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**Echinacoside, an Active Constituent of Cistanche Herba, Exerts a Neuroprotective Effect in a Kainic Acid Rat Model by Inhibiting Inflammatory Processes and Activating the Akt/ GSK 3β Pathway**

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Echinacoside is a major compound of *Cistanche Herb* and has glutamate release-inhibiting activity in the brain. Given the involvement of excitotoxicity caused by massive glutamate in the pathophysiology of epilepsy, we explored the antiepileptic effect of echinacoside on kainic acid–induced seizures in rats. The rats were intraperitoneally administrated echinacoside for 30 min prior to intraperitoneal injection with kainic acid. The results showed that kainic acid induced seizure-like behavioral patterns, increased glutamate concentrations, caused neuronal loss and microglial activation, and stimulated proinflammatory cytokine gene expression in the hippocampus. These kainic acid-induced alternations were found to be attenuated by echinacoside pretreatment. Furthermore, decreased Akt and glycogen synthase kinase 3β phosphorylation as well as Bcl-2 expression in the hippocampus was reversed by the echinacoside pretreatment. These results demonstrate that echinacoside exert its antiepileptic and neuroprotective actions in a kainic acid rat model through suppressing inflammatory response and activating the Akt/GSK3β signaling. Therefore, the present study suggest that echinacoside is the potentially useful in the prevention of epilepsy.

**Keywords:** echinacoside; kainic acid; epilepsy; neuroprotective effect; hippocampus
Epilepsy is a prevalent brain disorder worldwide. It affects more than 65 million people, nearly 1% of the world’s population. Synthetic antiepileptic drugs are widely available in the pharmaceutical market; however, these drugs are only effective in 60-70% of patients and have adverse side effects. Thus, there is an unmet need to search a drug with more efficacious and safer. Medicinal plants represent a potential source of such compounds.

In traditional Chinese herbal medicine, *Cistanche Herba* has long been used for the treatment of epilepsy. Echinacoside is a major component of *Cistanche Herba* and has many pharmacological properties, such as antioxidant, anti-inflammatory, antineoplastic, hepatoprotective, and immune modulatory activity. Moreover, it has been reported that echinacoside can entry the brain and elicit neuroprotection. We previously demonstrated that echinacoside reduces glutamate release in rat cerebrocortical nerve terminals. Because glutamate is a major excitatory neurotransmitter in the mammalian brain and plays a crucial role in the pathophysiology of epilepsy, we suggested that echinacoside has antiepileptic activity. To elucidate this hypothesis, a kainic acid rat model was used in this study.

Kainic acid is a glutamate derivative and its single systemic injection to rodents results in seizures, neuroinflammation, and neuronal degeneration or death on selective population of neurons in the brain. These pathological alternations are similar to human temporal lobe epilepsy. Administering kainic acid to rodents is generally considered to induce an adequate model of epilepsy. To date, no study has investigated the antiepileptic and neuroprotective properties of echinacoside in a kainic acid-injected animal model. Therefore, our study aims to evaluate the effect and its possible mechanism of echinacoside preadministration in kainic acid-treated rats.
MATERIALS AND METHODS

Animals and Seizure Activity  Male Sprague-Dawley rats (BioLASCO, Taipei, Taiwan) weighing 150-200g were used throughout the study. The rats were randomly assigned to four groups as follows: i) Group 1: dimethylsulfoxide-treated group (control); ii) Group 2: kainic acid-treated group; iii) Group 3: echinacoside 10 mg/kg + kainic acid group; iv) Group 4: echinacoside 50 mg/kg + kainic acid group. Echinacoside (ChemFaces, Wuhan, PRC) was injected (intraperitoneal administration) 30 min prior to kainic acid (15 mg/kg) intraperitoneal injection. Seizure activity was rated during a 4 h period after kainic acid injection according to the Racine's scale. The dose and schedule of administration were chosen on the basis of previous studies. The experiments on animals were approved by the Institutional Animal Care and Use Committee at the Fu Jen Catholic University, and carried out in accordance with the protocols issued, which followed National Institutes of Health Guide for the Care and Use of Laboratory (NAC 2011).

Glutamate Levels Determination  4 h after the kainic acid treatment, the rats were sacrificed by decapitation. The whole brain was dissected and the hippocampus of each rat was collected for analysis. The glutamate concentration of brain hippocampus tissue was determined according to the methods described by previous studies.

Neutral Red and Fluoro-Jade B Staining  3 days after the kainic acid injection, rats were anesthetized using chloral hydrate (650 mg/kg) and perfused transcardially with fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS, pH 7.4). Brains were removed immediately after perfusion and stored in 4% paraformaldehyde overnight at 4°C. After post-fixation, the brains were immersed with 30% sucrose at 4°C for 24 h. The specimens were frozen rapidly and 30-μm-thick sections were cut by using a frozen microtome. Free-floating sections were stained with neutral red (Sigma-Aldrich, St. Louis,
MO, USA) and Fluoro-Jade B (Millipore, CA, USA). Fluoro-Jade B-positive cells in the CA3 region of hippocampus was determined as previously described.30,31)

**Immunohistochemistry** The sections were washed with 0.1 M PBS and blocked with 0.5% normal goat serum in 0.1 M PBS for 1 h at room temperature. Primary antibody (mouse monoclonal anti-OX-42 antibody, 1:1000; AbD Serotec, Oxford, UK) incubation was performed two overnight at 4°C. After repeated washes with PBS, the sections were incubated with biotinylated anti-mouse IgG (1:200) for 90 min, treated with the ABC solution (1:1000) for 1 h at room temperature, and reacted with 0.025% 3, 3′-diaminobenzidine and 0.0025% H2O2 until the desired stain intensity was achieved. Sections were mounted on slides using standard protocols. The stained slides were examined and imaged under a microscope (Olympus).31) Analysis of microglial cell body and process length was carried out with ImageJ.32)

**Transmission Electron Microscopy** Transmission electron microscopy was performed as previous described.33) Briefly, the CA3 region of hippocampus was removed and fixed in a buffer containing 4% paraformaldehyde and 2.5% glutaraldehyde at 4°C for 24-36 h. After washes with PBS, CA3 regions were postfixed in 1% osmium tetroxide for 2 h, dehydrated with ethanol, and embedded in epoxy resin. Ultrathin sections (70 nm) were prepared by using an ultramicrotome (EM UC7, Leica Microsystems, Wetzlar, Germany). The sections were stained with uranyl acetate and lead citrate, and observed through transmission electron microscopy (ModelJEM-1400,JEOLLtd,Tokyo,Japan).

**Quantitative real-time PCR** RNA extraction and real-time quantitative PCR were performed as previously described.34,35) Total RNA was extracted from the hippocampus using mirVana™ miRNA Isolation Kit (Life Technologies, Grand Island, New York, USA)
following the manufacturer’s protocol. Genomic DNA was removed by incubation with DNase I. The cDNA was synthesized by (GoScript reverse transcription system kit; Promega, Madison, Wisconsin, USA) and used for the SYBR Green (Roche Diagnostics, Mannheim, Germany) quantitative real-time PCR amplification with specific primers using the LightCycler 480 System (Roche Diagnostics). The protocol consisted of an initial denaturation step at 95°C for 3 min, followed by 45 cycles of denaturing at 95°C for 10s, annealing at 56°C for 30s. The mRNA levels of each target gene were normalized to that of GAPDH mRNA. Fold-induction was calculated using the $2^{-\Delta\Delta CT}$ method as described in a previous study.\(^{34}\)

**Immunoblotting** 20 μg of protein extracts from the hippocampi were separated by 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) followed by electrophoretic transfer onto polyvinylidene difluoride (PVDF) membrane. Membrane was blocked using 5% skimmed milk for 1 h at room temperature and incubated with the appropriate antibodies in 1% skimmed milk overnight at 4°C, followed by incubation with a secondary antibody for 1h. Primary antibodies were rabbit monoclonal antibodies directed against Akt (1:2000), pAktSer473 (1:2000), GSK3β (1:2000), pGSK3βSer9 (1:500), Bcl-2 (1:1000), and β-actin (1:2000) obtained from Cell Signaling Technology (MA, USA). The secondary peroxidase-conjugated goat anti-rabbit antibody (1:5000) was obtained from Santa Cruz (CA, USA). Bands were visualized with enhanced chemiluminescence (Amersham, Buckinghamshire, UK) using a gel imaging analysis system. The intensity of each band was measured using the Syngene software (Synoptics, Cambridge, UK).

**Statistical Analysis** Data are presented as the mean ± SEM. Statistical analysis was carried out by the two-tailed Student’s $t$ test when comparing two groups and by one-way ANOVA with Tukey's multiple comparisons post hoc tests when comparing more than two groups.
Analysis was completed using SPSS software (Version 17.0; SPSS Inc., Chicago, IL, USA). P < 0.05 was considered statistically significant.

RESULTS

Effect of Echinacoside on Kainic Acid-Induced Seizures and Elevation of Hippocampal Glutamate Levels  To evaluate the neuroprotective effect of echinacoside against kainic acid-induced excitotoxicity, rats were pretreated with echinacoside (5, 10 and 50 mg/kg, intraperitoneal administration) for 30 min before kainic acid (15 mg/kg) intraperitoneal injection. Kainic acid (15 mg/kg) intraperitoneal injection resulted in seizures with latency and score of 92.5 ± 5.3 min and 4.9 ± 0.1, respectively (Fig. 1A and B). A significant increase in seizure latency [F (2, 43) = 40.2, p < 0.01; Fig. 1A] and a significant decrease in seizure score [F (2, 40) = 21.5, p < 0.001; Fig. 1B] were observed in echinacoside (10 or 50 mg/kg)-pretreated rats. However, echinacoside at 5 mg/kg had no significant effect on seizure latency and score (p > 0.05; Fig. 1A and B). Given the robust attenuation of seizure activity that was seen with 10 or 50 mg/kg echinacoside, these two dose of echinacoside were used in subsequent experiments to evaluate the mechanisms that underlie the ability of echinacoside to inhibit kainic acid-induced seizures. Fig. 1C shows that elevation of hippocampal glutamate levels was noted in kainic acid-treated rats at 4 h when compared with dimethylsulfoxide-treated group (control; p < 0.001). However, decreased hippocampal glutamate levels were obtained in the echinacoside group (10 or 50 mg/kg) [F(3,16) = 6.1, p < 0.01; Fig. 1C].

Effect of Echinacoside on Kainic Acid-Induced Neuronal Injury in the CA3 Region of Hippocampus  The degree of neuronal damage was evaluated 3 days after kainic acid
injection through neutral red staining or Fluoro-Jade B to detect surviving neurons and degenerating neurons, respectively. Neutral red staining revealed an apparent neuronal loss in CA3 of kainic acid-injected rats, compared with dimethylsulfoxide-treated rats (control; \( p < 0.001 \); Fig. 2A and C). However, echinacoside pretreatment (10 or 50 mg/kg) effectively reduced kainic acid-induced neuronal loss in CA3 \([F(2,13) = 11.6, p < 0.001; \text{Fig. 2A and C}]\). Similarly, echinacoside-treated animals presented a diminished Fluoro-Jade B fluorescence in the CA3 regions of hippocampus after kainic acid injection \((p < 0.001)\), compared with the strong damage observed in kainic acid-injected rats \([F(2,24) = 6.9, p < 0.001; \text{Fig. 2B and D}]\).

In addition, transmission electron microscopy shows that neuronal injury in CA3 was not obvious in the dimethylsulfoxide-treated (control) group. However, in the kainic acid-treated group, nerve cells had ultrastructural changes. Nerve cells exhibited autophagosome formations, deformed nuclei with condensed, and disruptive cell plasma membrane. In contrast, the neuronal injury in the echinacoside-pretreated group was apparently attenuated compared with the kainic acid-injected rats (Fig. 3).

**Effect of Echinacoside on Kainic Acid-Induced Microglia Activation and Gene Expression of Proinflammatory Cytokines in the Hippocampus**

In the model for kainic acid-induced seizures and hippocampal neuronal death, microglia activation and proinflammatory cytokine production are increased in the hippocampus.\(^{36,37}\) In order to investigate whether echinacoside affected inflammatory response in the hippocampus of kainic acid-injected rats, microglia activation was analyzed using the anti-OX42 antibody. In dimethylsulfoxide-treated rats (control group), the microglial cell bodies in the CA3 regions were small and their processes were long and thin (Fig. 4A). However, the number of activated microglial cells in the CA3 area of KA injected rats increased significantly, the microglial cell bodies were enlarged \((p < 0.01; \text{Fig. 4A and B})\), and their cellular processes
became shorter and thicker ($p < 0.001$; Fig. 4A and C). In the animals pretreated with echinacoside (10 or 50 mg/kg)-pretreated rats, the number of activated microglial cells was significantly decreased, and the microglial cell bodies were thin [$F(2,13) = 6.2, p < 0.01$; Fig. 4B] and their cytoplasmic processes were ramified [$F(2,28) = 47.5, p < 0.001$; Fig. 4C]. In addition, the expression levels of interleukin-1β, interleukin-6, and tumor necrosis factor-α mRNA in the hippocampus were apparently elevated at 1 day after kainic acid injection, compared to dimethylsulfoxide-injected animals (control) ($p < 0.001$). However, decreased mRNA levels for these proinflammatory cytokines were obtained in the echinacoside group [interleukin-1β, $F(3,18) = 154.7, p < 0.001$; interleukin-6, $F(3,17) = 17.9, p < 0.001$; tumor necrosis factor-α, $F(3,16) = 31.1, p < 0.001$; Fig. 5A-C].

**Effect of Echinacoside on Kainic Acid-Induced decrease in the levels of Hippocampal Phospho-Akt, Phospho-Glycogen Synthase Kinase3β, and Bcl-2**

The levels of phosphorylated Akt (pAkt) (Ser473), phosphorylated GSK3β (pGSK3β) (Ser9), and Bcl-2, was assayed in the hippocampus (Fig. 6). A substantial lowering in pAkt, pGSK-3β, and Bcl-2 expression in the hippocampus were observed in kainic acid-treated rats at 4 h when compared with dimethylsulfoxide-treated rats (control) ($p < 0.001$). However, pretreatment with echinacoside (10 or 50 mg/kg) significantly increased the levels of pAkt, pGSK3β, and Bcl-2 compared with kainic acid treatment alone [pAkt, $F(3,19) = 69.5, p < 0.001$; pGSK3β, $F(3,17) = 60.3, p < 0.001$; Bcl-2, $F(3,18) = 26.1, p < 0.001$; Fig. 6A-C).
DISCUSSION

Evidences indicated that glutamate plays a crucial role in the pathophysiology of epilepsy.\textsuperscript{17,18} The glutamatergic hypothesis of epilepsy suggests that epileptogenic process is related to an increased glutamate concentration in the brain and a reduction in the concentration of this neurotransmitter is able to produce antiepileptic action.\textsuperscript{38-40} Study from our laboratory demonstrated that echinacoside can reduce glutamate release from nerve terminals and suggested that echinacoside is likely to have an antiepileptic effect.\textsuperscript{17} This suggestion was confirmed in the current study using a kainic acid-injected rat model. Kainic acid, a glutamate derivative, is widely used for inducing epilepsy in animal studies, because its neuropathological and biochemical characteristics are similar to those in humans.\textsuperscript{23,24}

Intraperitoneal injection of kainic acid into rats causes seizures and neuronal damage, particularly in the CA3 regions of the hippocampus.\textsuperscript{41-43} Furthermore, these pathological alternations produced by kainic acid are related to the massive release of glutamate.\textsuperscript{41,44,45} In agreement with these findings, we observed that the intraperitoneal administration of kainic acid (15 mg/kg) induced seizure behavior, increased glutamate levels in the hippocampus, and caused pyramidal cell loss in the hippocampal CA3 region. These kainic acid-induced pathological alternations were attenuated by intraperitoneal pretreatment with echinacoside (10 or 50 mg/kg). Furthermore, the hippocampal CA3 nerve cell injury evaluated by ultrastructural alternation with transmission electron microscopy was alleviated by echinacoside in the kainic acid rats. In addition, an increased number of autophagosomes was observed in CA3 cells after kainic acid administration. Pretreatment with echinacoside suppressed the increased formation of autophagosomes. Previous studies have suggested that autophagy activation is involved in kainic acid-induced neuronal death and inhibition of autophagy process seems to be neuroprotective.\textsuperscript{46,47} Although the mechanism underlying the
antiepileptic effect of echinacoside requires further exploration, the neuroprotective effect of echinacoside in the kainic acid animal model may be resulted from antiepileptic activity through inhibition of glutamate excitotoxicity and autophagy activation.

Neuroinflammation contributes considerably to delayed brain damage after acute injury and exerts a detrimental effect on a neurological outcome. Inflammatory responses, such as microglia activation and inflammatory cytokine production, has been described in human epilepsy and in experimental models of epilepsy. Moreover, the release of these proinflammatory cytokines from the activated microglial cells promotes kainic acid-induced neuronal damage in the hippocampus. We showed that, while kainic acid increased the activation of microglia and gene expression of proinflammatory cytokines (interleukin-1β, interleukin-6, and tumor necrosis factor-α) in the hippocampus, these effects were suppressed by echinacoside pretreatment. Therefore, we suggest that echinacoside through suppressing inflammatory processes, may contribute to its antiepileptic and neuroprotective effects in the kainic acid animal model. As already observed by our previous studies, several drugs and natural products also exert antiepileptic effects and neuroprotection against kainic acid-induced seizures, by means of their anti-inflammatory effect.

Numerous protein kinase signaling cascades are known to be activated by kainic acid that play important roles in neuroprotection. Among those that appear to have a particularly significant participation is Akt. Akt mediates its neuroprotective effects by activating various downstream substrates, including GSK-3β and Bcl-2. Akt phosphorylates GSK-3β at Ser9, leading to its inactivation and the reinforcement of neuronal cell survival because the active form of GSK-3β activates the mitochondrial death pathway. Bcl-2 is a key member of the anti-apoptotic Bcl-2 family and plays a key role in suppressing mitochondrial-mediated apoptotic cell death. For example, Bcl-2 can protect the integrity of mitochondrial membrane.
and block cytochrome C release from mitochondria, leading to cell survival and the inhibition of apoptosis.\textsuperscript{54} It has also reported that the overexpression of Bcl-2 protects neurons against excitotoxic insults in vitro and in vivo.\textsuperscript{54,55} The present study observed a decreased Akt (Ser473) and GSK3\(\beta\) (Ser9) phosphorylation as well as Bcl-2 expression in the hippocampus of rats following kainic acid exposure, which is in accordance with previous studies.\textsuperscript{56,57} Furthermore, echinacoside pretreatment rescued kainic acid-induced decrease in pAkt and pGSK3\(\beta\) as well as Bcl-2 expression. Thus, the neuroprotective effects of echinacoside in the kainic acid animal model might be partly mediated by preventing the downregulation of the Akt/GSK-3\(\beta\)/Bcl-2 pathway, thus increasing neuron survival.

Echinacoside is a potential new therapeutic candidate. This is because echinacoside can entry the brain and exhibits numerous benefits.\textsuperscript{58} Numerous studies have demonstrated that echinacoside attenuates brain damage and alleviates motor or memory impairment in several experimental models \textit{in vivo}.\textsuperscript{12,14,15,59} Although the mechanisms that underlie the neuroprotective effects of echinacoside in the brain require elucidation, inhibited neuroinflammation, antioxidation, oxygen-free radical scavenging, and increased neurogenesis have been implicated.\textsuperscript{14-16} In addition to these possible mechanisms, our previous\textsuperscript{17} and present results suggest that the inhibition of glutamate-mediated overexcitation may partly contribute to the neuroprotective and antiepileptic effects of echinacoside in the brain. This is supported by the fact that kainic acid-induced seizure activity, neuroinflammation and brain damage are associated with the excessive release of glutamate and activation of glutamate receptors.\textsuperscript{44,45} Furthermore, several clinically used antiepileptic drugs have been shown to attenuate the seizure activity induced by kainic acid \textsuperscript{60,61} and decrease the glutamate release in human and rat brain tissues.\textsuperscript{62,63} These findings pointed out that reduced glutamate levels is important for pharmacotherapeutic effects of antiepileptic drugs.
In conclusion, our results demonstrate that echinacoside, through suppressing inflammatory processes, decreasing glutamate-mediated overexcitation, as well as increasing Akt/GSK-3β activation and Bcl-2 expression, have a significant antiepileptic and neuroprotective effect in kainic acid-injected rats. Although the role of echinacoside on patients with epilepsy requires further evaluation, our findings suggest that it could be a valuable approach in epilepsy therapy.

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Conflict of Interest The authors declare no conflict of interest.
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*Biological and Pharmaceutical Bulletin Advance Publication*


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Figure Legends

Figure 1. Echinacoside attenuates kainic acid-induced seizure behavior in rats. Echinacoside (5, 10 or 50 mg/kg) was intraperitoneally administered 30 min prior to kainic acid (15 mg/kg) intraperitoneal injection. The seizure behavior tests [seizure latency (A) and seizure score (B)] were evaluated during 1-4 h after kainic acid injection. (C) Glutamate levels in the hippocampus were measured with HPLC at 4 h after kainic acid treatment. Data are expressed as mean ± S.E.M. *** P < 0.001 compared with the control group. # P < 0.05 compared with the kainic acid-treated group.

Figure 2. Protective action of echinacoside against kainic acid-induced neuronal damage in CA3 region of hippocampus. Determination of neuronal damage was examined by neutral red (A) and Fluoro-Jade B (B) staining. Scale bar, 100 μm. The insets are high magnification micrographs of neurons. Scale bar, 25 μm. Numbers of surviving neurons (C) and Fluoro-Jade B-positive neurons (D) were counted in the CA3 regions of hippocampus and presented as mean ± S.E.M. (n = 6-11). *** P < 0.001 compared with the control group. # P < 0.05 compared with the kainic acid-treated group.

(Color figure can be accessed in the online version.)

Figure 3. The hippocampal CA3 nerve cell injury measured by transmission electron micrograph. (A), the control group; (B), the kainic acid group; (C, D), the echinacoside group. Arrows indicate autophagosomes and arrowheads indicate nucleolus. Scale bar, 2 μm.

Figure 4. Echinacoside suppresses kainic acid-induced microglial activation in the hippocampal CA3 region. (A) Representative coronal sections of the hippocampal CA3 region following OX-42 immunostaining 3 days after kainic acid treatment. Representative picture from five
independent experiments were presented. Scale bar, 100 μm. The insets are high magnification micrographs of microglial cells. Scale bar, 25 μm. Measurements of cell body size (B) and longest processes (C) and presents as mean ± S.E.M. (n = 5-11). *** P < 0.001, * P < 0.01 as compared with the control group. # P < 0.05 as compared with the kainic acid-treated group. (Color figure can be accessed in the online version.)

Figure 5. Echinacoside reduces kainic acid-induced proinflammatory cytokine expression in the hippocampus. Relative quantification of interleukin-1β (A), interleukin-6 (B), and tumor necrosis factor-α (C) mRNA expression in the hippocampus were measured at 1 day after kainic acid treatment with RT-PCR. The expression levels of interleukin-1β, interleukin-6, and tumor necrosis factor-α were normalized to that of control. Data are expressed as mean ± S.E.M. *** P < 0.001 compared with the control group. # P < 0.05 compared with the kainic acid-treated group.

Figure 6. Echinacoside rescues kainic acid-induced inhibition of pAkt, pGSK3β, and Bcl-2 expression in the hippocampus. The expression levels of pAkt (A), pGSK3β (B), and Bcl-2 (C) in the whole hippocampus were determined with immunoblotting at 4 h after kainic acid treatment. Data are expressed as mean ± S.E.M. *** P < 0.001 compared with the control group. # P < 0.05 compared with the kainic acid-treated group.
Seizure latency (min)

Kainic acid
Echinacoside 5 mg/kg + Kainic acid
Echinacoside 10 mg/kg + Kainic acid
Echinacoside 50 mg/kg + Kainic acid

Glutamate concentration (μM)

Control
Kainic acid
Echinacoside 10 mg/kg + Kainic acid
Echinacoside 50 mg/kg + Kainic acid

Seizure score

Control
Kainic acid
Echinacoside 5 mg/kg + Kainic acid
Echinacoside 10 mg/kg + Kainic acid
Echinacoside 50 mg/kg + Kainic acid

Fig. 1
**Fig. 2**

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**C**

![Graph showing number of surviving neurons](C)

**D**

![Graph showing number of Fluoro-Jade B positive neurons](D)
Fig. 4

A

Control

Kainic acid

Echinacoside 10 mg/kg + Kainic acid

Echinacoside 50 mg/kg + Kainic acid

B

Cell body size ($\mu m^2$)

Control

Kainic acid

Echinacoside 10 mg/kg + Kainic acid

Echinacoside 50 mg/kg + Kainic acid

C

Process length ($\mu m$)

Control

Kainic acid

Echinacoside 10 mg/kg + Kainic acid

Echinacoside 50 mg/kg + Kainic acid

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