INTRODUCTION

Epilepsy is one of the most common and serious neurological conditions, with an annual incidence of 50 people per 100,000 (Poole et al., 2000). Seizures are controlled in nearly 70% of patients with epilepsy, mostly through drug effects on membrane ion channels or on GABAergic or glutamatergic transmission. However, for the remaining 20-30% with intractable seizures, recent advances in systemic antiepileptic drug development have had little impact. Refractory epilepsy is associated with considerable medical, social, and psychiatric morbidity and enormous financial costs (Sander, 2003). Thus, despite increasing interest in alternative medicine use, there are limited data on alternative medicine use by patients with epilepsy (Gidal et al., 1999). Therefore, we investigated several plants to discover whether they have anticonvulsant activities.

As one of the famous traditional Chinese medicine, Artemisia capillaris Herba (AC) is listed officially in the Chinese pharmacopoeia and used as a choleretic, anti-inflammatory and diuretic agent in the treatment of epidemic hepatitis (Tang and Eisenbrand, 1992). Esculetin (ECT), which is main constituent of AC, has multiple pharmacological activities including the inhibition of xanthine oxidase activity (Egan et al., 1990), platelet aggregation (Okada et al., 1995) and antioxidant activity (Paya et al., 1992; Lin et al., 2000). Although AC and ECT have various biological effects, their anticonvulsant effects have not been reported.

In the present study, we examined the anticonvulsant effect of the 70% ethanol extract of AC and its mechanism. The objective of this study was to evaluate the possibility of AC as an anticonvulsant drug and to find out which constituent ex-
erts this activity. First, behavioral tests, electroshock seizure, chemical induced seizure were studied in vivo in ICR mice. Secondly, influx of Cl\(^-\) was studied in vitro using neuroblastoma cells.

**MATERIALS AND METHODS**

**Materials**

70% ethanol extract of AC was supplied by the National Center for Standardization of Herbal Medicine. ECT was purchased from Wako Pure Chemical Industries (Japan). Bicuculline, diazepam and other materials were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). N-(6-methoxyquinolyl) acetoxethylester (MQAE) was purchased from Invitrogen Co. (Carlsbad, USA). 70% ethanol extract of Artemisia capillaris, diazepam, strychnine and pentylenetetrazol (PTZ) were dissolved in sterile distilled water before injection. AC was orally administered in a doses of 50, 100, 200, 400 mg/kg and ECT was intraperitoneally injected in doses of 1, 2 and 5mg/kg. 5mg/kg of diazepam was intraperitoneally injected to mice to serve the positive control group. Animals of the negative control group were administered saline. Bicuculline was dissolved in dimethyl sulfoxide (DMSO) and the maximum concentration of DMSO was 0.1%. Cells were cultured in MEM (Gibco BRL, Rockville, MD) supplemented with 10% fetal bovine serum(FBS) and 5% CO\(_2\) at 37°C.

**Animals and treatments**

The male ICR mice (20-25 g) used in this study were obtained from Hanlim Laboratory Animals Co. (Hwaseong, Korea). They were housed in animal room which was maintained at temperature (22 ± 2°C) and humidity (55 ± 5%) under a 12/12-hr light/dark cycle with lights on from 7:00 AM. Food and water were available ad libitum. All animals were acclimated to their home cages for at least 6 days before testing. The experimental groups, consisting of 8-10 animals per drug and dose, were chosen by means of a randomized schedule and all mice were used only once. All tests took place between 10:00 and 16:00 h. Animal treatment and maintenance were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23 revised 1985) and the Animal Care and Use Guidelines of Sahmyook University, Korea.

**Locomotor activity**

Computerized EthoVision system (Noldus IT b.v., Netherlands) was used to evaluate changes in locomotor activity. The observation apparatus consisted of five plastic boxes (42×42 cm) with a field bordered by 42-cm-high sidewalls. The total distance moved, total movement time, and total turn angle degree were monitored for 10 min after administration (Noldus et al., 2001; Kim et al., 2003).

**Rota-rod test**

The rota-rod test was used to assess whether materials caused myorelaxation or gross motor impairment in the animals. Twenty-four hours before the experiment, all mice were habituated to running in a rota-rod at a speed of 36 rpm for 3 minutes. The latency to fall and falling frequency were recorded 30 min after administration (Farkas et al., 2005; Lee et al., 2006).

**Measurement of electroshock seizure threshold**

Seizure was evoked by constant current stimulator and the resulting seizure was determined by overt hindlimb extension. To determine the electroshock seizure threshold, convulsive current 50 (CC\(_{50}\)) which elicits convulsion in 50% of animals was calculated by a ‘staircase’ procedure (Browning et al., 1990). Individual animals was treated with electroshocks of 1 second stimulus duration to determine the current-convulsion relationship. If an animal showed convulsion, the next animal was given with 3 mA decrements in current intensity. If an animal did not show convulsion, the next animal was given with 3mA increments in current intensity. In this way, the current-convulsion relationship was generated and CC\(_{50}\) value was determined by Litchfield-Wilcoxon II method (Litchfield and Wilcoxon, 1949). For each treatment group, 20-30 pups were prepared and the animals were sacrificed right after the determination of the electroshock seizure threshold.

**Test for anticonvulsant potency (PTZ model)**

The different experimental groups of mice (n=10/group) orally treated with 50 mg/kg and 100 mg/kg of AC were challenged with PTZ (70 mg/kg, i.p.) 30 min. after the administration of AC (Novack et al., 2005; Obniska et al., 1978). Control group received saline. The percentage of seizure response induced by PTZ in mice was recorded and compared with the respective control group.

**Test for anticonvulsant potency (strychnine model)**

The different experimental groups of mice (n=10/group) treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of AC were challenged with strychnine (1 mg/kg, i.p.) 30 min. after the administration of AC (Ngo Bum et al., 2001). Control group received saline. The percentage of seizure response induced by strychnine in mice was recorded and compared with the respective control group.

**Intracellular Cl\(^-\) measurement assay**

Relative changes in intracellular Cl\(^-\) concentration ([Cl\(^-\)]\_i\) in SH-SY5Y human neuroblastoma cells were monitored using the Cl\(^-\)-sensitive indicator, N-(6-methoxyquinolyl) acetoxetylester (MQAE), developed by Verkman et al. (1989). Experiments were performed, as described by West and Molly (1996). Briefly, cells were washed twice and resuspended at a concentration of 4×10\(^5\) cells/ml in Hank's solution. For loading MQAE into the cells, cells were incubated with the dye overnight at a final concentration of 5 mM at room temperature. Fluorescence (excitation wavelength set at 365 nm and the emission wavelength at 450 nm) was monitored in a well-stirred cuvette. Experiments were performed at room temperature to minimize fluorescent dye loss. Data are presented as relative fluorescence F/F\(_0\), where F\(_0\) is the fluorescence without Cl\(^-\) ions and F is the fluorescence as a function of time. The F/ F\(_0\) values are directly proportional to [Cl\(^-\)]\_i. All fluorescence values were corrected for background fluorescence which was separately determined using a HEPES-buffered KSCN solution containing 5 μM valinomycin to maximally quench the MQAE ion-selective signal (Shumaker et al., 1999). In separate experiments the F\(_0\) value was determined by bathing the cells with Cl\(^-\)-free (KNO\(_3\)) solution containing 10 mM tributyltin and 10 mM nigericin.