Anti-ischemic Activities of *Aralia cordata* and Its Active Component, Oleanolic Acid

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*Aralia* has been reported to exhibit various pharmacological properties, including anti-inflammatory, antidiabetic and antioxidant activities. We performed *in vitro* and *in vivo* analyses on the neuroprotective effects of an ethanolic extract of the aerial parts of *Aralia cordata* Thunb. (Araliaceae). In cultured cortical neurons from rats, *A. cordata* (5-20 µg/mL) inhibited 100 µM hydrogen peroxide (H$_2$O$_2$)-induced apoptotic neuronal death, elevation of intracellular calcium concentration ([Ca$^{2+}$]) and generation of reactive oxygen species (ROS). Since oleanolic acid isolated from *A. cordata* also inhibited H$_2$O$_2$-induced neuronal death, increase in [Ca$^{2+}$], and ROS generation in cultured cortical neurons, some of the neuroprotective effects of *A. cordata* might be attributable to this compound. In rats, *A. cordata* prevented cerebral ischemic injury induced by 3 h of middle cerebral artery occlusion, followed by 24 h of reperfusion. Ischemic infarct and edema volumes were significantly reduced in rats that received *A. cordata* (50 mg/kg, orally). These animals exhibited a corresponding improvement in neurological function and a reduction of neuronal death, as determined histologically from the cortex and hippocampal regions. It is possible that the anti-oxidative properties of *A. cordata* may be responsible for its neuroprotective effects against focal cerebral ischemic injury. In future, *A. cordata* might play a therapeutic role in the prevention and treatment of neurodegeneration in stroke.

**Key words:** *Aralia cordata*, Cortical neurons, Hydrogen peroxide, Ischemia, Neuroprotection, Oleanolic acid

**INTRODUCTION**

*Aralia cordata* Thunb. (Araliaceae) is a medicinal plant distributed widely in Korea, China and Japan. The *A. cordata* root has been used as traditional Chinese medicine in the treatment of rheumatism, lumbago and lameness (Kim, 1998), and it has also demonstrated effectiveness with respect to analgesia, hypothermia and the prolongation of pentobarbital-induced anesthesia (Okuyama et al., 1991). Biological studies have shown that diterpenes are the active constituents of *A. cordata*, responsible for its analgesic and anti-inflammatory activities (Han, 1983; Okuyama et al., 1991). Recent studies have indicated that extracts from the aerial parts of *A. cordata* inhibit cyclooxygenase (COX)-1, COX-2 and COX-2-dependent prostaglandin E$_2$ (PGE$_2$) generation, as well as hyperalgesia during peripheral inflammation (Dang et al., 2005; Lee et al., 2006b; Park et al., 2005). Oleanolic acid (OA) is an active constituent found in the aerial parts of *A. cordata*; it is also found in various traditional medicinal herbs. OA inhibits the

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activity of COX-1 and COX-2 (Lee et al., 2006b). In rats, OA has been shown to afford protection against myocardial ischemia reperfusion injuries by enhancing a mitochondrial antioxidant mechanism mediated by glutathione and α-tocopherol (Du and Ko, 2006). Aralosides from A. elata have been demonstrated to prevent myocardial ischemic damage in rats (Deng et al., 1988). Since reactive oxygen species (ROS) and inflammatory pathways are involved in a complex series of pathophysiological events that occur during neurodegenerative brain diseases (Cherubini et al., 2005), researchers have focused on free radical scavengers and anti-inflammatory agents in their search for potential neuroprotective molecules.

Ischemic stroke results from a transient or permanent reduction in cerebral blood flow caused by the occlusion of a cerebral artery via an embolus or local thrombosis (De Keyser et al., 1999; Dirnagl et al., 1999). Loss of blood flow results in depletions of metabolic substrates such as oxygen and glucose. Oxidative stress is believed to exacerbate the damage caused by cerebral ischemia (Chan, 2001). Since the aerial parts of A. cordata exhibit antioxidant and anti-inflammatory activities, we hypothesized that an ethanolic extract of this plant might protect neurons against ischemia.

In the present study, we investigated the neuroprotective effects of an ethanolic extract of the aerial parts of A. cordata in vivo and in vitro using a rat model and cultured cortical neurons respectively. For this, we tested the effects of A. cordata on cerebral ischemic injuries induced by middle cerebral artery occlusion (MCAO), as well as on hydrogen peroxide (H2O2)-induced cell death in cultured neuronal cells. We show here that OA is an active component of A. cordata that contributes to its neuroprotective effects during H2O2-induced neurotoxicity.

**MATERIALS AND METHODS**

**Chemicals**

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), trypsin (from bovine pancreas), Dulbecco’s modified Eagle’s medium (DMEM), Joklik-modified MEM, poly-L-lysine, H2O2 and 2,3,5-triphenyltetrazolium chloride (TTC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hoechst 33342, Fluo-4 AM, and 2’,7’-dichlorodihydrofluorescein diacetate (H2DCF-DA) were purchased from Molecular Probes Inc. (Eugene, OR, USA). Fetal bovine serum (FBS) was purchased from JRH Biosciences (Lenexa, KS, USA). All other chemicals used were of the highest grade available.

**Plant material, preparation, and identification**

The aerial parts of A. cordata were collected in 2006 in Daejeon, Korea, and identified by one of the authors (Dr. KiHwan Bae). A voucher specimen (CNU 1499) was deposited in the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea. The dried aerial parts of A. cordata (4 kg) were extracted 3 times with ethanol at room temperature for 3 days, filtered, and concentrated to yield an ethanol extract (300 g; yield: 7.5%), which was then stored at 20°C until required. A. cordata was standardized based on its oleic acid contents. Oleic acid was determined using reverse-phase high-performance liquid chromatography (HPLC; Shimadzu, Japan) equipped with a UV detector. Separation was carried out using a YMC-pack pack C18 column (5 μm, 10 × 250 mm; Shisheido, Tokyo) at 30°C with a linear gradient of acetonitrile:water (20:80, v/v) and acetonitrile:water (30:70, v/v) as the mobile phase at a flow rate of 1.5 mL/min. The detector wavelength was set at 210 nm. Oleic acid was found in the aerial parts of A. cordata at a mean level of 2.2 ± 0.08% (n = 3).

The ethanolic extract of A. cordata was suspended in H2O (2 L) and then partitioned successively with hexane (2 L × 3), ethyl acetate (2 L × 3), and butanol (2 L × 3) for activity-guided purification. Since the hexane-soluble fraction exhibited considerable activity, this fraction was investigated extensively. The hexane-soluble fraction (85 g) was chromatographed on a silica gel column (30.0 × 10.0 cm) and eluted with a gradient of hexane-acetone (100:1) for activity-guided purification. Since the hexane-soluble fraction exhibited considerable activity, this fraction was investigated extensively. The hexane-soluble fraction (85 g) was chromatographed on a silica gel column (30.0 × 10.0 cm) and eluted with a gradient of hexane-acetone (100:1→1:2) to produce 4 fractions. Repeated silica gel column chromatography (30.0 × 5.0 cm) of fraction 4 using hexane-acetone (4:1) gave compound 1 (25 mg).

**Compound 1 (oleanolic acid):** white powder; m.p. 196-198°C; IR (KBr) νmax cm−1: 3430 (OH), 1705 (COOH); 1H-NMR (300 MHz, CDCl3) δ (ppm): 0.74, 0.79, 0.90, 0.92, 0.93, 0.99 and 1.12 (each 3H, s), 3.22 (1H, dd, J = 4.0, 9.5 Hz, H-3), 5.28 (1H, m, H-12); 13C-NMR (75 MHz, CDCl3) (ppm): 38.9 (C-1), 28.4 (C-2), 79.8 (C-3), 39.6 (C-4), 56.0 (C-5), 19.0 (C-6), 33.3 (C-7), 40.0 (C-8), 48.4 (C-9), 37.5 (C-10), 23.8 (C-11), 122.8 (C-12), 144.2 (C-13), 42.0 (C-14), 28.4 (C-15), 23.7 (C-16), 46.5 (C-17), 42.0 (C-18), 46.5 (C-19), 31.1 (C-20), 34.5 (C-21), 33.2 (C-22), 28.7 (C-23), 16.5 (C-24), 16.0 (C-25), 18.0 (C-26), 26.0 (C-27), 184.2 (C-28), 33.2 (C-29), 23.8 (C-30).

**Animals**

Sprague-Dawley (SD) rats (Daehan Biolink Co., Ltd., Chungbuk, Korea) were housed in an environmentally controlled room at 22 ± 2°C, with a relative humidity of 55 ± 5%, a 12-h light/dark cycle, and food and water ad libitum. The procedures involving ex-