INTRODUCTION

Astrocytes, the major non-neuronal cells in the brain, play a pivotal role by interacting with neurons to provide structural, metabolic, and trophic support (Dallerac et al., 2013). In particular, astrocytes maintain a homeostatic environment for neurons by controlling oxidative stress. Various antioxidant enzymes are prominently expressed in astrocytes, enabling efficient detoxification and protection against reactive oxygen species (ROS) (Ryter et al., 2006; de Vries et al., 2008; Vargas and Johnson, 2009).

Hemeoxygenase-1 (HO-1) is the rate limiting-enzyme that catalyzes the oxidation of heme to carbon monoxide (CO), biliverdin, and free iron (Fe²⁺). HO-1 belongs to phase II antioxidant enzymes that are mainly involved in detoxification and cytoprotection mechanisms of astrocytes, the expression of which is controlled by Nrf2-ARE signaling pathways (Otterbein et al., 2003). Considering that brain is highly vulnerable to oxidative stress, development of agents that can control oxidative stress by modulating HO-1 expression is considered to be a potential therapeutic approach for treatment of various neurodegenerative diseases (Syapin, 2008).

Arctigenin, the phenylpropanoid dibenzylbutyrolactone lignan isolated from the seeds of Arctium lappa, has been reported to have a variety of pharmacological activities including antitumor, anti-viral, anti-inflammatory, diuretic, and detoxifying effects (Kou et al., 2011; Li et al., 2014; Shi et al., 2014). In addition, arctigenin decreased H₂O₂-induced ROS production and elevated antioxidant capacity of the skeletal muscles (Wu et al., 2014). Recent studies have demonstrated on the neuroprotective effects of arctigenin in the brain. Arctigenin protected cortical neurons from glutamate-induced toxicity by binding to kainate receptor (Jang et al., 2002). Arctigenin exerted protective effects in ischemic stroke through inhibition of neuroinflammation, and ameliorated memory impairment in Alzheimer’s disease mouse models (Fan et al., 2012; Zhu et al., 2013). Despite previous studies, the antioxidant effects of arctigenin in brain glial cells have not been reported and detailed molecular mechanism remains obscure. In this study, we found that arctigenin inhibited ROS production and increased HO-1 expression in rat primary astrocytes. Further mechanistic studies revealed that arctigenin increased the phosphorylation of AKT, a downstream substrate of phosphatidylinositol 3-kinase (PI3K). Treatment of cells with a PI3K-specific inhibitor, LY294002, suppressed the HO-1 expression, Nrf2 DNA binding and ARE-mediated transcriptional activities in arctigenin-treated astrocyte cells. The results collectively suggest that PI3K/AKT signaling pathway is at least partly involved in HO-1 expression by arctigenin via modulation of Nrf2/ARE axis in rat primary astrocytes.

Key Words: Arctigenin, Astrocyte, Hemeoxygenase-1, PI3K/AKT, Nrf2/ARE axis

Arctigenin Increases Hemeoxygenase-1 Gene Expression by Modulating PI3K/AKT Signaling Pathway in Rat Primary Astrocytes

Yeon-Hui Jeong¹, Jin-Sun Park¹, Dong-Hyun Kim² and Hee-Sun Kim¹,*

¹Department of Molecular Medicine, Tissue Injury Defense Research Center, Ewha Womans University Medical School, Seoul 158-710, ²Life and Nanopharmaceutical Sciences, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea

Abstract
In the present study, we found that the natural compound arctigenin inhibited hydrogen peroxide-induced reactive oxygen species (ROS) production in rat primary astrocytes. Since hemeoxygenase-1 (HO-1) plays a critical role as an antioxidant defense factor in the brain, we examined the effect of arctigenin on HO-1 expression in rat primary astrocytes. We found that arctigenin increased HO-1 mRNA and protein levels. Arctigenin also increases the nuclear translocation and DNA binding of Nrf2/c-Jun to the antioxidant response element (ARE) on HO-1 promoter. In addition, arctigenin increased ARE-mediated transcriptional activities in rat primary astrocytes. Further mechanistic studies revealed that arctigenin increased the phosphorylation of AKT, a downstream substrate of phosphatidylinositol 3-kinase (PI3K). Treatment of cells with a PI3K-specific inhibitor, LY294002, suppressed the HO-1 expression, Nrf2 DNA binding and ARE-mediated transcriptional activities in arctigenin-treated astrocyte cells. The results collectively suggest that PI3K/AKT signaling pathway is at least partly involved in HO-1 expression by arctigenin via modulation of Nrf2/ARE axis in rat primary astrocytes.

Key Words: Arctigenin, Astrocyte, Hemeoxygenase-1, PI3K/AKT, Nrf2/ARE axis
Rat primary astrocyte cell culture

Reagents

Artogenic was purchased from Enzo Life Sciences (Farmingdale, NY, USA). The chemical structure of artogenic is as shown in Fig. 1A. All reagents used for cell culture were purchased from Thermo Scientific (Fremont, CA, USA). Antibodies against HO-1, Nrf-2, c-Jun, and lamin A were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Antibodies against phospho- or total forms of ERK, JNK, p38, and AKT were obtained from Cell Signaling Technology (Danvers, MA, USA). All reagent used for RT-PCR were purchased from Promega (Madison, WI, USA).

Rat primary astrocyte cell culture

Rat primary astrocyte cultures were prepared from mixed glial cultures using a previous method with modifications (Park et al., 2011b). In brief, after cortices were dissected from 2-day-old rats, cells were dissociated by pipetting and resuspended in minimal essential medium containing 10% fetal bovine serum, streptomycin (10 μg/mL), penicillin (10 U/mL), 2 mM glutamine, and 10 mM HEPES. Cell suspensions were plated on poly-D-lysine (1 μg/mL)-coated T75 flasks and incubated for 7-10 days. After the primary cultures reached confluence, the culture flasks were shaken at 280 rev/min for 16 h to remove microglia and oligodendrocytes. The purity of the astrocyte-enriched cultures (>95%) were confirmed by staining with antibodies against the astrocyte-specific marker glial fibrillary acidic protein.

Measurement of intracellular ROS levels

The intracellular accumulation of ROS was measured with H$_2$DCF-DA (Sigma-Aldrich) by modifying a previously reported method (Lee and Kim, 2011). In brief, astrocyte cells were stimulated with H$_2$O$_2$ for 1 h and stained with 20 μM H$_2$DCF-DA in PBS for 30 min at 37°C. DCF fluorescence intensity was measured at the 488 nm excitation and 535 nm emission on a fluorescence plate reader.

RT-PCR

Rat primary astrocytes (2.5×10$^5$ cells on a 6 well plate) were treated with artogenic, and total RNA was extracted with TRI reagent (Invitrogen). For RT-PCR, total RNA (1 μg) was reverse-transcribed in a reaction mixture that contains 1 U RNase inhibitor, 500 ng random primers, 3 mM MgCl$_2$, 0.5 mM dNTP, and 10 U reverse transcriptase (Promega). The synthesized cDNA was used as a template for PCR reaction using GoTaq polymerase (Promega) and primers, as below. 5'-TGT CAC CCT GTG CTT GAC CT-3' and 5'-ATA CCC GCT ACC TGG GTG AC-3' for HO-1; 5'-GTG CTG AGT ATG TCG C-3' for ATG0; 5'-GC-3' for 02-05 05 00 51 02 05 0 M arctigenin for 1 h, followed by treat-

nistic studies revealed that PI3K/AKT signaling pathway is involved in HO-1 expression by artogenic via modulation of Nrf2/ARE axis. Our data collectively suggest that artogenic may have therapeutic potential for various neurodegenerative diseases that are accompanied by oxidative stress.

MATERIALS AND METHODS

Western blot analysis

Cells were appropriately treated and total cell lysates were prepared, as previously described (Jung et al., 2010). Proteins (20-100 μg) were heated with 4×SDS sample buffer and separated by SDS-PAGE gel electrophoresis and transferred to nitrocellulose membranes. After blocking, the membranes were incubated with primary antibodies (1:1000) and then, horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution in TBST; New England Biolabs, Ipswich, MA) were applied and the blots were developed using an enhanced chemiluminescence detection kit (Thermo Fisher Scientific, Waltham, MA).

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared from astrocytes as previously described (Woo et al., 2003). The double-stranded DNA oligonucleotides containing the ARE consensus sequences (Promega) were end-labeled by [γ-32P]ATP. Five micrograms of the nuclear proteins were incubated with 32P-labeled ARE probes on ice for 30 min and resolved on a 5% acrylamide gel as previously described (Woo et al., 2003).

Transient transfection and luciferase assays

Rat primary astrocytes were plated in 12 well plate at the density of 2.5×10$^5$ cells, and transfected with 0.5 μg of plasmid DNA using Convoy™ Platinum transfection reagent (CellTA-Gen, Korea). To determine the effect of artogenic on ARE promoter activity, cell were treated with artogenic and incubated for 16 h prior to harvesting cells and luciferase assay was performed as previously described (Park et al., 2007).
Statistical analysis

Unless otherwise stated, all experiments were performed with triplicate samples and repeated at least three times. The data are presented as mean ± S.E.M. and statistical comparisons between groups were performed by using one-way analysis of variance, followed by Newman-Keuls test. A p value < 0.05 was considered significant.

RESULTS

Arctigenin inhibited ROS production in H2O2-treated astrocytes

To determine the antioxidant capacity of arctigenin, we measured intracellular ROS scavenging activity of arctigenin in H2O2-treated astrocyte cells. We found that arctigenin significantly inhibited H2O2-induced ROS production in a dose-dependent manner (Fig. 1B). MTT assay data showed that arctigenin was not cytotoxic at least up to 100 μM (data not shown). The results suggest the strong antioxidant effects of arctigenin in rat primary astrocytes.

Arctigenin increased the expression of antioxidant enzyme HO-1 in astrocytes

To investigate the molecular mechanism underlying antioxidant effects of arctigenin, we examined the effect of arctigenin on the expression of HO-1, which plays a critical role as an antioxidant defense factor in the brain. Western blot and RT-PCR analyses showed that arctigenin increased HO-1 expression at the protein and mRNA levels (Fig. 2). We observed that 5-20 μM of arctigenin upregulated HO-1 expression in
a concentration-dependent manner (Fig. 2A, C). In addition, arctigenin (20 μM) induced HO-1 mRNA and protein expression at 1 h, the level of which was increased at least up to 6 h (Fig. 2B, D).

**Arctigenin increased the nuclear translocation and DNA binding of Nrf2/c-Jun to ARE, and ARE-mediated transcripional activities in rat primary astrocytes**

We performed EMSA to determine whether arctigenin increases nuclear factor binding to ARE on HO-1 promoter. As shown in Fig. 3A, arctigenin increased ARE-nuclear protein binding complex. We have recently demonstrated that Nrf2 and c-Jun bind to ARE and coordinately regulate HO-1 expression in astrocytes (Park et al., 2011b; Park and Kim, 2014). Thus, we examined the effects of arctigenin on Nrf-2 and c-Jun translocation to nucleus. Western blot analysis showed that arctigenin increased the protein amount of Nrf2 and c-Jun in nuclear extracts of astrocyte cells (Fig. 3B). In addition, arctigenin increased ARE-mediated transcriptional activities as shown by ARE-luc reporter gene assay (Fig. 3C). The data suggest that arctigenin increases HO-1 gene expression by enhancing the transcription factor Nrf2/c-Jun binding to ARE on HO-1 promoter.

**Arctigenin increased AKT phosphorylation, and treatment of LY294002 inhibited HO-1 expression by modulating Nrf2/ARE axis**

Previous studies reported that HO-1 expression is under the control of MAPK or PI3K signaling pathways in various cell types (Mates, 2000; Chen et al., 2012; Wang et al., 2012). To identify the signaling pathways modulating HO-1 induction by arctigenin, we examined the effects of arctigenin on MAPK and AKT phosphorylation. As shown in Fig. 4A, arctigenin increased AKT phosphorylation, but it did not affect the phosphorylation of three types of MAP kinases. To investigate whether PI3K/AKT pathway is involved in HO-1 upregulation by arctigenin, we examined the effect of LY294002 (a PI3K inhibitor) on HO-1 expression. As shown in Fig. 4B, C, LY294002 diminished arctigenin-induced HO-1 expression at the protein and mRNA levels. In addition, LY204002 suppressed Nrf2
DNA binding and ARE-luc reporter gene activities (Fig. 4D, E). The data suggest that PI3K/AKT signaling pathway is at least partly involved in HO-1 expression by arctigenin by modulating Nrf2/ARE axis in rat primary astrocytes.

**DISCUSSION**

In the present study, we have demonstrated that the natural compound arctigenin inhibited ROS production in H₂O₂-treated rat primary astrocytes (Fig. 1). We have also demonstrated that arctigenin increased HO-1 expression by enhancing the nuclear translocation and ARE binding of Nrf2 and c-Jun transcription factors (Fig. 2, 3). Finally, PI3K/AKT signaling pathway was identified to be involved in HO-1 expression by modulating Nrf2/ARE axis in arctigenin-treated astrocyte cells (Fig. 4).

Recent studies have suggested Nrf2-ARE signaling pathways as key therapeutic targets for treatment of neuroinflammation and brain disorders (Syapin, 2008). Moreover, Nrf2 is known as a multi-organ protector (Lee et al., 2005). Nrf2 protects various cell types such as liver, kidney, lung, and brain by increasing ARE-driven detoxification and antioxidant genes, such as HO-1, NQO-1, and γ-glutamylcysteine ligase (Lee et al., 2005). Natural compounds, such as curcumin, sulforaphane, resveratrol, and caffeic acid phenyl ester have been reported to induce HO-1 expression by modulating Nrf2 signaling pathways (Lee and Surh, 2005). Our group recently reported that isoflavone metabolites such as tectorigenin and glycinin exert antioxidant effects in hydrogen peroxide-stimulated rat primary astrocytes by upregulating HO-1 and NQO1 expression (Park et al., 2011b). In addition, we demonstrated that tertiary-butylhydroquinone upregulates HO-1 expression by inducing the coordinate interaction of Nrf2 and c-Jun in astrocytes (Park and Kim, 2014). In the present study, we report for the first time the antioxidant effects of arctigenin in astrocytes, and that Nrf2-mediated HO-1 expression may be involved in antioxidant effects of arctigenin.

There are some studies linking the activation of PI3K/AKT signaling pathways to the up-regulation of phase II antioxidant enzymes (Surh, 2003; Li et al. 2006). Oxidative stress activates PI3K/AKT, which subsequently phosphorylate Nrf2 and facilitate Nrf2 release from its cytosolic inhibitor Keap1. Then, Nrf2 is translocated into the nucleus and binds to ARE, inducing the expression of downstream antioxidant genes (Surh, 2003). In addition, there are reports that LY294002 blocked HO-1 expression through Nrf2 pathway against oxidative stress (Martin et al., 2004; Lim et al., 2008). In support of these previous reports, our present study demonstrated that inhibition of PI3K/AKT by LY294002 blocked Nrf2 activation and subsequent HO-1 expression in arctigenin-treated astrocytes, suggesting that AKT phosphorylation by arctigenin plays an important role in Nrf2 activation.

The therapeutic effects of arctigenin have been reported in the brain and peripheral systems. Arctigenin ameliorates inflammation in lipopolysaccharide-induced acute lung injury in rats via activation of AMPK and suppression of NF-xB signaling pathway (Shi et al., 2014). Arctigenin also attenuated TNBS-induced cytokine expressions by inhibiting PI3K, AKT and IKKβ phosphorylations in mice (Hyam et al., 2013). They suggested arctigenin may ameliorate inflammatory disease by inhibiting PI3K/AKT and polarizing M1 macrophages to M2-like macrophages. Thus, the roles of PI3K/AKT appear to be somewhat different in arctigenin-treated astrocytes and inflammatory macrophage cells. The anti-inflammatory effects of arctigenin have been also reported in Raw264.7 macrophage cells, peritoneal macrophages, and brain microglia (Cho et al., 2004; Kou et al., 2011; Park et al., 2011a). A recent study reported that oral administration of arctigenin lowered blood glucose levels and improved lipid metabolism in ob/ob mice, suggesting the therapeutic potential of arctigenin for type 2 diabetes (Huang et al., 2012). In addition, the neuroprotective activity of arctigenin has been demonstrated in various in vitro and in vivo models of neuronal disorders. Arctigenin improved the movement behavior and upregulated dopamine levels in MPTP-injected mouse, an animal model of Parkinson’s disease (Li et al., 2014). Furthermore, the neuroprotective effects of arctigenin have been reported in cerebral ischemia rats and Alzheimer’s disease mouse models (Fan et al., 2012; Zhu et al., 2013).

Considering that oxidative stress is one of the factors contributing to development of neurodegenerative diseases, the antioxidant effects and HO-1 upregulation by arctigenin may support the therapeutic potential of arctigenin for treatment of various neurodegenerative diseases.

**ACKNOWLEDGMENTS**

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (Grant #2012R1A5A2A32671866).

**REFERENCES**

Chen, J. H., Huang, S. M., Tan, T. W., Lin, H. Y., Chen, P. Y., Yeh, W. L., Chou, S. C., Tsai, C. F., Wei, I. H. and Lu, D. Y. (2012) Berberine induces heme oxygenase-1 up-regulation through phosphatidylinositol 3-kinase/AKT and NF-E2-related factor-2 signaling pathway in astrocytes. *Int. Immunopharmacol.* 12, 94-100.

Cho, M. K., Jang, Y. P., Kim, Y. C. and Kim, S. G. (2004) Arctigenin, a phenylpropionan dibenzylbutyrolactone lignan, inhibits MAP kinases and AP-1 activation via potent MKK inhibition: the role in TNF-alpha inhibition. *Int. Immunopharmacol*. 4, 1419-1429.

Dallerac, G., Chever, O. and Rouach, N. (2013) How do astrocytes shape synaptic transmission? Insights from electrophysiology. *Front. Cell. Neurosci.* 7, 159.

de Vries, H. E., Witte, M., Hondius, D., Rozemuller, A. J., Dukarich, B., Hoozemans, J. and van Horssen, J. (2008) Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease? *Free Radic. Biol. Med.* 45, 1375-1383.

Fan, T., Jiang, W. L., Zhu, J. and Feng Zhang, Y. (2012) Arctigenin protects focal cerebral ischemia-reperfusion rats through inhibiting neuroinflammation. *Biol. Pharm. Bull.* 35, 2004-2009.

Huang, S. L., Yu, R. T., Gong, J., Feng, Y., Dai, Y. L., Hu, F., Hu, Y. H., Tao, Y. D. and Leng, Y. (2012) Arctigenin, a natural compound, activates AMP-activated protein kinase via inhibition of mitochondrial complex I and ameliorates metabolic disorders in ob/ob mice. *Diabetologia* 55, 1469-1481.

Hyam, S. R., Lee, I. A., Gu, W., Kim, K. A., Jeong, J. J., Jang, S. E., Han, M. J. and Kim, D. H. (2013) Arctigenin ameliorates inflammation in vitro and in vivo by inhibiting the PI3K/AKT pathway and polarizing M1 macrophages to M2-like macrophages. *Eur. J. Pharmacol.* 708, 21-29.

Jang, Y. P., Kim, S. R., Choi, Y. H., Kim, J., Kim, S. G., Markelonis, G.
J., Oh, T. H. and Kim, Y. C. (2002) Arctigenin protects cultured cortical neurons from glutamate-induced neurodegeneration by binding to kainate receptor. J. Neurosci. Res. 68, 233-240.

Jung, J. S., Shin, J. A., Park, E. M., Lee, J. E., Kang, Y. S., Min, S. W., Kim, D. H., Hyun, J. W., Shin, C. Y. and Kim, H. S. (2010) Anti-inflammatory mechanism of ginsenoside Rh1 in lipopolysaccharide-stimulated microglia: critical role of the protein kinase A pathway and hemeoxygenase-1 expression. J. Neurochem. 115, 1668-1680.

Kou, X., Qi, S., Dai, W., Luo, L. and Yin, Z. (2011) Arctigenin inhibits lipopolysaccharide-induced iNOS expression in RAW264.7 cells through suppressing JAK-STAT signaling pathway. Int. Immunopharmacol. 11, 1095-1102.

Lee, E. J. and Kim, H. S. (2011) Inhibitory mechanism of MMP-9 gene expression by ethyl pyruvate in lipopolysaccharide-stimulated BV2 microglial cells. Neurosci. Lett. 493, 38-43.

Lee, J. M., Li, J., Johnson, D. A., Stein, T. D., Kraft, A. D., Calkins, M. J., Jakel, R. J. and Johnson, J. A. (2005) Nrf2, a multi-organ protector? FASEB J. 19, 1061-1066.

Lee, J. S. and Surh, Y. J. (2005) Nrf2 as a novel molecular target for chemoprevention. Cancer Lett. 224, 171-184.

Li, D., Liu, Q., Jia, D., Dou, D., Wang, X. and Kang, T. (2014) Protective effect of arctigenin against MPP+ and MPTP-induced neurotoxicity. Planta Med. 80, 48-55.

Li, M. H., Cha Y. N. and Surh Y. J. (2006) Peroxynitrite induces HO-1 expression via ethyl pyruvate in lipopolysaccharide-stimulated BV2 microglial cells. Biochem. Biophys. Res. Commun. 347, 672-677.

Lim, J. H., Kim, K. M., Kim, S. W., Hwang, O. and Choi, H. J. (2008) Bromocriptine activates NQO1 via Nrf2-Pi3K/Akt signaling: novel cytoprotective mechanism against oxidative damage. Pharmacol. Res. 57, 325-331.

Martin, D., Rojo, A. I., Salinas, M., Diaz, R., Gallardo, G., Alam, J., De Galarreta, C. M. and Cuadrado, A. (2004) Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical camosol. J. Biol. Chem. 279, 8919-8929.

Mates, J. M. (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicity. Toxicology 153, 85-104.

Otterbein, L. E., Soares, M. P., Yamashita, K. and Bach, F. H. (2003) Heme oxygenase-1: unleashing the protective properties of heme. Trends Immunol. 24, 449-455.

Park, J. H., Hong, Y. J., Moon E., Kim S. A., and Kim, S. Y. (2011a) Forsythiae fructus and its active component, arctigenin, provide neuroprotection by inhibiting neuroinflammation. Biomol. Ther. 19, 425-430.

Park, J. S., Jung, J. S., Jeong, Y. H., Hyun, J. W., Le, T. K., Kim, D. H., Choi, E. C. and Kim, H. S. (2011b) Antioxidant mechanism of isoflavone metabolites in hydrogen peroxide-stimulated rat primary astrocytes: critical role of hemeoxygenase-1 and NQO1 expression. J. Neurochem. 119, 909-919.

Park, J. S. and Kim, H. S. (2014) Regulation of hemeoxygenase-1 gene expression by Nrf2 and c-Jun in tertiary butylhydroquinone-stimulated rat primary astrocytes. Biochem. Biophys. Res. Commun. 447, 672-677.

Park, J. S., Woo, M. S., Kim, D. H., Hyun, J. W., Kim, W. K., Lee, J. C. and Kim, H. S. (2007) Anti-inflammatory mechanisms of isoflavone metabolites in lipopolysaccharide-stimulated microglial cells. J. Pharmacol. Exp. Ther. 320, 1237-1245.

Ryter, S. W., Alam, J. and Choi, A. M. (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol. Rev. 86, 583-650.

Shi, X., Sun, H., Zhou, D., Xi, H. and Shan, L. (2014) Arctigenin attenuates lipopolysaccharide-induced acute lung injury in rats. Inflammation [Epub ahead of print]

Surh Y. J. (2003) Cancer chemoprevention with dietary phytochemicals. Nat. Rev. Cancer 3, 768-780.

Syapin, P. J. (2008) Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders. Br. J. Pharmacol. 155, 623-640.

Vargas, M. R. and Johnson, J. A. (2009) The Nrf2-ARE cytoprotective pathway in astrocytes. Expert Rev. Mol. Med. 11, e17.

Wang, H. Q., Xu, Y. X. and Zhu, C. Q. (2012) Upregulation of heme oxygenase-1 by acteoside through ERK and PI3 K/Akt pathway confers neuroprotection against beta-amyloid-induced neurotoxicity. Neurotox. Res. 21, 368-378.

Woo, M. S., Jiang, P. G., Park, J. S., Kim, W. K., Joh, T. H. and Kim, H. S. (2003) Selective modulation of lipopolysaccharide-stimulated cytokine expression and mitogen-activated protein kinase pathways by dibutyryl-cAMP in BV2 microglial cells. Brain Res. Mol. Brain Res. 113, 86-96.

Wu, R. M., Sun, Y. Y., Zhou, T. T., Zhu, Z. Y., Zhuang, J. J., Tang, X., Chen, J., Hu, L. H. and Shen, X. (2014) Arctigenin enhances swimming endurance of sedentary rats partially by regulation of antioxidant pathways. Acta Pharmacol. Sin. 35, 1274-1284.

Zhu, Z., Yan, J., Jiang, W., Yao, X. G., Chen, J., Chen, L., Li, C., Hu, L., Jiang, H. and Shen, X. (2013) Arctigenin effectively ameliorates memory impairment in Alzheimer's disease model mice targeting both beta-amyloid production and clearance. J. Neurosci. 33, 13138-13149.