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Anticonvulsant Effect of *Artemisia capillaris* Herba in Mice

Tae Seon Woo[1], Seo Young Yoon[1,5], Ike Campomayor dela Peña[1], Ji Young Choi[1], Hye Lim Lee[1], Yoon Jung Choi[1], Yong Soo Lee[2], Jong Hoon Ryu[3], Jae Sue Choi[4] and Jae Hoon Cheong[1,*]

[1]*Uimyung Research Institute for Neuroscience, Sahmyook University, Seoul 139-742,*
[2]*Department of Pharmacology, College of Pharmacy, Duksung Women's University, Seoul 132-714,*
[3]*Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130-701,*
[4]*Department of Food Science and Nutrition, College of Fisheries Science, Pukyong National University, Busan 608-739,*
[5]*Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea*

Abstract

In the present study, the anticonvulsant effects of *Artemisia capillaris* Herba (AC) and its major constituent, esculetin (ECT), were tested and the mechanism studied. Locomotion, Myorelaxation, motor coordination and electroshock seizure experiment were conducted in mice. To identify the anticonvulsant mechanism effect of this drug, chemical-induced seizure in mice and the ionic movement in neuroblastoma cells were also observed. The ethanol extract of AC was orally administered to mice 30 min. prior to testing and ECT was intraperitoneally injected. AC and ECT treatment did not change locomotor activities as well as activities on the rota-rod, which indicates that they did not cause a sedative and myorelaxation effect. AC and ECT treatment increased threshold of convulsion induced by electroshock. AC treatment also inhibited convulsion induced by pentylenetetrazole. In the case of strychnine however, only high dose of AC treatment inhibited convulsion. AC and ECT treatment increased the Cl^- influx into the intracellular area in a dose-dependent manner. On the other hand, bicuculline, a GABA antagonist, inhibited the Cl^- influx induced by AC and ECT. These results indicate that ECT induces the anticonvulsive effect of AC extract through the GABAergic neuron.

Key Words: *Artemisia capillaris*, Esculetin, Anticonvulsion, Seizure, GABA

INTRODUCTION

Epilepsy is one of the most common 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 the beneficial effect of the currently available drugs, there is still a need for broadly acting anticonvulsant drugs possessing multiple mechanisms of action with decreased adverse effect, preferably originated from natural products (Park *et al.*, 2007).

Herbal medicine is one of the most common forms of alternative medicine, and patients generally consider this form of treatment to be both safe and effective (Eisenberg *et al.*, 1998). 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 Eisenbrand1992). 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-

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***Corresponding Author**
E-mail: cheongjh@syu.ac.kr
Tel: +82-2-3399-1605, Fax: +82-2-3399-1619

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) acetoethylester (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% fatal 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-Wilcoxons 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) acetoetylester (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/F0, where F0 is the fluorescence without Cl^- ions and F is the fluorescence as a function of time. The F/F0 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 F0 value was determined by bathing the cells with Cl^--free (KNO3) solution containing 10mM tributyltin and 10 mM nigericin.

Statistical analysis

Data were expressed as the mean ± S.E.M. For statistical evaluation of data, one-way ANOVA was used. When statistically significant differences were found, Newman-Keul's test was used as a post-hoc test to determine the statistical differences between groups. Differences were considered statistically significant when $p<0.05$.

RESULTS

Locomotor activity

Locomotor activities were determined based on the total distance moved and the duration of movement. Fig. 1 and Fig. 2 show that AC and ECT did not change the locomotor activity while diazepam significantly decreased locomotor activity in mice.

Rota-rod test

Motor coordination was evaluated by rota-rod test. Fig. 3 and Fig. 4 show that AC and ECT did not change the running time and falling frequency on the rota-rod. Diazepam, however, significantly decreased running time and increased falling frequency on the rota-rod.

Measurement of electroshock seizure threshold

The effects of AC and ECT on the electroshock seizure threshold are shown in Fig. 5. To determine the electroshock seizure threshold, we calculated the convulsive current 50 (CC_{50}), which elicits convulsion in 50% of animals. Electro-

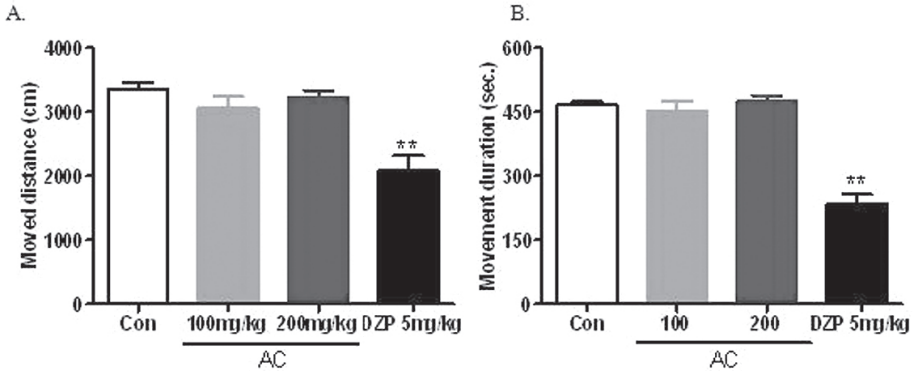

Fig. 1. Effects of AC on Locomotor activity in mice (n=9-10). (A) Each bar represents the mean ± S.E.M of the moved distance in AC treatment for 10 minutes. (B) Each bar represents the mean ± S.E.M of the movement duration in AC treatment for 10 minutes (**$p<0.01$ compared to control group).

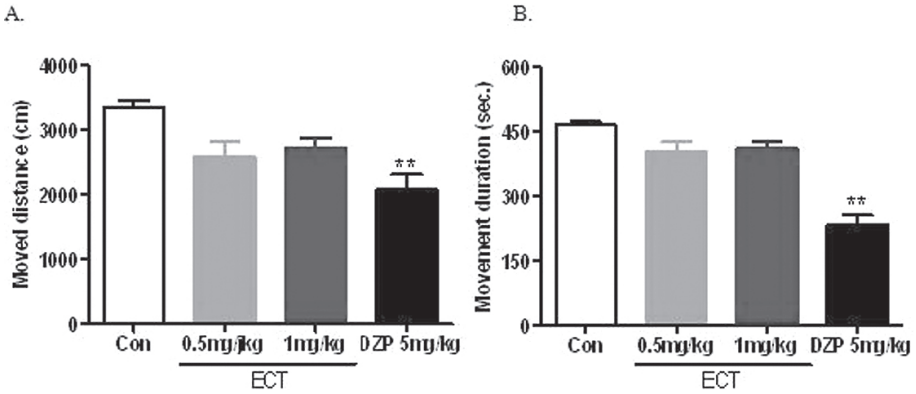

Fig. 2. Effects of ECT on Locomotor activity in mice (n=9-10). (A) Each bar represents the mean ± S.E.M of the moved distance in AC treatment for 10 minutes. (B) Each bar represents the mean ± S.E.M of the movement duration in AC treatment for 10 minutes (**$p<0.01$ compared to control group).

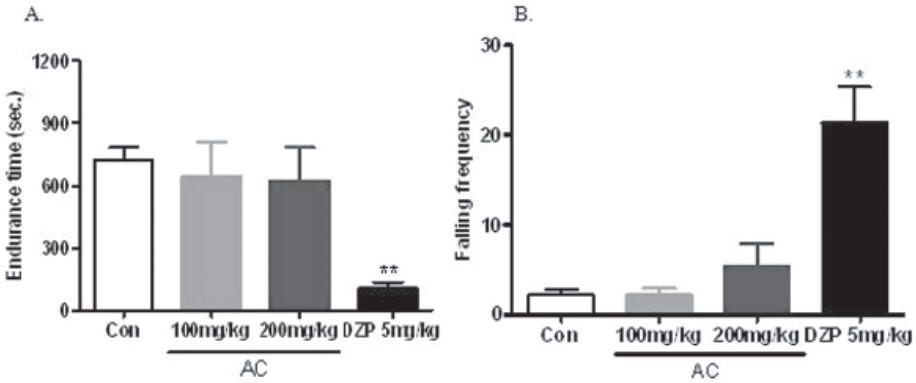

Fig. 3. Effects of AC on activity on the rotating rod in mice (n=9-10). (A) Each bar represents the mean ± S.E.M of endurance time on the rotating rod. (B) Each bar represents the mean ± S.E.M of falling frequency from rotating rod (**$p<0.01$ compared control group).

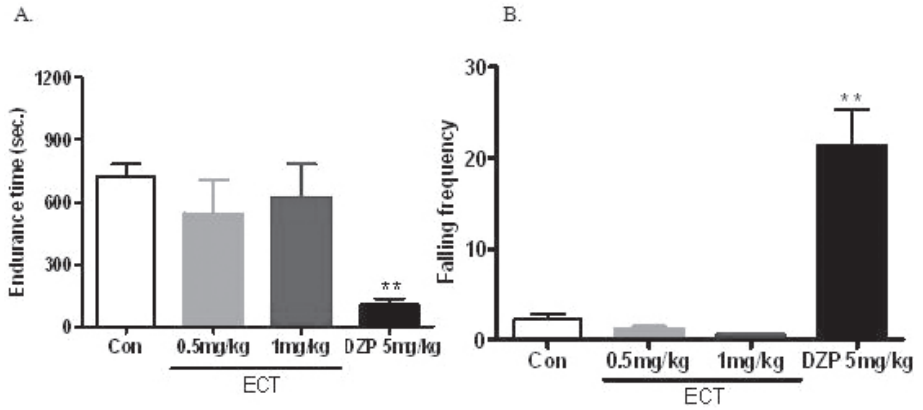

Fig. 4. Effects of ECT on activity on the rotating rod in mice (n=9-10). (A) Each bar represents the mean ± S.E.M of endurance time on the rotating rod. (B) Each bar represents the mean ± S.E.M of falling frequency from rotating rod (**p<0.01 compared control group).

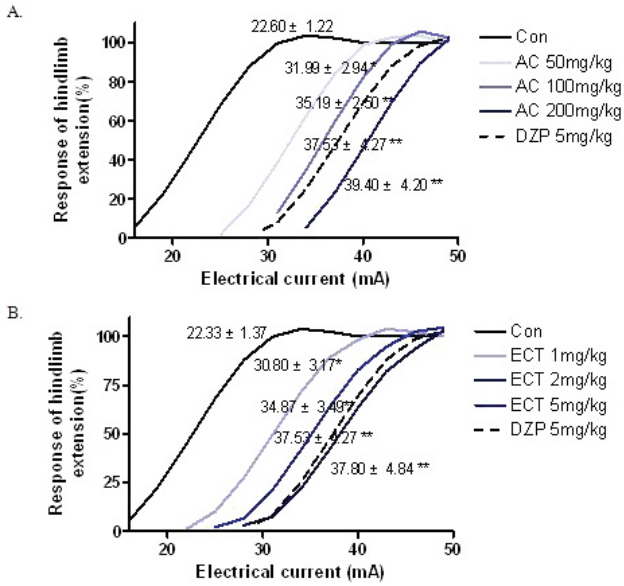

Fig. 5. Effects of AC and ECT on seizure induced by electroshock in mice. Each number on the graph represents CC_{50} ± 95% confidence intervals (*p<0.05 **p<0.01 compared to control group).

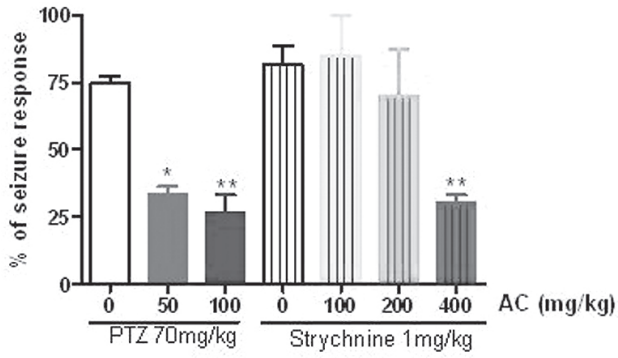

Fig. 6. Effects of AC on seizure induced by chemicals in mice. Each bar represents mean ± SEM of seizure response % (*p<0.05, **p<0.01 compared to control group).

shock seizure thresholds of the AC-treated mice were significantly higher than those of the control group. CC_{50} of the AC-treated mice increased in a dose-dependent manner. The CC_{50} of the 100 and 200 mg/kg of AC-treated mice were 35.19 ± 2.50 and 39.40 ± 4.20 mA, respectively, and the CC_{50} of the diazepam-treated group was 37.53 ± 4.27 mA. The CC_{50} of the 200 mg/kg AC group was significantly higher than that of the diazepam group. ECT treatment also significantly increased electroshock seizure thresholds. The CC_{50} of the 1 mg/kg and 2 mg/kg of ECT treatment were 30.80 ± 3.17 and 34.87 ± 3.49. The CC_{50} 5 mg/kg of ECT treatment was 37.80 ± 4.84 which is higher than that of the same dose of the diazepam treated group.

Test for anticonvulsant potency in PTZ or strychnine model

Fig. 6 shows that administration of AC decreased the percentage of seizure response induced by PTZ in a dose-dependent manner. However, in Fig. 6, only 400 mg/kg AC treatment significantly change the percentage of seizure response induced by 1 mg/kg of strychnine.

Intracellular Cl^- measurement assay

Fig. 7 and Fig. 8 show the electrophysiological exchange induced by AC and ECT treatment. AC and ECT treatment increased Cl^- influx into the intracellular area in a dose-dependent manner. Conversely, bicuculiline inhibited the Cl^- influx induced by AC and ECT treatment.

DISCUSSION

In the present study, we found out that ethanol extract of AC has significant anticonvulsant activity in both electroshock and PTZ seizure models. Unlike diazepam, AC (100 and 200 mg/kg) did not change the locomotor activity and running time on the rota-rod which indicates that it does not cause sedation and myorelaxation. Such difference between AC and diazepam is beneficial considering that sedation is a common side effect of some GABAergic neuronal anticonvulsnats. ECT, one of the major compounds in AC, exerts anticonvulsant activity similar to that of AC, which indicates that the effect of AC is due to ECT.

AC significantly decreased seizure response induced by electroshock and PTZ but did not decrease seizure response induced by strychnine except at its highest dose (400 mg/

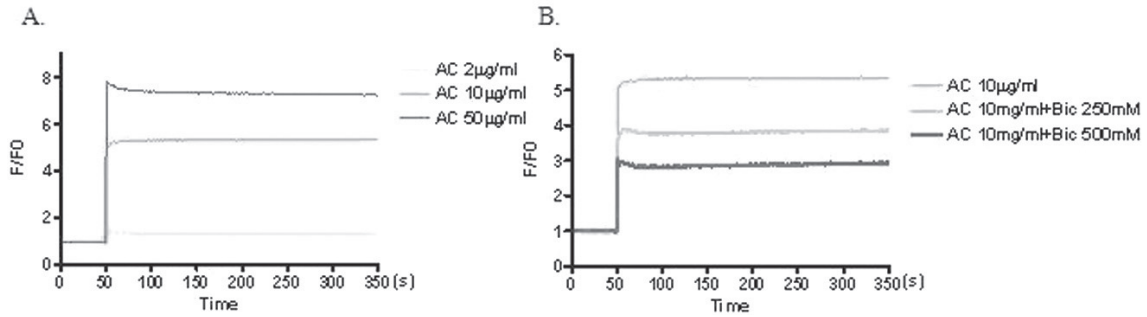

Fig. 7. Effects of AC on [Cl^-]i in neuroblastoma cells. Fluorescence was monitored in the excitation wavelength at 365 nm and the emission wavelength at 450 nm using the Cl^--sensitive indicator, N-(6-methoxyquinolyl) acetoetylester (MQAE) Contents of influx Cl^- ion was expressed as a peak (a.u.).

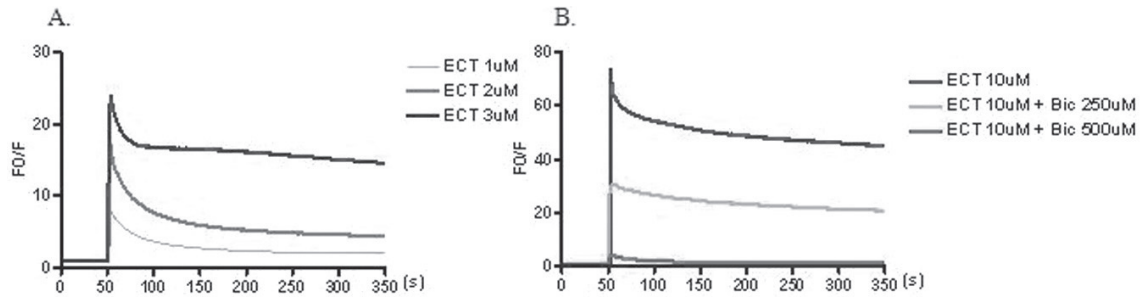

Fig. 8. Effects of ECT on [Cl^-]i in neuroblastoma cells. Fluorescence was monitored in the excitation wavelength at 365 nm and the emission wavelength at 450 nm using the Cl^--sensitive indicator, N-(6-methoxyquinolyl) acetoetylester (MQAE). Contents of influx Cl^- ion was expressed as a peak (a.u.).

kg). AC reduced CC_{50} and PTZ-induced seizure response in a dose-dependent manner. PTZ is an uncompetitive GABA receptor antagonist and strychnine is a glycine receptor antagonist (Takeuchi and Takeuchi, 1969). Although animal models based on PTZ have still been widely used for drug screening, the mechanism by which PTZ elicits its action has not been completely understood. One generally accepted mechanism by which PTZ exerts its action is by acting as an antagonist at the picrotoxin sensitive site of GABAA receptor complex (Ramanjaneyulu and Ticku, 1984). GABA is a principal inhibitory neurotransmitter in the mammalian central nervous system, producing inhibitory post-synaptic potentials in both feedforward and feedback circuits. Impairment of GABA-mediated inhibitory circuits has been implicated in different forms of epilepsy in experimental animal models and in human studies (Hansen *et al.*, 2004; Morimoto *et al.*, 2004).

The result of inhibiting PTZ-induced seizure indicate that the effects of AC may be related to the enhancement of GABA function in the brain, whereas failure to inhibit strychnine-induced seizures when 100 mg/kg or 200 mg/kg of AC was treated indicates that AC does not have much constituents having effects on the glycine-related response in the spinal cord (Takeuchi and Takeuchi, 1969). This suggests that the anticonvulsant effect of AC is more related with the Cl^- channel of the GABA: benzodiazepine (BDZ) receptor complex. However, as AC also inhibited strychnine-induced seizures at its high dose, it indicates that its anticonvulsant effect is not solely related to GABAergic neuron.

The anticonvulsant effect of BDZ agonist is due to its facilitating effect on the inhibitory GABAergic neurotransmission in the central nervous system. The mechanism of this facilitating action of BDZ agonist is via the activation of the BDZ binding site on the GABAA receptor to increase the frequency of the chloride (Cl^-) channel openings in response to a given concentration of GABA (Study and Barker, 1982). Therefore, the present study investigated the Cl^- influx using the neuroblastoma cells. Bicuculline, GABA antagonists, inhibited the Cl^- influx induced by AC in a dose-dependent manner. This suggests that the anticonvulsant effect of AC was mediated by enhancing the GABA function.

ECT, the main constituent of AC, significantly increased the CC_{50} and Cl^- influx in a dose-dependent manner. Like AC, bicuculline also inhibited Cl^- influx induced by ECT. This similar activities indicate that ECT also exerts anticonvulsant effect through the GABAergic neuron. In the course of our study, separating constituents from ethanol fraction of AC, we found out the ECT accounts for 2% among the constituents and only ECT exhibited anticonvulsant effect through the GABAergic neuron. Thus we concluded that the GABAergic neuronal anticonvulsant effect of AC is due to ECT.

This study demonstrated that AC treatment resulted in a dose-dependent increase of the electroshock seizure threshold. Unlike diazepam, AC did not cause sedation and myorelaxation in locomotor activity and the rota-rod test. These results indicate that the anticonvulsant effect of AC cannot be attributed only by interaction with GABA function, although the action of AC on the GABA receptor complex contributes to the anticonvulsant effect of AC. Besides, when highest dose (400

mg/kg) of AC was treated, AC also decreased the seizure response induced by strychnine. Although ECT exerts similar activities with AC, the GABAergic anticonvulsant effect, there may be still other kinds of constituents which exert anticonvulsant effect through the non-GABAergic neuron. Further studies are required to find out which constituent is responsible for the non-GABAergic anticonvulsant effect.

In conclusion, AC possesses anticonvulsant effect which can be attributed to the potentiation of the activity of GABA and its activity is mediated by ECT. However, more studies are required to find out any other constituents which exerts other mechanisms of anticonvulsant activity.

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