

Ethanol extract of *Oenanthe javanica* increases cell proliferation and neuroblast differentiation in the adolescent rat dentate gyrus

Bai Hui Chen^{1,#}, Joon Ha Park^{2,#}, Jeong Hwi Cho², In Hye Kim², Bich Na Shin¹, Ji Hyeon Ahn², Seok Joon Hwang³, Bing Chun Yan⁴, Hyun Jin Tae⁵, Jae Chul Lee², Eun Joo Bae⁶, Yun Lyul Lee¹, Jong Dai Kim³, Moo-Ho Won^{2,*}, Il Jun Kang^{7,*}

1 Department of Physiology, College of Medicine, and Institute of Neurodegeneration and Neuroregeneration, Hallym University, Chuncheon, South Korea

2 Department of Neurobiology, School of Medicine, Kangwon National University, Chuncheon, South Korea

3 Department of Food Science and Biotechnology, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, South Korea

4 Department of Integrative Traditional & Western Medicine, Medical College, Yangzhou University, Yangzhou, Jiangsu Province, China

5 Department of Biomedical Science and Research Institute for Bioscience and Biotechnology, Hallym University, Chuncheon, South Korea

6 Department of Pediatrics, Chuncheon Sacred Heart Hospital, College of Medicine, Hallym University, Chuncheon, South Korea

7 Department of Food Science and Nutrition, Hallym University, Chuncheon, South Korea

Abstract

Oenanthe javanica is an aquatic perennial herb that belongs to the *Oenanthe* genus in Apiaceae family, and it displays well-known medicinal properties such as protective effects against glutamate-induced neurotoxicity. However, few studies regarding effects of *Oenanthe javanica* on neurogenesis in the brain have been reported. In this study, we examined the effects of a normal diet and a diet containing ethanol extract of *Oenanthe javanica* on cell proliferation and neuroblast differentiation in the subgranular zone of the hippocampal dentate gyrus of adolescent rats using Ki-67 (an endogenous marker for cell proliferation) and doublecortin (a marker for neuroblast). Our results showed that *Oenanthe javanica* extract significantly increased the number of Ki-67-immunoreactive cells and doublecortin-immunoreactive neuroblasts in the subgranular zone of the dentate gyrus in the adolescent rats. In addition, the immunoreactivity of brain-derived neurotrophic factor was significantly increased in the dentate gyrus of the *Oenanthe javanica* extract-treated group compared with the control group. However, we did not find that vascular endothelial growth factor expression was increased in the *Oenanthe javanica* extract-treated group compared with the control group. These results indicate that *Oenanthe javanica* extract improves cell proliferation and neuroblast differentiation by increasing brain-derived neurotrophic factor immunoreactivity in the rat dentate gyrus.

Key Words: nerve regeneration; *Oenanthe javanica* extract; cell proliferation; neuroblast differentiation; brain-derived neurotrophic factor; vascular endothelial growth factor; rat; neural regeneration

Funding: This study was supported by Hallym University Specialization Fund, No. HRF-S-12.

Chen BH, Park JH, Cho JH, Kim IH, Shin BN, Ahn JH, Hwang SJ, Yan BC, Tae HJ, Lee JC, Bae EJ, Lee YL, Kim JD, Won MH, Kang IJ (2015) Ethanol extract of *Oenanthe javanica* increases cell proliferation and neuroblast differentiation in the adolescent rat dentate gyrus. *Neural Regen Res* 10(2):271-276.

*Correspondence to:

Moo-Ho Won, D.V.M., Ph.D. or

Il-Jun Kang, Ph.D.,

mhwon@kangwon.ac.kr or

ijkang@hallym.ac.kr.

These authors contributed equally to this work.

doi:10.4103/1673-5374.152382

<http://www.nrronline.org/>

Accepted: 2014-12-10

Introduction

The hippocampus is involved in cognitive processes such as declarative memory, context-dependent and spatial learning processes (Wang et al., 2011; Gil-Mohapel et al., 2013; Jelenkovic et al., 2014). It has been reported that two regions in the brain, the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricle, can improve self-renewal activity throughout the animal's life (Yamaguchi et al., 2000; Yoo et al., 2012; Yang et al., 2014). In the SGZ of the hippocampal DG, neural precursor cells undergo cell proliferation, maturation, migrate into the granule cell layer, and integrate into pre-existing neural circuitry (Kempermann, 2008; Chen et

al., 2013).

It has been reported that neurogenesis in the DG can be altered by various factors, such as pharmacological drugs, aging and neurodegenerative diseases (Choi et al., 2012). Many studies have shown that brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) are important neurotrophic factors (Pencea et al., 2001; Li et al., 2012; Kim et al., 2014), and they are widely studied in various research areas, such as neurogenesis in the hippocampal DG (Ji et al., 2014; Zeng et al., 2014). In particular, some researchers have reported that chronic stress in animal models inhibits neurogenesis, suggesting a lower expression of BDNF in the DG (Roceri et al., 2002; Garza et

al., 2012). In addition, VEGF is an angiogenic and vascular permeability factor, and blood vessels influence neurogenesis in the SGZ of the DG in the adult rat (Palmer et al., 2000). Jin et al. (2002) have reported that VEGF can stimulate neurogenesis in major neuroproliferative zones (SGZ and SVZ) of the adult rat brain.

Oenanthe javanica is an aquatic perennial herb that belongs to the *Oenanthe* genus in Apiaceae family, and it has well-known medicinal properties (Jiang et al., 2014). The fresh stems and leaves are widely used as a seasoning in diet in Korea (Seo and Baek, 2005), and they are used in traditional Chinese medicine for the treatments of fever, leucorrhea, mumps, difficult urination and hypertension (Kim et al., 2013). Previous studies indicate that *Oenanthe javanica* has effects of anti-inflammatory (Yang et al., 2013), antioxidant and antigenotoxic activities (Kwon et al., 2006). In clinical applications, it may have some efficacy on acute liver diseases (Yang et al., 2014), sepsis, and septic shock (Kim et al., 2013). It has been also reported that persicarin, a main constituent of *Oenanthe javanica*, significantly protects primary cultured rat cortical cells from glutamate-induced toxicity (Ma et al., 2010). However, few studies regarding effects of *Oenanthe javanica* on neurogenesis in the brain have been reported.

Therefore, in the present study, we investigated the effects of *Oenanthe javanica* extract (OJE) on cell proliferation and neuroblast differentiation in the DG of the adolescent rat using Ki-67 (an endogenous marker for cell proliferation) and doublecortin (DCX, a marker for neuroblast). In addition, we examined the effects of OJE on immunoreactivities of BDNF and VEGF in the DG of the adolescent rats.

Materials and Methods

Experimental animals

Fourteen male Wistar rats, weighing 110–130 g, aged 5 weeks, were purchased from Orient Bio Inc. (Seongnam, South Korea). They were housed in a conventional state under adequate temperature (23°C) and humidity (60%) control with a 12-hour light/dark cycle, and free access to food and water. The procedures for handling and caring for the animals adhered to the guidelines that are in compliance with the current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996), and were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University. All of the experiments were conducted with an effort to minimize the number of animals used and the suffering caused by the procedures used in the present study.

Preparation of OJE

Oenanthe javanica was collected by Professor Jong Dai Kim in Kangwon province (South Korea), in March 2013 and kept in a deep freezer (−70°C). *Oenanthe javanica* was extracted with 10 vol(v/w) of 70% ethanol at 70°C for 4 hours, and extraction was repeated three times. The extracts were filtered through Whatman filter paper (No.2), concentrated with a vacuum evaporator, and completely dried with

a freeze-drier and then ground into fine powder. The extraction yield was 14.5%. OJE was mixed by 0.5% in normal composition diet. Compositions of experimental diets are represented in **Table 1**.

Treatment of OJE

The rats were divided into two experimental groups: (1) vehicle-treated group as the control ($n = 7$) and (2) OJE-treated group ($n = 7$). As previously described (Tae et al., 2014), rats of the control group were fed with a normal diet, and rats of the OJE-treated group were fed with a diet containing OJE (**Table 1**) for 4 weeks, because, in traditional Chinese medicine, extracts from plants have been taken orally and there are no data about the absorption and metabolism of OJE. Each rat was housed and fed individually in plastic cage (40 cm × 25 cm × 15 cm) during experiment. Each rat was given experimental diets (approximately 50 g in total) every other day. The body weight was measured before diet or after diet for 4 weeks, and food intake was recorded at regular time as 2 days interval for 4 weeks. There were no significant differences in body weight and food intake between the control and OJE-treated groups (**Table 2**).

Tissue processing for histology

For histological analysis, the rats ($n = 7$ in each group) were anesthetized with 30 mg/kg Zoletil 50 (Virbac, Carros, France) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brains were removed and postfixed in the same fixative for 4 hours. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, frozen tissues were serially sectioned on a cryostat (Leica, Wetzlar, Germany) into 30- μ m coronal sections, and they were then collected into 6-well plates containing PBS for further process.

Immunohistochemistry

To obtain accurate data for immunohistochemistry, the sections were carefully processed under the same conditions. The tissue sections were selected between −3.00 and −4.08 mm posterior to the bregma in reference to the rat. The sections were sequentially treated with 0.3% hydrogen peroxide (H₂O₂) in PBS for 30 minutes and 10% normal goat serum in 0.05 M PBS for 30 minutes. The sections were next incubated with diluted rabbit anti-Ki-67 (1:100; Abcam, Cambridge, UK), goat anti-DCX (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-BDNF (1:400; Abcam), rabbit anti-VEGF (1:200; Santa Cruz Biotechnology) overnight at 4°C. Thereafter, the sections were exposed to biotinylated goat anti-rabbit or rabbit anti-goat IgG (1:200; Vector, Burlingame, CA, USA) for 2 hours at room temperature and streptavidin peroxidase complex (1:200; Vector) for 1 hour at room temperature. They were visualized with 3,3'-diaminobenzidine tetrahydrochloride in 0.1 M Tris-HCl buffer and mounted on the gelatin-coated slides. After dehydration, the sections were mounted in canada balsam (Kanto Chemical, Japan).

Table 1 Composition of experimental diets

| Ingredients | Control | OJE-treated |
|-----------------------------|---------|-------------|
| Casein | 20.0% | 20.0% |
| Corn oil | 5.0% | 5.0% |
| Cholesterol | 0.5% | 0.5% |
| Corn starch | 15.0% | 15.0% |
| Cellulose | 5.0% | 5.0% |
| Mineral mix (AIN-76) | 3.5% | 3.5% |
| Vitamin mix (AIN-76) | 1.0% | 1.0% |
| Methione | 0.3% | 0.3% |
| Choline bitartrate | 0.2% | 0.2% |
| Sucrose | 49.5% | 49% |
| 70% ethanol extracts of OJE | – | 0.5% |

OJE: *Oenanthe javanica* extract.

Images of Ki-67, DCX, BDNF and VEGF-immunoreactive structures were taken from the dentate gyrus through AxioM1 light microscope (BX53, Olympus, Germany) equipped with digital camera (DP72, Olympus) connected to a PC monitor. Total number of Ki-67 or DCX positive cells in all the groups was counted in 15 sections/rat using an image analyzing system equipped with a computer-based CCD camera (software: Optimas 6.5, CyberMetrics, Scottsdale, AZ, USA). Cell counts were obtained by averaging the counts from the sections taken from each rat. In addition, the staining intensity of BDNF and VEGF-immunoreactive structures was evaluated on the basis of an optical density (OD), which was obtained after the transformation of the mean gray level using the formula: $OD = \log(256/\text{mean gray level})$. The OD of background was taken from areas adjacent to the measured area. After the background density was subtracted, a ratio of the OD of image file was calibrated as % (relative OD, ROD) using Adobe Photoshop version 8.0 and then analyzed using NIH Image 1.59 software. All measurements were performed in order to ensure objectivity under blind conditions, by two observers for each experiment, and experimental samples were measured under the same conditions.

Statistical analysis

The data shown here are expressed as the mean \pm SEM. Differences of the means among the groups were statistically analyzed by one-way analysis of variance with Duncan's *post hoc* test. Statistical significance was considered at $P < 0.05$.

Results

Effects of OJE on cell proliferation

Ki-67-immunoreactive cells were found in the SGZ of the DG in all the experimental groups (Figure 1). In the control group, Ki-67-immunoreactive cells were easily observed in the SGZ, and the mean number of them was 5.7 ± 0.41 per section (Figure 1A, 1a and 1C). In the OJE-treated group, the number of Ki-67-immunoreactive cells was significantly increased in the DG compared with the control group, and the mean number of them was 16.2 ± 0.12 per section (about

Table 2 The effects of *Oenanthe javanica* extract (OJE) diet on body weight and food intake

| Group | Body weight (g) | | Food intake (g/4 weeks) |
|-------------|-------------------|------------------|-------------------------|
| | 0 day | 4 weeks | |
| Control | 110.7 \pm 17.47 | 160.7 \pm 9.65 | 434.1 \pm 29.67 |
| OJE-treated | 110.2 \pm 11.54 | 164.7 \pm 13.2 | 442.9 \pm 52.55 |

284% of the control group) ($P < 0.05$; Figure 1B, 1b and 1C).

Effects of OJE on neuronal differentiation

In all the experimental groups, DCX-immunoreactive neuroblasts were mainly detected in the SGZ of the DG (Figure 2). In the control group, DCX-immunoreactive neuroblasts were easily observed in the SGZ of the DG, and some of them had poorly developed processes (Figure 2A and 2a). The mean number of DCX-immunoreactive neuroblasts was 13.7 ± 2.24 per section (Figure 2A and 2C). In the OJE-treated group, the number of DCX-immunoreactive neuroblasts was significantly increased in the SGZ of the DG, and their processes were very long and thick compared with those in the control group (Figure 2B and 2b). In addition, the mean number of DCX-immunoreactive neuroblasts was 33.4 ± 3.19 per section and significantly higher (about 244 % of the control group) compared with the control group ($P < 0.05$; Figure 2B and 2C).

Effects of OJE on immunoreactivities of BDNF and VEGF

In the control group, weak BDNF immunoreactivity was observed in the granule cell layer of the DG (Figure 3A). In the OJE-treated group, BDNF immunoreactivity was significantly increased in the granule cell layer of the DG compared with the control group (Figure 3B and 3E).

On the other hand, weak VEGF immunoreactivity was detected in the granule cell layer of the DG in the control group, and the VEGF immunoreactivity in the OJE-treated group was similar to that in the control group (Figure 3C, 3D and 3E).

Discussion

In the present study, we examined the effects of OJE on cell proliferation and neuroblast differentiation in the SGZ of the DG of the adolescent rats. Our results showed markedly increased numbers of Ki-67-immunoreactive cells and DCX-immunoreactive neuroblasts in the OJE-treated group compared with those in the control group. Furthermore, in the OJE-treated group, the neuroblasts displayed well-developed processes.

Recently, some researchers demonstrated that dendrite maturation was closely related to the survival of newborn neurons (Plumpe et al., 2006). In addition, Snyder et al. (2005) reported that newly generated cells might be associated with spatial learning and memory of normal hippocampal function in the adult hippocampus. It is well known that DCX is a specific marker for neuronal precursors and neurogenesis in the development of the adult CNS (Bonfanti

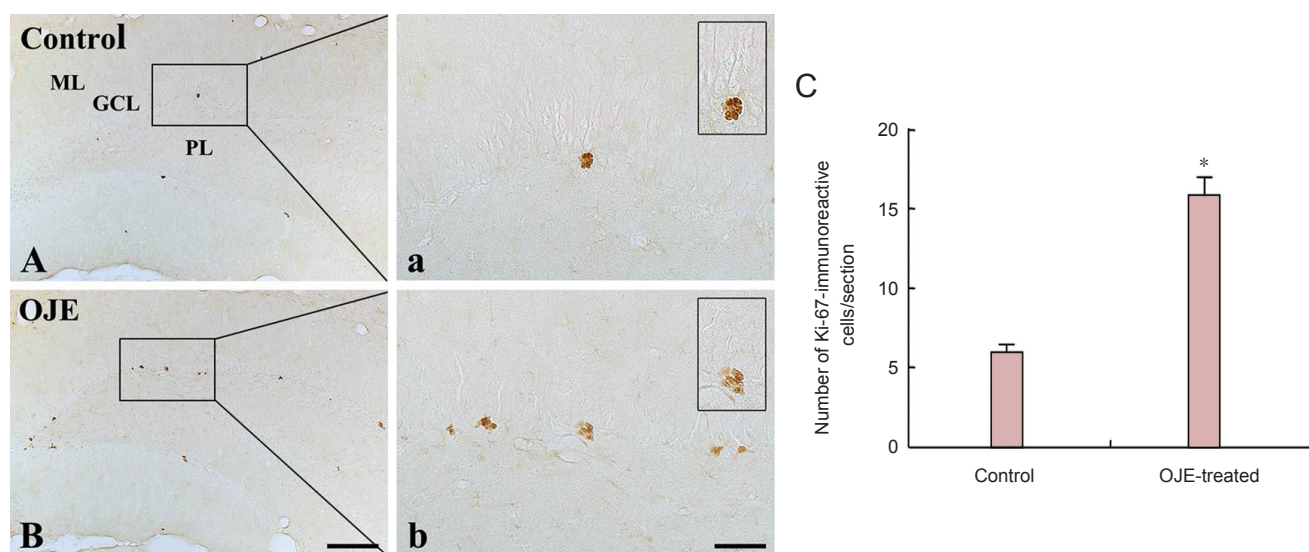


Figure 1 Ki-67 immunohistochemistry in the dentate gyrus of rats in the control and *Oenanthe javanica* extract (OJE)-treated groups. Immunohistochemistry for Ki-67 in the DG of the control (A and a) and OJE- (B and b) treated groups. Ki-67-immunoreactive cells are significantly increased in the OJE-treated group compared with the control group. ML: Molecular layer; GCL: granule cell layer; PL: polymorphic layer. Scale bars: 200 μ m (A and B), 40 μ m (a and b). Insets show higher magnification to point out Ki-67-immunoreactive cells. (C) Analysis of Ki-67-immunoreactive cells per section of each group ($n = 7$ per group; $*P < 0.05$, significantly different from the control group; one-way analysis of variance with Duncan's *post hoc* test). The bars indicate the mean \pm SEM.

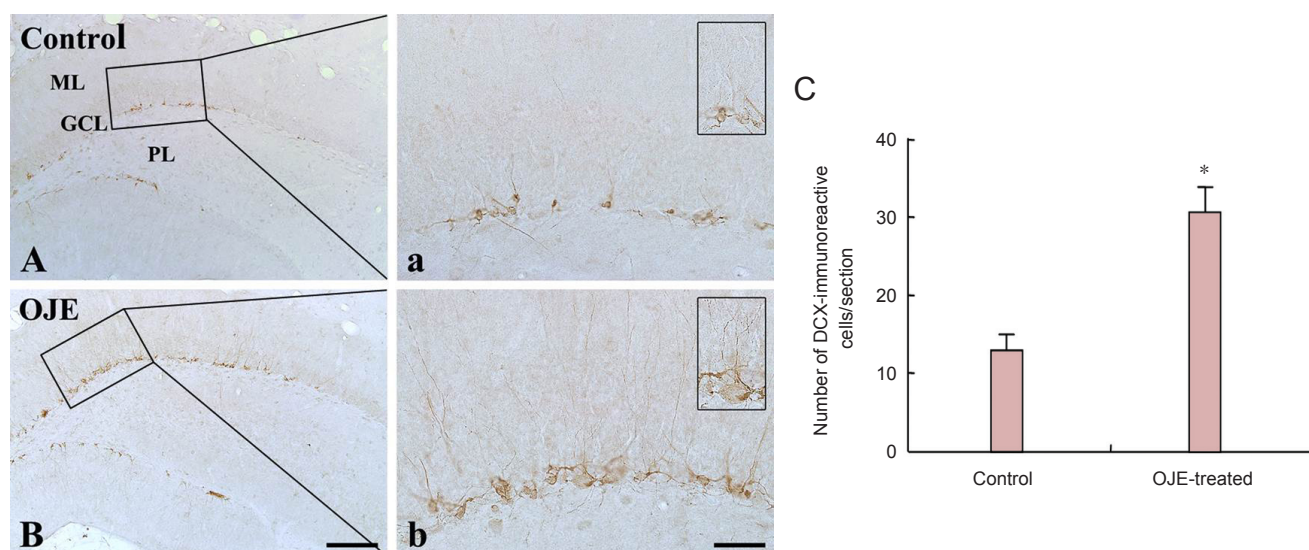


Figure 2 Doublecortin (DCX) immunohistochemistry in the dentate gyrus of the control and *Oenanthe javanica* extract (OJE)-treated groups. DCX immunohistochemistry in the DG of the control (A and a) and OJE- (B and b) treated groups. In the OJE-treated group, the number of DCX-immunoreactive neuroblasts is significantly increased, and their processes are much longer and thicker than those in the control group. ML: Molecular layer; GCL: granule cell layer; PL: polymorphic layer. Scale bar: 200 μ m (A and B), 40 μ m (a and b). Insets show higher magnification to point out DCX-immunoreactive neuroblasts. (C) Analysis of DCX-immunoreactive neuroblasts per section of the control and OJE-treated groups ($n = 7$ per group; $*P < 0.05$, significantly different from the control group; one-way analysis of variance with Duncan's *post hoc* test). The bars indicate the mean \pm SEM.

and Theodosios, 1994; Brown et al., 2003; Jin et al., 2004). It was reported that the increase of DCX immunoreactivity could result in increasing branch points and complexity of the dendrites of DCX-immunoreactive neuroblasts (Cohen et al., 2008; Yoo et al., 2011). Therefore, our present finding in the OJE-treated-group indicates that OJE may be involved in neuroblast maturation.

Some plants have ability to create hippocampal neurogenesis. For example, *Rosa damascena* enhances adult neu-

rogenesis, hippocampal volume and synaptic plasticity in a rat model of Alzheimer's disease (Esfandiary et al., 2014). In this study, we used *Oenanthe javanica*, which is an aquatic perennial herb, and our results showed that OJE improved cell proliferation and neuroblast differentiation in the SGZ of the DG.

We also detected the immunoreactivities of BDNF and VEGF in the DG of the OJE-treated group, and we found that the immunoreactivity of BDNF, not VEGF, was signifi-

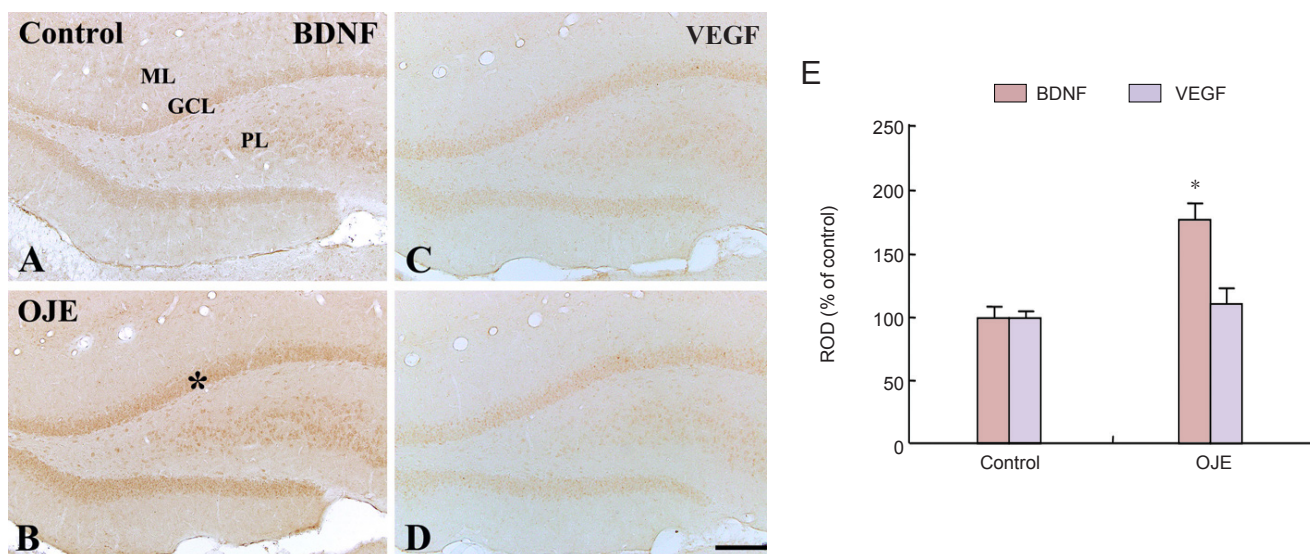


Figure 3 Brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) immunohistochemistry in the dentate gyrus of the control and *Oenanthe javanica* extract (OJE)-treated groups.

BDNF (A and B) and VEGF (C and D) immunohistochemistry in the dentate gyrus of the control and OJE-treated groups. BDNF immunoreactivity (asterisk) is significantly increased in the OJE-treated group compared with the control group. However, VEGF immunoreactivity in the OJE-treated group is not changed. ML: Molecular layer; GCL: granule cell layer; PL: polymorphic layer. Scale bar: 200 μ m. (E) Relative optical density (ROD) as percentage of BDNF- and VEGF-immunoreactive structures in the control and OJE-treated groups ($n = 7$ per group; $*P < 0.05$, significantly different from the control group; one-way analysis of variance (ANOVA) with Duncan's *post hoc* test). The bars indicate the mean \pm SEM.

cantly increased after OJE treatment. It was reported that the expression of BDNF was increased in the DG of the hippocampus, and the elevation might enhance neurogenesis in the brain (Kaloustian et al., 2008; Hwang et al., 2009). In addition, Lee et al. (2002) showed that neurogenesis was significantly increased after the administration of BDNF, and, recently, Dong et al. (2014) reported that the induction of hippocampal neurogenesis was linked with the activation of ERK1/CREB signaling pathway by upregulating the level of BDNF.

It is well known that VEGF is a signal protein linked with the regulation of physiological and pathological angiogenesis (Ferrara and Davis-Smyth, 1997; Nowacka and Obuchowicz, 2013). Administration of VEGF could markedly reduce neuronal death (Qiu et al., 2003) and increase neurogenesis and angiogenesis in the ischemic brain *in vitro* and *in vivo* (Jin et al., 2002). However, in this study, we did not find that the immunoreactivity of VEGF was increased in the DG after OJE treatment. This finding indicates that OJE does not increase the expression of endogenous VEGF, although we cannot exactly explain why VEGF immunoreactivity is increased in the rat DG after OJE treatment.

In brief, in this study, we showed that cell proliferation and neuroblast differentiation were markedly increased in the DG of the OJE-treated adolescent rats. In addition, BDNF immunoreactivity was significantly increased after OJE administration. These results suggest that OJE has a potential to significantly increase cell proliferation and neuroblast differentiation. These effects of OJE might be associated with the increase of BDNF immunoreactivity in the rat DG.

Acknowledgments: We would like to thank Mr. Sung Uk Lee from the Kangwon National University, South Korea for his technical help in this study.

Author contributions: All authors participated in the design, implementation and evaluation of the study, and approved the final version of this article.

Conflicts of interest: None declared.

References

- Bonfanti L, Theodosios DT (1994) Expression of polysialylated neural cell adhesion molecule by proliferating cells in the subependymal layer of the adult rat, in its rostral extension and in the olfactory bulb. *Neuroscience* 62:291-305.
- Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol* 467:1-10.
- Chen BH, Yan BC, Park JH, Ahn JH, Lee DH, Kim IH, Cho JH, Lee JC, Kim SK, Lee B, Cho JH, Won MH, Lee YL (2013) Aripiprazole, an atypical antipsychotic drug, improves maturation and complexity of neuroblast dendrites in the mouse dentate gyrus via increasing superoxide dismutases. *Neurochem Res* 38:1980-1988.
- Choi JH, Yoo KY, Lee CH, Park JH, Yan BC, Kwon SH, Seo JY, Cho JH, Hwang IK, Won MH (2012) Comparison of neurogenesis in the dentate gyrus between the adult and aged gerbil following transient global cerebral ischemia. *Neurochem Res* 37:802-810.
- Cohen D, Segal M, Reiner O (2008) Doublecortin supports the development of dendritic arbors in primary hippocampal neurons. *Dev Neurosci* 30:187-199.
- Dong H, Gao Z, Rong H, Jin M, Zhang X (2014) beta-Asarone reverses chronic unpredictable mild stress-induced depression-like behavior and promotes hippocampal neurogenesis in rats. *Molecules* 19:5634-5649.
- Esfandiary E, Karimipour M, Mardani M, Alaei H, Ghannadian M, Kazemi M, Mohammadnejad D, Hosseini N, Esmaeili A (2014) Novel effects of *Rosa damascena* extract on memory and neurogenesis in a rat model of Alzheimer's disease. *J Neurosci Res* 92:517-530.

- Ferrara N, Davis-Smyth T (1997) The biology of vascular endothelial growth factor. *Endocr Rev* 18:4-25.
- Garza JC, Guo M, Zhang W, Lu XY (2012) Leptin restores adult hippocampal neurogenesis in a chronic unpredictable stress model of depression and reverses glucocorticoid-induced inhibition of GSK-3 β /beta-catenin signaling. *Mol Psychiatry* 17:790-808.
- Gil-Mohapel J, Brocardo PS, Choquette W, Gothard R, Simpson JM, Christie BR (2013) Hippocampal neurogenesis levels predict WAT-TERMAZE search strategies in the aging brain. *PLoS One* 8:e75125.
- Hwang IK, Yoo KY, Han TH, Lee CH, Choi JH, Yi SS, Lee SY, Ryu PD, Yoon YS, Won MH (2009) Enhanced cell proliferation and neuroblast differentiation in the rat hippocampal dentate gyrus following myocardial infarction. *Neurosci Lett* 450:275-280.
- Jelenkovic A, Jovanovic MD, Stevanovic I, Petronijevic N, Bokonic D, Zivkovic J, Igic R (2014) Influence of the green tea leaf extract on neurotoxicity of aluminium chloride in rats. *Phytother Res* 28:82-87.
- Ji JF, Ji SJ, Sun R, Li K, Zhang Y, Zhang LY, Tian Y (2014) Forced running exercise attenuates hippocampal neurogenesis impairment and the neurocognitive deficits induced by whole-brain irradiation via the BDNF-mediated pathway. *Biochem Biophys Res Commun* 443:646-651.
- Jiang Q, Wang F, Li MY, Ma J, Tan GE, Xiong AS (2014) Selection of Suitable Reference Genes for qPCR Normalization under Abiotic Stresses in *Oenanthe javanica* (BL.) DC. *PLoS One* 9:e92262.
- Jin K, Mao XO, Greenberg DA (2004) Proteomic analysis of neuronal hypoxia in vitro. *Neurochem Res* 29:1123-1128.
- Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA (2002) Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc Natl Acad Sci U S A* 99:11946-11950.
- Kaloustian S, Wann BP, Bah TM, Girard SA, Apostolakis A, Ishak S, Mathieu S, Rylvlin P, Godbout R, Rousseau G (2008) Apoptosis time course in the limbic system after myocardial infarction in the rat. *Brain Res* 1216:87-91.
- Kempermann G (2008) The neurogenic reserve hypothesis: what is adult hippocampal neurogenesis good for? *Trends Neurosci* 31:163-169.
- Kim TH, Ku SK, Bae JS (2013) Anti-inflammatory activities of isorhamnetin-3-O-galactoside against HMGB1-induced inflammatory responses in both HUVECs and CLP-induced septic mice. *J Cell Biochem* 114:336-345.
- Kim YR, Kim HN, Ahn SM, Choi YH, Shin HK, Choi BT (2014) Electroacupuncture promotes post-stroke functional recovery via enhancing endogenous neurogenesis in mouse focal cerebral ischemia. *PLoS One* 9:e90000.
- Kwon D, Yoon S, Carter O, Bailey GS, Dashwood RH (2006) Antioxidant and antigenotoxic activities of *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* in the *Salmonella* mutagenicity assay and in HCT116 human colon cancer cells. *BioFactors* 26:231-244.
- Lee J, Duan W, Mattson MP (2002) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J Neurochem* 82:1367-1375.
- Li LF, Lu J, Li XM, Xu CL, Deng JM, Qu R, Ma SP (2012) Antidepressant-like effect of magnolol on BDNF up-regulation and serotonergic system activity in unpredictable chronic mild stress treated rats. *Phytother Res* 26:1189-1194.
- Ma CJ, Lee KY, Jeong EJ, Kim SH, Park J, Choi YH, Kim YC, Sung SH (2010) Persicarin from water dropwort (*Oenanthe javanica*) protects primary cultured rat cortical cells from glutamate-induced neurotoxicity. *Phytother Res* 24:913-918.
- Nowacka M, Obuchowicz E (2013) BDNF and VEGF in the pathogenesis of stress-induced affective diseases: an insight from experimental studies. *Pharmacol Rep* 65:535-546.
- Palmer TD, Willhoite AR, Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425:479-494.
- Pencea V, Bingaman KD, Wiegand SJ, Luskin MB (2001) Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. *J Neurosci* 21:6706-6717.
- Plumpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Romer B, Rodriguez GR, Kronenberg G, Kempermann G (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci* 7:77.
- Qiu MH, Zhang R, Sun FY (2003) Enhancement of ischemia-induced tyrosine phosphorylation of Kv1.2 by vascular endothelial growth factor via activation of phosphatidylinositol 3-kinase. *J Neurochem* 87:1509-1517.
- Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Mol Psychiatry* 7:609-616.
- Seo WH, Baek HH (2005) Identification of characteristic aroma-active compounds from water dropwort (*Oenanthe javanica* DC.). *J Agric Food Chem* 53:6766-6770.
- Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM (2005) A role for adult neurogenesis in spatial long-term memory. *Neuroscience* 130:843-852.
- Tae HJ, Park JH, Cho JH, Kim IH, Ahn JH, Lee JC, Kim JD, Park J, Choi SY, Won MH (2014) *Oenanthe javanica* extract increases immunoreactivities of antioxidant enzymes in the rat kidney. *Chin Med J (Engl)* 127:3758-63.
- Wang Z, Dai J, Chen L, Huang Y, Zhao Y (2011) Preventive action of Panax ginseng roots in hypercortisolism-induced impairment of hippocampal neurons in male C57BL/6N mice. *Phytother Res* 25:1242-1245.
- Yamaguchi M, Saito H, Suzuki M, Mori K (2000) Visualization of neurogenesis in the central nervous system using nestin promoter-GFP transgenic mice. *Neuroreport* 11:1991-1996.
- Yang JH, Kim SC, Shin BY, Jin SH, Jo MJ, Jegal KH, Kim YW, Lee JR, Ku SK, Cho IJ, Ki SH (2013) O-Methylated flavonol isorhamnetin prevents acute inflammation through blocking of NF-kappaB activation. *Food Chem Toxicol* 59:362-372.
- Yang SA, Jung YS, Lee SJ, Park SC, Kim MJ, Lee EJ, Byun HJ, Jhee KH, Lee SP (2014) Hepatoprotective effects of fermented field water-dropwort (*Oenanthe javanica*) extract and its major constituents. *Food Chem Toxicol* 67:154-160.
- Yoo DY, Nam SM, Kim W, Lee CH, Won MH, Hwang IK, Yoon YS (2011) N-acetylserotonin increases cell proliferation and differentiating neuroblasts with tertiary dendrites through upregulation of brain-derived neurotrophic factor in the mouse dentate gyrus. *J Vet Med Sci* 73:1411-1416.
- Yoo DY, Kim W, Yoo KY, Nam SM, Chung JY, Yoon YS, Won MH, Hwang IK (2012) Effects of pyridoxine on a high-fat diet-induced reduction of cell proliferation and neuroblast differentiation depend on cyclic adenosine monophosphate response element binding protein in the mouse dentate gyrus. *J Neurosci Res* 90:1615-1625.
- Zeng L, He X, Wang Y, Tang Y, Zheng C, Cai H, Liu J, Wang Y, Fu Y, Yang GY (2014) MicroRNA-210 overexpression induces angiogenesis and neurogenesis in the normal adult mouse brain. *Gene Ther* 21:37-43.

Copyedited by Park S, Numakawa T, Chuah MI, Li CH, Song LP, Zhao M