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Neuroprotective effects of *Eleutherococcus senticosus* bark on transient global cerebral ischemia in rats

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ABSTRACT

Ethnopharmacological relevance: Eleutherococcus senticosus Maxim., classified into the family of Araliaceae, is used in a variety of diseases in traditional Korean medicine including ischemic heart disease.
Aim of the study: To determine the neuroprotective effects of *Eleutherococcus senticosus* on global cerebral ischemia.
Materials and methods: A four-vessel occlusion (4-VO) rat model was used to evaluate the potential protective effects against transient global cerebral ischemia ethanol extracts of *Eleutherococcus senticosus* was orally administered at doses of 3, 30, and 300 mg/kg twice at times of 0 and 90 min after reperfusion. The effects on memory deficit were investigated by using a Y-maze neurobehavioral test after brain ischemia, and the effects on hippocampal neuronal damage were measured 7 days after ischemia. The expressions of glial fibrillary acid protein (GFAP), CD11b antibody (OX-42), and cyclooxygenase-2 (COX-2) were investigated by immunohistochemistry.
Results: Oral administration of *Eleutherococcus seticosus* at 30, 100 and 300 mg/kg significantly reduced hippocampal CA1 neuronal death by 3.5%, 25.9% and 53.1%, respectively, compared with a vehicle-treated group. Oral administration of *Eleutherococcus senticosus* at 300 mg/kg inhibited 81.9% of the decrease in spontaneous alternation induced by 4-VOin the Y-maze test, and also attenuated ischemia-induced activation of COX-2, GFAP and OX-42 in the hippocampal CA1 region.
Conclusion: Eleutherococcus senticosus protects delayed neuronal death in the CA1 region of the hippocampus against global cerebral ischemia in rats with the recovery of spatial memory, which can be considered as the normal functioning of the hippocampus. Regarding the immunohistochemical study, the effect of *Eleutherococcus senticosus* may be attributable to its anti-inflammatory properties through the inhibition of COX-2 expression, microglia and astrocyte expression.

1. Introduction

Transient global cerebral ischemia, arising in humans, can be a consequence of cardiac arrest or severe systemic hypotension. It leads to major neuropsychological dysfunctions, including learning and memory disabilities (Peskine et al., 2004). Although cardiac arrest and cerebral ischemia remain as leading causes of adult disability in industrialised countries, little progress has been achieved in translation of promising experimental therapies into clinical practice (O'Collins et al., 2006; Rosamond et al., 2008). The main focus of drug development to protect ischemia-induced injury has been the investigation of neuroprotective sources capable of protecting salvageable neurons from ischemic cell death. Natural products, especially medicinal plants, could be an ideal source to develop safe and effective agents for neuroprotection of ischemia-induced injury (Kim, 2005).

Eleutherococcus senticosus Maxim., known as Siberian Ginseng, is a medicinal herb with a long history of use including ischemic heart disease due to its traditional Korean medical effects such as tonify *qi*, strengthen muscle and bone, and tranquilize (Yi et al., 2001; Wang et al., 2010). *Eleutherococcus senticosus* has also been reported to possess anti-stress, anti-tumor, hypoglycemic, and anti-arrhythmic effects (Hibasami et al., 2000; Kimura and Sumiyoshi, 2004; Park et al., 2006; Maslov and Guzarova, 2007). The major active components of *Eleutherococcus senticosus* are eleutherosides, chiisanoside, acanthosides, daucosterin, β-sitosterol, sesamin, and they are responsible for its diverse biological activities (Davydov and Krikorian, 2000). It has been reported that eleutheroside E, liriodendrin, isofraxidin, chiisanoside and β-sitosterol have been reported to have

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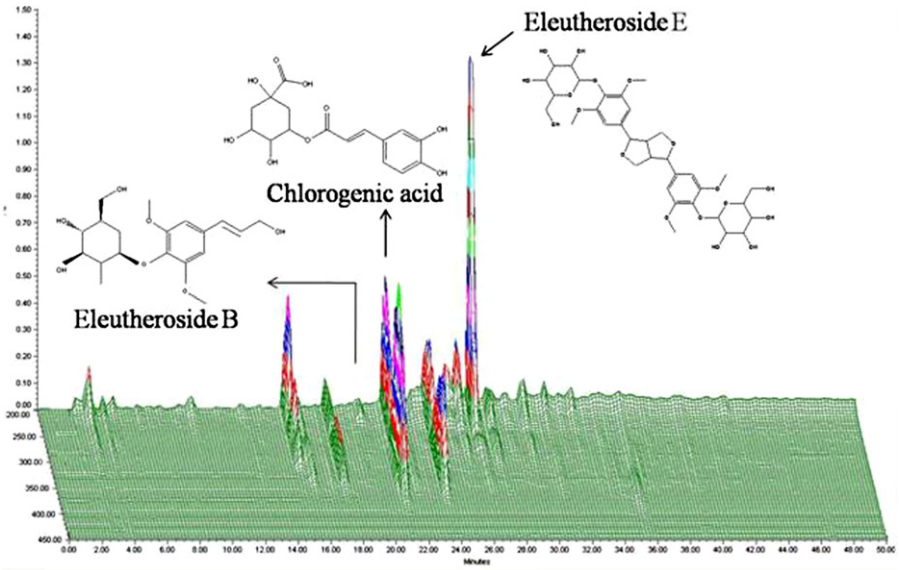

Fig. 1. 3-D HPLC chromatogram for standardization of *Eleutherococcus senticosus*. Detection was performed by using a photodiode array detector. *X*-axis is retention time; *Y*-axis is wavelength, and *Z*-axis is absorbance unit. Analytical conditions were as follows: column, C_{18} Φ4 250 mm; mobile phase, solvent A (1% H_3PO_4) and solvent B (CH_3CN); flow rate, 1 ml/min; program, 0–60 min 5–50% B; 60–61 min 50–70% B; 61–85 min 70% B.

anti-inflammatory effects (Tokiwa et al., 2006; Jung et al., 2003; Yamazaki et al., 2007). Especially, *Eleutherococcus senticosus* has been reported to protect against neuronal death induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and amyloid beta (Fujikawa et al., 2005; Tohda et al., 2008).

Recently, our group found that *Eleutherococcus senticosus* reduces the infarct volume in transient focal cerebral ischemia (Bu et al., 2005). However focal ischemia is a far more complex insult than global ischemia with several unresolved issues such as extracellular edema, vascular damage while global ischemia involves a short very intense insult in which ATP is severely lowered and is quite uniform as a delayed neuronal death (Lipton, 1999). Global ischemia is characterized by a slow development of cell death during reperfusion, which shows great selectivity. There is no intimation of involvement of the vasculature, although there is a real possibility that microglia/macrophages play a role in the process (Swan et al., 1988).

The aim of the present study was to determine the neuroprotective effects of *Eleutherococcus senticosus* in global cerebral ischemia in rats. We used a four-vessel occlusion (4-VO) rat model to evaluate the potential protective effects against transient global cerebral ischemia (Katsuta et al., 2003). Y-maze test was used to investigate spatial memory impairment. We observed the expression of glial fibrillary acid protein (GFAP), CD11b antibody (OX-42), and COX-2 by immunohistochemistry to find out the inhibitory effects on microglia activation, astrocyte activation and COX-2 upregulation which are related in inflammation.

2. Materials and methods

2.1. Plant material

The dried stem bark of *Eleutherococcus senticosus* was purchased from *Yaksudang* Co. (Seoul, Korea). It was identified by Dr. Ho-Young Choi, Department of Herbal Pharmacology, College of Oriental Medicine, Kyung Hee University where the voucher specimen (#HP060) is deposited.

2.2. Sample preparation

Eleutherococcus senticosus (150 g) was extracted with 70% ethanol (3000 ml) for 3 h at 80 °C in a reflux apparatus. The extract was filtrated and concentrated under reduced pressure, then, lyophilized to yield a dark brown powder. The yield of extract was 9.26%. The sample was then stored at −20 °C for further use. The quantitative authentication of *Eleutherococcus senticosus* was performed by a high performance liquid chromatography (HPLC) analysis system equipped with a Waters 600 controller, a 717 autosampler and a 996 PDA detector. The chromatic separation was achieved at room temperature on Hypersil Gold C_{18} column (250 mm × 4 mm i.d., 5 μm particle size). Mobile phases A and B were 1% H_3PO_4 (v/v) and CH_3CN, respectively. Gradient elution was as follows: 0–60 min 5–50% B; 60–61 min 50–70% B; 61–85 min 70% B. The flow rate was 1.0 ml/min and the sample injection volume was 10.0 μl. The isolated compounds were monitored with a photodiode array detector (926; Waters, Milford, MA, USA). In HPLC analysis, 3 compounds were identified in *Eleutherococcus senticosus*: eleutheroside E, eleutheroside B and chlorogenic acid. Among them, the content of eleutheroside E was calculated for standardization. *Eleutherococcus senticosus* was standardized to contain 0.486 ± 0.046% eleutheroside E. A 3-D HPLC chromatogram and the structures of the constituent compounds are shown in Fig. 1.

2.3. Animals

Male Wistar rats (170–190 g) were obtained from Samtako Co. (Osan, Korea). Animals were allowed to have an access to water and food *ad libitum*, and maintained under a constant temperature (23 ± 1 °C), humidity (60 ± 10%) and a 12 h light/dark cycle (light on 07:00–19:00 h). Animal treatment and maintenance were carried out in accordance with the Principle of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University.

2.4. Surgery

Transient global cerebral ischemia was induced by 4-VO, as described in previous (Pulsinelli and Brierley, 1979). Briefly, under 1.5–2.0% isoflurane anesthesia in a mixture of 70% N_2O/30%O_2, vertebral arteries were electrocauterized and common carotid arteries were exposed. On the following day, both carotid arteries were occluded with aneurysm clips to induce cerebral ischemia. After