially

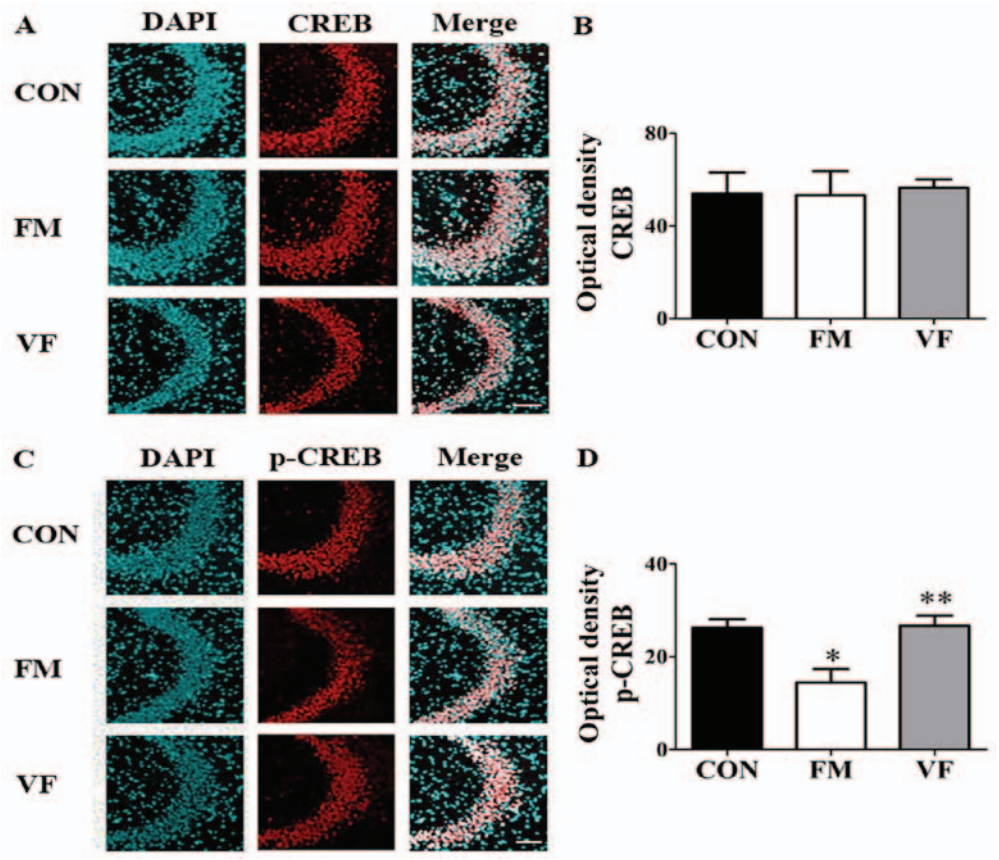

Figure 8. Patterns of p-CREB expression across the hippocampus in the FM model and VF-administered FM model groups (n=4 mice/group). (A) Immunohistochemistry was performed to determine CREB immunoreactivity in the hippocampus. Confocal microscopy image presents immunofluorescent staining for CREB (Cy3 anti-CREB; red) with DAPI (blue) in the hippocampus. Scale bar, 50 μm. (B) Optical density values of CREB signals in immunostained hippocampus. (C) Confocal microscopy image showing immunofluorescent staining of p-CREB (Cy3 anti-p-CREB; red) with DAPI (blue) in the hippocampus. Scale bar, 50 μm. (D) Optical density values of p-CREB signals in immunostained hippocampus. *P<0.05 vs. Con group; **P<0.05 vs. FM group. Con, control mice; CREB, cAMP response element-binding protein; FM, fibromyalgia mouse model; p-CREB, phosphorylated-CREB; VF, *Valeriana fauriei* extract-administered FM mouse model.

expressed in the immunofluorescent-stained brain images of the control, FM and VF-administered FM groups (P<0.05; Fig. 8).

Discussion

In order to evaluate the extent to which treatment with VF alters behavioral activity and protein expression that may be associated with the pathophysiology of FM, the present study determined the effects of VF treatment on behavioral phenotypes using the TST, TFT, PWL and PWT. The present study demonstrated that BDNF and p-CREB expression were significantly decreased in the medial prefrontal cortex and hippocampus of mice in the FM group. The protein expression levels of BDNF and p-CREB were increased in the VF-administered FM model, as determined by western blotting and immunohistochemistry. In addition, mice in the FM model group exhibited a transient increase in serum corticosterone levels. These results provide evidence of a possible association between the BDNF-CREB pathway and FM susceptibility and pathophysiology.

Previous interest in herbal medicine has focused on the mechanisms underlying neuroendocrinological abnormalities, including the hypothalamic-pituitary-adrenal axis, cortisol production and BDNF, as well as impaired endogenous opioid function, alterations in GABAergic and/or glutamatergic transmission, cytokine or steroidal alterations, and abnormal circadian rhythm (50,51). It has been previously reported that BDNF, a key protein in the BDNF pathway, is a member of the neurotrophin family, which includes nerve growth factor, neurotrophin (NT)-3, NT-4 and BDNF (52). BDNF is involved in the development and survival of neurons and in modulating the activity of neurotransmitter systems (53), particularly serotonin and dopamine (52), which are abnormal in FM (54). Furthermore, increased BDNF-related hyperalgesia is dependent on an NMDA receptor-mediated mechanism (55). This study has reported that BDNF produces an acute, dose-dependent thermal hyperalgesic response in normal mice while antisense directed against either BDNF or its receptor, prevent inflammation-induced hyperalgesia. Furthermore, our FM model showed a thermal hyperalgesic response with decreased BDNF expression. There is growing evidence indicating that BDNF also serves a role in major depressive disorders and that antidepressant treatments increase serum BDNF levels (56,57). In animal and human studies, antidepressant treatments may increase central, as well as peripheral, BDNF levels (23,24). Liu *et al* reviewed studies that had been performed to identify additional modes of action of various herbal medicines

on several mood disorders and antidepressants (57). The results suggested that Fuzi total alkaloid increased the phosphorylation levels of CREB and the expression of BDNF in the frontal cortex and hippocampus of an animal model. In addition, in murine models it was reported that the Fuzi total alkaloid-induced generation of antidepressant-like effects may involve the CREB-BDNF pathway (58). Furthermore, hydrophilic constituents of *Morinda citiforlia* are well known in folk medicine for a wide range of health purposes, including anti-inflammatory, antioxidative, detoxifying and cell-rejuvenating properties (59). In the present study, it was suggested that the BDNF-CREB pathway may be associated with FM-related pain. In addition, VF was revealed to exert anti-depressive and anti-hyperalgesic effects via the BDNF-CREB pathway.

Several studies have indicated that herbal hypnotics and sedatives, including *Valeriana* spp. (valerian) and *Humulus lupulus* (hops), usually work via modulation of adenosine receptors and via melatoninergic effects (60-62). In a previous study, valerian and its primary active component, valerenic acid, produced anxiolytic and sedative effects mainly via GABA-ergic mechanisms, similar to benzodiazepine drugs (62,63). Numerous studies have reported that the phenomenons of anxiety, psychological distress and depression are associated with the chronicity of pain (64,65), rather than tissue damage (66). The relationship between physical disease, psychiatric disorders and chronic pain is likely caused by a complex interaction. Ammer and Melnizky assessed the effects of pine oil and valerian on pain, sleep and tender point count in FM. This previous study indicated that valerian baths were associated with improved sleep, pine oil baths with increased sensitivity to pain in certain body areas, and plain water baths appeared to reduce pain intensity (67).

In the present study, the decrease in the protein expression levels of BDNF and p-CREB following ICS and CRS procedures was revealed to be reversed by treatment with VF. In addition, the results confirmed that the FM animal model was able to induce FM-like pain via abnormalities in BDNF signaling. The present study demonstrated the beneficial effects of VF on FM-associated symptoms, including depression and hyperalgesia. However, further research using cellular and animal model systems or human patients is required to fully characterize the pharmacological functions of VF on FM.

Acknowledgements

The present study was carried out with the support of the Cooperative Research Program for Agriculture Science and Technology Development (grant no. PJ01158203), Rural Development Administration, Republic of Korea.

References

1. Lindell L, Bergman S, Petersson IF, Jacobsson LT and Herrström P: Prevalence of fibromyalgia and chronic widespread pain. Scand J Prim Health Care 18: 149-153, 2000.
2. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, *et al*: The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 33: 160-172, 1990.
3. Montserrat-de la Paz S, García-Giménez MD, Ángel-Martín M and Fernández-Arche A: Validation and additional support for an experimental animal model of fibromyalgia. Mod Rheumatol 25: 116-122, 2015.
4. Schmidt-Wilcke T and Clauw DJ: Pharmacotherapy in fibromyalgia (FM) - implications for the underlying pathophysiology. Pharmacol Ther 127: 283-294, 2010.
5. Green PG, Alvarez P, Gear RW, Mendoza D and Levine JD: Further validation of a model of fibromyalgia syndrome in the rat. J Pain 12: 811-818, 2011.
6. Schur EA, Afari N, Furberg H, Olarte M, Goldberg J, Sullivan PF and Buchwald D: Feeling bad in more ways than one: Comorbidity patterns of medically unexplained and psychiatric conditions. J Gen Intern Med 22: 818-821, 2007.
7. Wilson HD, Starz TW, Robinson JP and Turk DC: Heterogeneity within the fibromyalgia population: Theoretical implications of variable tender point severity ratings. J Rheumatol 36: 2795-2801, 2009.
8. Anderson RJ, McCrae CS, Staud R, Berry RB and Robinson ME: Predictors of clinical pain in fibromyalgia: Examining the role of sleep. J Pain 13: 350-358, 2012.
9. Balasubramaniam R, de Leeuw R, Zhu H, Nickerson RB, Okeson JP and Carlson CR: Prevalence of temporomandibular disorders in fibromyalgia and failed back syndrome patients: A blinded prospective comparison study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 104: 204-216, 2007.
10. Sluka KA, Kalra A and Moore SA: Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. Muscle Nerve 24: 37-46, 2001.
11. Khasar SG, Miao JP, Jänig W and Levine JD: Modulation of bradykinin-induced mechanical hyperalgesia in the rat by activity in abdominal vagal afferents. Eur J Neurosci 10: 435-444, 1998.
12. Khasar SG, Dina OA, Green PG and Levine JD: Sound stress-induced long-term enhancement of mechanical hyperalgesia in rats is maintained by sympathoadrenal catecholamines. J Pain 10: 1073-1077, 2009.
13. Nagakura Y, Oe T, Aoki T and Matsuoka N: Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: A putative animal model of fibromyalgia. Pain 146: 26-33, 2009.
14. Nishiyori M and Ueda H: Prolonged gabapentin analgesia in an experimental mouse model of fibromyalgia. Mol Pain 4: 52, 2008.
15. Nishiyori M, Uchida H, Nagai J, Araki K, Mukae T, Kishioka S and Ueda H: Permanent relief from intermittent cold stress-induced fibromyalgia-like abnormal pain by repeated intrathecal administration of antidepressants. Mol Pain 7: 69, 2011.
16. Xu Y, Lin D, Yu X, Xie X, Wang L, Lian L, Fei N, Chen J, Zhu N, Wang G, *et al*: The antinociceptive effects of ferulic acid on neuropathic pain: Involvement of descending monoaminergic system and opioid receptors. Oncotarget 7: 20455-20468, 2016.
17. Uçar M, Sarp Ü, Karaaslan Ö, Gül AI, Tanik N and Arik HO: Health anxiety and depression in patients with fibromyalgia syndrome. J Int Med Res 43: 679-685, 2015.
18. Alok R, Das SK, Agarwal GG, Salwahan L and Srivastava R: Relationship of severity of depression, anxiety and stress with severity of fibromyalgia. Clin Exp Rheumatol 29 (Suppl 69): S70-S72, 2011.
19. Marco EM, Granstrem O, Moreno E, Llorente R, Adriani W, Laviola G and Viveros MP: Subchronic nicotine exposure in adolescence induces long-term effects on hippocampal and striatal cannabinoid-CB1 and mu-opioid receptors in rats. Eur J Pharmacol 557: 37-43, 2007.
20. Kivinummi T, Kaste K, Rantamäki T, Castrén E and Ahtee L: Alterations in BDNF and phospho-CREB levels following chronic oral nicotine treatment and its withdrawal in dopaminergic brain areas of mice. Neurosci Lett 491: 108-112, 2011.
21. Geng SJ, Liao FF, Dang WH, Ding X, Liu XD, Cai J, Han JS, Wan Y and Xing GG: Contribution of the spinal cord BDNF to the development of neuropathic pain by activation of the NR2B-containing NMDA receptors in rats with spinal nerve ligation. Exp Neurol 222: 256-266, 2010.
22. Masaki E, Mizuta K, Ohtani N and Kido K: Early postoperative nociceptive threshold and production of brain-derived neurotrophic factor induced by plantar incision are not influenced with minocycline in a rat: Role of spinal microglia. Neurosignals 24: 15-24, 2016.
23. Yuluğ B, Ozan E, Gönül AS and Kilic E: Brain-derived neurotrophic factor, stress and depression: a minireview. Brain Res Bull 78: 267-269, 2009.

24. Rybakowski JK: BDNF gene: Functional Val66Met polymorphism in mood disorders and schizophrenia. Pharmacogenomics 9: 1589-1593, 2008.
25. Licinio J, Dong C and Wong ML: Novel sequence variations in the brain-derived neurotrophic factor gene and association with major depression and antidepressant treatment response. Arch Gen Psychiatry 66: 488-497, 2009.
26. Russell IJ, Mease PJ, Smith TR, Kajdasz DK, Wohlreich MM, Detke MJ, Walker DJ, Chappell AS and Arnold LM: Efficacy and safety of duloxetine for treatment of fibromyalgia in patients with or without major depressive disorder: Results from a 6-month, randomized, double-blind, placebo-controlled, fixed-dose trial. Pain 136: 432-444, 2008.
27. Hobbs C: Valerian: A literature review. HerbalGram 21: 19-34, 1989.
28. Liu XG, Gao PY, Wang GS, Song SJ, Li LZ, Li X, Yao XS and Zhang ZX: In vivo antidepressant activity of sesquiterpenes from the roots of *Valeriana fauriei* Briq. Fitoterapia 83: 599-603, 2012.
29. Kang BS, Kang SS, Kang SK, Kang CK, Ko WC and Ko HG: Dictionary of traditional Chinese medicines. Jungdam Publ Korea 10: 5171-5174, 1985.
30. Yao M, Ritchie HE and Brown-Woodman PD: A developmental toxicity-screening test of valerian. J Ethnopharmacol 113: 204-209, 2007.
31. Ortiz JG, Nieves-Natal J and Chavez P: Effects of *Valeriana officinalis* extracts on [3H]flunitrazepam binding, synaptosomal [3H]GABA uptake, and hippocampal [3H]GABA release. Neurochem Res 24: 1373-1378, 1999.
32. Sudati JH, Vieira FA, Pavin SS, Dias GR, Seeger RL, Golombieski R, Athayde ML, Soares FA, Rocha JB and Barbosa NV: *Valeriana officinalis* attenuates the rotenone-induced toxicity in Drosophila melanogaster. Neurotoxicology 37: 118-126, 2013.
33. de Oliveria DM, Barreto G, De Andrade DV, Saraceno E, Aon-Bertolino L, Capani F, Dos Santos El Bachá R and Giraldez LD: Cytoprotective effect of *Valeriana officinalis* extract on an in vitro experimental model of Parkinson disease. Neurochem Res 34: 215-220, 2009.
34. Malva JO, Santos S and Macedo T: Neuroprotective properties of *Valeriana officinalis* extracts. Neurotox Res 6: 131-140, 2004.
35. Pereira RP, Fachinetto R, de Souza Prestes A, Wagner C, Sudati JH, Boligon AA, Athayde ML, Morsch VM and Rocha JB: *Valeriana officinalis* ameliorates vacuous chewing movements induced by reserpine in rats. J Neural Transm Vienna 118: 1547-1557, 2011.
36. Barnes J, Anderson LA and Phillipson JD: Herbal Medicines. A Guide for Heathcare Professionals. 2nd edition. Pharmaceutical Press, London, 2002.
37. Pizzorno JE and Murray MT: Textbook of Natural Medicine. 2nd edition. Churchill Livingstone, London, 1999.
38. National Research Council: Guide for the Care and Use of Laboratory Animals. The National Academies Press, Washington, DC, 1996
39. Lee H, Won H, Im J, Kim YO, Lee S, Cho IH, Kim HK, Kwon JT and Kim HJ: Effect of *Valeriana fauriei* extract on the offspring of adult rats exposed to prenatal stress. Int J Mol Med 38: 251-258, 2016.
40. Ejchel-Cohen TF, Wood GE, Wang JF, Barlow K, Nobrega JN, S McEwen B and Trevor Young L: Chronic restraint stress decreases the expression of glutathione S-transferase pi2 in the mouse hippocampus. Brain Res 1090: 156-162, 2006.
41. Magariños AM, Li CJ, Gal Toth J, Bath KG, Jing D, Lee FS and McEwen BS: Effect of brain-derived neurotrophic factor haploinsufficiency on stress-induced remodeling of hippocampal neurons. Hippocampus 21: 253-264, 2011.
42. Keyhanfar F, Shamsi Meymandi M, Sepehri G, Rastegaryanzadeh R and Heravi G: Evaluation of antinociceptive effect of pregabalin in mice and its combination with tramadol using Tail Flick test. Iran J Pharm Res 12: 483-493, 2013.
43. Meymandi MS, Sepehri G and Mobasher M: Gabapentin enhances the analgesic response to morphine in acute model of pain in male rats. Pharmacol Biochem Behav 85: 185-189, 2006.
44. Steru L, Chermat R, Thierry B and Simon P: The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology (Berl) 85: 367-370, 1985.
45. Hargreaves K, Dubner R, Brown F, Flores C and Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32: 77-88, 1988.
46. Inoue M, Rashid MH, Fujita R, Contos JJ, Chun J and Ueda H: Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. Nat Med 10: 712-718, 2004.
47. Rashid MH, Inoue M, Toda K and Ueda H: Loss of peripheral morphine analgesia contributes to the reduced effectiveness of systemic morphine in neuropathic pain. J Pharmacol Exp Ther 309: 380-387, 2004.
48. Morley-Fletcher S, Darnaudery M, Koehl M, Casolini P, Van Reeth O and Maccari S: Prenatal stress in rats predicts immobility behavior in the forced swim test. Effects of a chronic treatment with tianeptine. Brain Res 989: 246-251, 2003.
49. Joo J, Lee S, Nah SS, Kim YO, Kim DS, Shim SH, Hwangbo Y, Kim HK, Kwon JT, Kim JW, *et al*: Lasp1 is down-regulated in NMDA receptor antagonist-treated mice and implicated in human schizophrenia susceptibility. J Psychiatr Res 47: 105-112, 2013.
50. Hindmarch I: Expanding the horizons of depression: Beyond the monoamine hypothesis. Hum Psychopharmacol 16: 203-218, 2001.
51. Raison CL, Capuron L and Miller AH: Cytokines sing the blues: Inflammation and the pathogenesis of depression. Trends Immunol 27: 24-31, 2006.
52. Sebastião AM, Assaife-Lopes N, Diógenes MJ, Vaz SH and Ribeiro JA: Modulation of brain-derived neurotrophic factor (BDNF) actions in the nervous system by adenosine A(2A) receptors and the role of lipid rafts. Biochim Biophys Acta 1808: 1340-1349, 2011.
53. Pillai A: Brain-derived neurotropic factor/TrkB signaling in the pathogenesis and novel pharmacotherapy of schizophrenia. Neurosignals 16: 183-193, 2008.
54. Laske C, Stransky E, Eschweiler GW, Klein R, Wittorf A, Leyhe T, Richartz E, Köhler N, Bartels M, Buchkremer G, *et al*: Increased BDNF serum concentration in fibromyalgia with or without depression or antidepressants. J Psychiatr Res 41: 600-605, 2007.
55. Groth R and Aanonsen L: Spinal brain-derived neurotrophic factor (BDNF) produces hyperalgesia in normal mice while antisense directed against either BDNF or trkB, prevent inflammation-induced hyperalgesia. Pain 100: 171-181, 2002.
56. Xu Y, Ku B, Tie L, Yao H, Jiang W, Ma X and Li X: Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res 1122: 56-64, 2006.
57. Liu L, Liu C, Wang Y, Wang P, Li Y and Li B: Herbal medicine for anxiety, depression and insomnia. Curr Neuropharmacol 13: 481-493, 2015.
58. Liu L, Li B, Zhou Y, Wang L, Tang F, Shao D, Jiang X, Zhao H, Cui R and Li Y: Antidepressant-like effect of Fuzi total alkaloid on ovariectomized mice. J Pharmacol Sci 120: 280-287, 2012.
59. Deng S, West BJ, Palu AK, Zhou BN and Jensen CJ: Noni as an anxiolytic and sedative: A mechanism involving its gamma-aminobutyric acidergic effects. Phytomedicine 14: 517-522, 2007.
60. Sarris J: Herbal medicines in the treatment of psychiatric disorders: A systematic review. Phytother Res 21: 703-716, 2007.
61. Sarris J and Byrne GJ: A systematic review of insomnia and complementary medicine. Sleep Med Rev 15: 99-106, 2011.
62. Murphy K, Kubin ZJ, Shepherd JN and Ettinger RH: *Valeriana officinalis* root extracts have potent anxiolytic effects in laboratory rats. Phytomedicine 17: 674-678, 2010.
63. Dietz BM, Mahady GB, Pauli GF and Farnsworth NR: Valerian extract and valerenic acid are partial agonists of the 5-HT5a receptor in vitro. Brain Res Mol Brain Res 138: 191-197, 2005.
64. No authors listed: Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the study of pain, subcommittee on taxonomy. Pain Suppl 3: S1-S226, 1986.
65. Livingston G, Watkin V, Milne B, Manela MV and Katona C: Who becomes depressed? The Islington community study of older people. J Affect Disord 58: 125-133, 2000.
66. McBeth J and Silman AJ. Role Psychiatr Disord Fibromyalgia. 3: 157-166, 2001.
67. Ammer K and Melnizky P: Medicinal baths for treatment of generalized fibromyalgia. Forsch Komplementarmed 6: 80-85, 1999 (In German).