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The Anti-oxidant Effects of the Water Extracts of *Poris Cum, Semen Zizyphi Spinosae, Semen Biotae Orientalis* and *Acanthopanacis* Cortex on Astrogliocytes

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***Abstract*—Traditional Chinese herb medicines such as *Poris Cum* (PC), *Semen Zizyphi Spinosae* (SZS), *Semen Biotae Orientalis* (SBO) and *Acanthopanacis* Cortex (AC) have been used as functional herbs for calming, promoting sleep and anti-epilepsy for a long time. The objective of this study is to evaluate the anti-oxidant effects of the water extracts of these four herbs on the the cell viability, the ROS production and apoptosis of C6, a mouse astroglioma cell line, under electrical impulse injury as epileptic cells. The results showed that electrical impulse significantly reduced cell viability to 55.4% of control cells. Pre-treaments of the water extracts of PC, SZS, SBO and AC significantly increased cell viability of the electric impulsed-cells up to 79.2, 67.6, 70.6, 75.7% of control cells, respectively. The water extracts of PC significantly suppressed ROS production in the electric impulsed-cells as compared to that in the control electric impulsed-cells. Thus, the water extracts of PC, SZS, SBO and AC may have the neuroprotective effects on astrogliocytes by suppressing the free radical synthesis in treatment of electric impulse.**

Keywords- cell viability; ROS production; apoptosis; epileptic cells; C6 mouse astroglioma cell

I. INTRODUCTION

Epilepsy is induced by abnormal excitatory postsynaptic potential. Free radicals is contributing the damage in the epileptic brain including neuronal injury in cerebral ischemia and hemorrhage and may be involved in neuronal degeneration in schizophrenia, aging, and brain diseases [1]. In addition, reactive oxygen species (ROS) are responsible for the induction for peroxidation of neural lipids, which may injure neuronal membranes, and stimulation on the release of the exceeded excitatory or inhibitory neurotransmitters [2]. Thus, many antioxidants, such as alpha-tocopherol, and condensed tannins, including (-)-epigallocatechin and (-)-epigallocatechin-3-O-gallate, adenosine and its derivative, melatonin, uyaku (*Lindera Strychnifolia*), fermented papaya preparations, Gastrodia elata BI., and Guilingji, have been known to scavenge ROS and/or reactive nitrogen species and therefore can be used as the supplementary therapeutic components to attenuate seizure activities [3].

Epileptic seizure-mediated excitotoxicity is a primary contributing mechanism in seizure-activating apoptotic pathways. Neurons are more vulnerable to free radical damage than glial cells [4]. Reactive oxygen and nitrogen species play important roles in the initiation of apoptotic mechanisms and in mitochondrial permeability transition during the process of neuronal death [5].

Among thousands and thousands of the Chinese traditional herbs, Senhyetang (SHT) consisted of nine herbs has been used for more than two thousand years mainly for memory strengthening and physiological activities. The SHT is consisted of *Rehmanniae Radix*, *Corni Fructus*, *Polygalae Radix*, *Poris Cum* (PC), *Semen Zizyphi Spinosae* (SZS), *Semen Biotae Orientalis* (SBO), *Ginseng Radix*, *Acori Graminei Rhizoma*, *Sinapis Semen.* A recent report has demonstrated that SHT administration may modify the NMDA receptor-dependent response and therefore affect the associative learning and memory [6]. The methanol extracts of *Acanthopanacis* Cortex (AC) had considerable protective effects against CT105-induced cell death [7]. However,

research on the mechanisms of these herbs is still very limited. In hence, the objective of this research is to investigate the anti-oxidant effects of the water extracts of the SZS, SBO, PC and AC on the cell viability, the ROS production and apoptosis of C6, a mouse astroglioma cell line, under electrical impulse injury as epileptic cells.

II. Materials And Methods

A. *Water extracts of SZS, SBO, PC and AC*

The 300g of each SZS, SBO, PC and AC were boiled with 1L of steamed water for 2h, cooled down to the room temperature and then evaporated to dryness under vacuum (Buchi Rotavapor R-200, BÜCHI Labortechnik AG, Switzerland) as the water extracts.

B. *In vitro treatment of C6*

C6 cells, obtained from the American Type Culture Collection (Rockville, MD, U.S.A.), were grown in minimum essential medium (MEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco® BRL, Grand Island, NY) at 37 °C, 5% CO_2 in fully humidified air, and sub-cultured twice a week (Fig. 1).

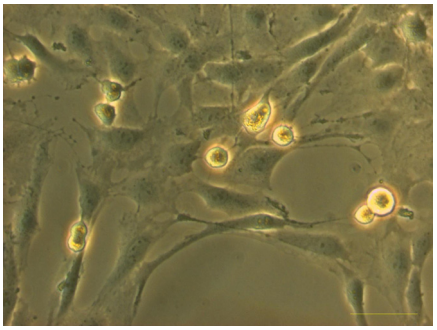

Figure 1. The photograph of the cultured C6 cells (bar=50μm).

C6 cells (1×10^5 cells/100μL/well) seeded in a 96-well plate was pre-treated with 1μL of water extracts of SZS, SBO, PC and AC (10μg/μL) for 24 h and then treated with or without electrical impulses (5mV/50 pulses) using a electroporator (BTX ECM830, BTX Bioscience company, USA). Then, the cell viability, the ROS production and apoptosis of the resultant cells were detected as following procedures.

C. *Cell viability assay*

The cell viability was estimated using the commercially available methylthiazoletetrazolium (MTT) kit (Sigma Chemical Co., St. Louis, MO, USA). The resultant cells were treated with 50μL of MTT reagent at 37 °C, 5% CO_2 for 1h. After the supernatant was evacuated, 100μL of dimethyl sulfoxide (DMSO) was added to solve the formazan reduction product of MTT and then the absorbance was read at the 595nm using an ELISA reader in this colorimetric assay.

D. *ROS production assay*

For evaluation of ROS production, the resultant cells were treated with 10μM of acetylated 2,7-dichlorofluorescein diacetate ($DCFH_2DA$) (Invitrogen Corp., Carlsbad, CA, USA), a fluorescent dye, for 30 min at 37°C. Then, the ROS production within epileptic cells stained with the $DCFH_2DA$ was determined using the flow cytometer (Coulter Epics Altra Flow Cytometry, Beckman Coulter, CA).

E. *Apoptosis assay*

The apoptosis of the resultant cells were determined using the commercial Apoptosis Kit (Invitrogen Corp.). In each well of the resultant cells, 5 μL Alexa Fluor® 488 annexin V and 1 μL 5 μM SYTOX® Green working solution were added and incubated at room temperature for 15 min. After the incubation period, 400 μL 1X annexin-binding buffer was mixed and then the fluorescence-staining apoptotic cells were immediately analyzed by using the flow cytometry (Coulter Epics Altra Flow Cytometry).

III. Results

After the treatment of electrical impulse, the survival rate of the epileptic cell was significantly reduced to 58.3±12.2% as compared to that of the normal cells. However, the pre-treatment of water extracts of PC, SZS, SBO and AC for 24 h, the cell viabilities of C6 cells were significantly increased to 79.2±12.5%, 67.6±9.3%, 70.6±9.5% and 75.7±9.1%, respectively (Fig. 2).

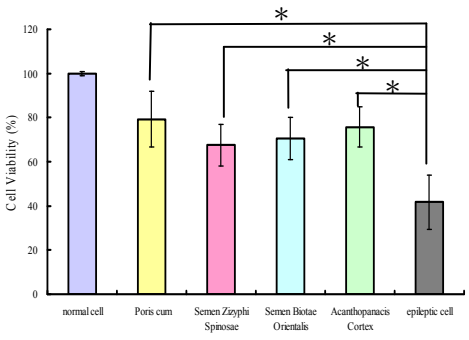

Figure 2. The effects of water extracts of PC, SZS, SBO and AC on cell viability of the C6 cells with electrical stimulation.

The treatment of electrical impulse increased the ROS production within epileptic cells up to 67.6±0.9% as compared to that of the normal controls. The water extracts of PC, SZS, SBO and AC significantly reduce the ROS production to 33.5±10.1%, 23.1±9.3%, 31.9±2.7% and 28.3±8.1% within epileptic cells, respectively (Fig. 3).

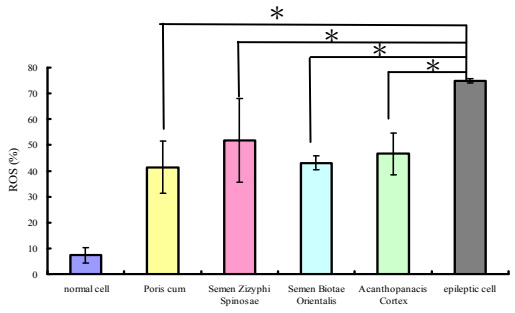

Figure 3. The effects of water extracts of PC, SZS, SBO and AC on ROS production of the C6 cells with electrical stimulation.

The electrical stimulation induces 15.0±3.1% of cell apoptosis of C6 (Fig. 4). The water extracts of PC, SZS, SBO and AC significantly reduced the percentages of apoptotic cells to 11.7±1.2%, 8.8±2.7%, 11.3±0.4% and 7.1±1.1%, respectively (Fig. 5).

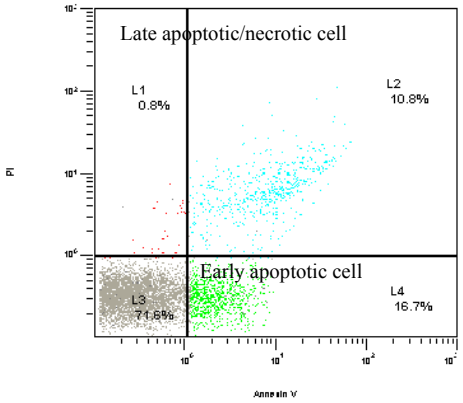

Figure 4. The apoptosis of the C6 cells with electrical stimulation.

IV. Discussions

The main aim of the present study was to test whether the water fractions isolated from *Poris Cum*, *Semen Zizyphi Spinosae*, *Semen Biotae Orientalis* and *Acanthopanacis Cortex* protect C6 cells against electrical impulse-induced cell damage. Among of the tested water extract of several plants, results show the water extracts of *Poris Cum*, *Semen Zizyphi Spinosae*, *Semen Biotae Orientalis* and *Acanthopanacis Cortex* can significantly decrease the electrical stimulation-induced cell death. The neuro-protective effects of the water extracts of *Poris Cum*, *Semen Biotae Orientalis* and *Acanthopanacis Cortex* may be due to the prevention of ROS production induced by electrical stimulation. It has been reported that natural products, such as danthron, a component of Rumex japonicus, senna, and aloe, attenuates β-amyloid-induced neurotoxicity in a murine cortical culture system and thus might be beneficial for the therapeutic agent of Alzheimer's disease [7]. However, the mechanism and the mode of action of these natural products are not well understood.

In the occurrence of epileptic seizures, excitatory amino acid receptor activation by glutamate or N-methyl-D-aspartic acid (NMDA) has been known to accompany generation of ROS, e.g., superoxide anion radical, hydrogen peroxide, and hydroxyl radical [3]. These ROS may exert injurious effects in the brain.

There are many well known radical-scavenging antioxidants, such as vitamin E (tocopherol), vitamin C, carotenoids and glutathione. In addition, many natural phenolic antioxidants have been found in plants, including vegetables, teas, and Chinese herbal medicines. Ita has been reported that pre-treatment with a free radical scavenger or antioxidant may prevent the development of epileptic activity in rat brain by decreasing the formation of peroxides at the injury sites of brain tissues [8]. The results of the present study also approve that the water extracts of PC, SZS, SBO and AC may have the neuroprotective effects on astrogliocytes by suppressing the free radical synthesis in treatment of electric impulse; these results not only demonstrate new neuroprotective components from plant extracts but also interpret part of the mechanism of their protection effects.

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References

[1] J.A. Jesberger, and J.S. Richardson, "Oxygen free radicals and brain dysfunction," Int J Neuro Sci, vol. 57, Mar 1991, pp. 1-17.

[2] S. Waldbaum, and M. Patel, "Mitochondria, oxidative stress, and temporal lobe epilepsy," Epilepsy Res, vol. 88, Jan 2010, pp. 23-45, doi:10.1016/j.eplepsyres.2009.09.020

[3] A. Mori, I. Yokoi, Y. Noda, and L.J. Willmore, "Natural antioxidants may prevent posttraumatic epilepsy: a proposal based on experimental animal studies," Acta Med Okayama, vol. 58, Jun 2004, pp.111-118.

[4] K. Blomgren, and H. Hagberg, "Free radicals, mitochondria, and hypoxia-ischemia in the developing brain," Free Radic Biol Med, vol. 40, Feb 2006, pp. 388-397, doi:10.1016/j.freeradbiomed.2005.08.040

[5] J.L. Franklin, "Redox Regulation of the Intrinsic Pathway in Neuronal Apoptosis," Antioxid Redox Signal, , Sep 2010, [Epub ahead of print] doi:10.1089/ars.2010.3596

[6] U.H. Jin, J.H. Kim, G.T. Chang, J.K. Kim, K.H. Chung, and C.H. Kim, "Enhancement of learning and memory by a medicinal formulation, Saenhyetang, in mice," J Ethnopharmacol, vol. 109, Jan 2007, pp. 271-280, doi:10.1016/j.jep.2006.07.030

[7] S.T. Kim, J.D. Kim, Y.S. Lyu, M.Y. Lee, and H.W. Kang, "Neuroprotective effect of some plant extracts in cultured CT105-induced PC12 cells," Biol Pharm Bull, vol. 29, Oct 2006, pp. 2021-2024, doi:10.1248/bpb.29.2021

[8] L.J. Willmore, and J.J. Rubin, "Antiperoxidant pretreatment and ironinduced epileptiform discharges in the rat: EEG and histopathologic studies," Neurology, vol. 31, 1981, pp. 63-69.

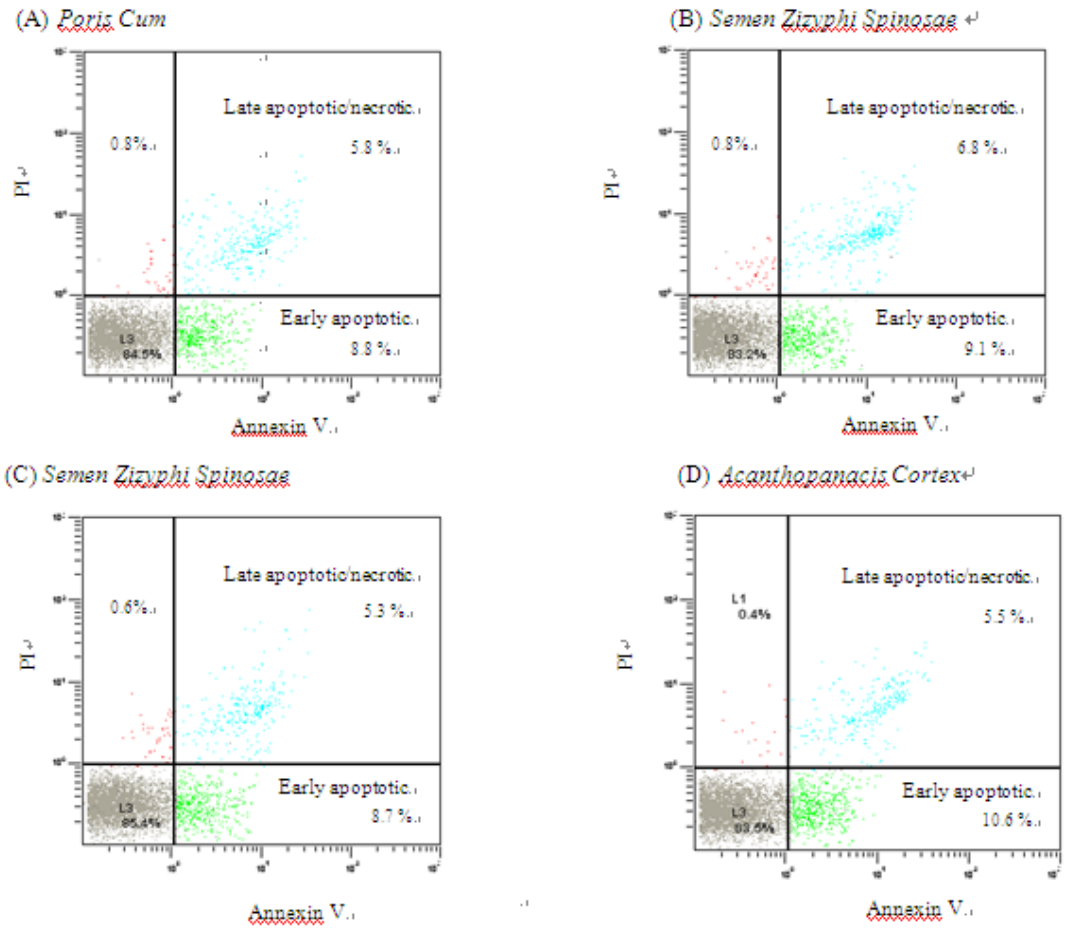

Figure 5. The effects of water extracts of PC, SZS, SBO and AC on apoptosis of the C6 cells treated with electrical stimulation.