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Original Article

Acute and Sub-chronic Anticonvulsant Effects of Edaravone on Seizure Induced by Pentylenetetrazole or Electroshock in Mice, Nitric Oxide Involvement

Leila Moezi, PhD; Fatema Pirsalami, BSc; Mona Dastgheib, PhD; Somayeh Oftadehgan, PhD; Azar Purkhosrow, MSc; Elahe Sattarinezhad, PhD

Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:
Elahe Sattarinezhad, PhD;
Department of Pharmacology, School of Medicine, Zand Blvd., Postal Code: 71348-53185, Shiraz, Iran
Tel/Fax: +98 71 32307591
Email: elahesat@yahoo.com
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Abstract

Background: Edaravone is an anti-stroke medication that may have nitric oxide (NO) modulating properties. This study evaluated the role of NO in the acute and sub-chronic anticonvulsant effects of edaravone in murine models of seizures induced by intraperitoneal (IP) or intravenous (IV) injections of pentylenetetrazole (PTZ) or electroshock (maximal electroshock seizure [MES]).

Methods: 132 male albino mice were randomly divided into 22 groups (n=6) and given IP injections of vehicle or edaravone either acutely or for eight days (sub-chronically). The seizure was induced by electroshock or PTZ (IP or IV). The following edaravone doses were used: 7.5, 10, 12.5 (acute); 5, 7.5, 10 (sub-chronic) in IP PTZ model; 5, 7.5, 10 in IV PTZ model; and 5, 10 mg/Kg in the MES. To evaluate NO involvement, 216 mice were randomly divided into 36 groups (n=6) and pretreated with vehicle, edaravone, a non-specific nitric oxide synthase (NOS) inhibitor: N(ω)-nitro-L-arginine methyl ester (L-NAME) (5 mg/Kg), a specific nNOS inhibitor: 7-nitroindazole (7-NI) (60 mg/Kg), or a combination of edaravone plus L-NAME or 7-NI, either acutely or for eight days before seizure induction. Doses of edaravone were as follows: in IP PTZ model: 12.5 (acute) and 10 (sub-chronic); in IV PTZ model: 10; and in the MES: 5 mg/Kg. Data were analyzed using the one-way analysis of variance (ANOVA) followed by Tukey's test (SPSS 18). $P \leq 0.05$ was considered statistically significant.

Results: In the IP PTZ model, edaravone increased time latencies to seizures ($P<0.001$), prevented tonic seizures, and death. Edaravone increased the seizure threshold ($P<0.001$) in the IV PTZ model and shortened the duration of tonic hind-limb extension (THE) in the MES model ($P<0.001$). In comparison to mice treated with edaravone alone, adding L-NAME or 7-NI reduced seizure time latencies ($P<0.001$), reduced seizure threshold ($P<0.001$), and increased THE duration ($P<0.001$).

Conclusion: Edaravone (acute or sub-chronic) could prevent seizures by modulating NO signaling pathways.

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Keywords ● Edaravone ● Epilepsy ● Pentylenetetrazole ● Electroshock ● Nitric oxide

What's Known

- Edaravone has acute anti-seizure efficacy in numerous animal models of convulsion.
- Edaravone has nitric oxide modulating properties.

What's New

- This study investigated the following subjects for the first time:
- The sub-chronic anticonvulsant activity of edaravone and its anti-seizure effects in intravenous pentylenetetrazole and maximal electroshock tests
- The involvement of neuronal nitric oxide synthase in anti-seizure effects of edaravone

Introduction

Epilepsy is considered one of the most prevalent and disabling neurologic disorders worldwide. Epilepsy patients have recurring spontaneous seizures caused by the abnormal and excessive electrical activity of the brain.[1] These patients are at risk of reduced life expectancy as well as a high risk of various injuries and psychological dysfunctions.[1]

Despite the development of various antiepileptic medications, about one-third of patients are resistant to the current pharmacological therapies and remain uncured.[2, 3] Furthermore, the emergence of typical antiepileptic medications' side effects has a significant negative impact on the patient's compliance. Due to the side effects, a considerable number of patients may stop taking their prescriptions. On the other hand, many patients, particularly the elderly, have multiple underlying diseases and take numerous drugs, which increases the chance of drug interactions.[4] Therefore, developing new antiepileptic medications with innovative mechanisms of action, increased efficacy, fewer adverse effects, and fewer drug interactions is an inevitable necessity.

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) is a potent free radical scavenger with neuroprotective properties. It has been used to treat stroke and amyotrophic lateral sclerosis (ALS) with no serious side effects or drug interactions.[5] Edaravone protects neurons by quenching free radicals and inhibiting lipid peroxidation. On the other hand, recent findings suggested that edaravone, in addition to its free radical scavenging activity, may have modulating effects on nitric oxide (NO) production.[6, 7] In addition to mediating several physiological functions, NO is a key mediator in maintaining central nervous system (CNS) homeostasis.[8] Various studies emphasized the role of NO in epilepsy.[9-11] NO has the potential to either act as an anticonvulsant or a proconvulsant agent.[12, 13] Diverse roles of NO in epilepsy result from the type of seizure, the source of NO, its concentration, and other neurotransmitter systems that may be involved.[14, 15] The precise mechanism by which NO acts as an endogenous anti-seizure mediator is still unknown. However, it was proposed that NO has a negative modulatory effect on N-methyl-D-aspartate (NMDA) receptor functions.[16] Furthermore, NO may inhibit the gamma-aminobutyric acid-transaminase enzyme and increase the Gamma-aminobutyric acid (GABA) concentration.[17]

NO is synthesized from its precursor L-arginine by three non-specific nitric oxide synthase (NOS) isoforms, all of which are present in the CNS. Two isoforms (eNOS and nNOS) are constitutively expressed, while one isoform (iNOS) is inducibly expressed.[18, 19] In pathological conditions, iNOS catalyzes the synthesis of high levels of NO.[20] NO is a free radical that easily interacts with other reactive oxygen species, mainly superoxide, to produce peroxynitrite, a highly reactive free radical which mediates inflammatory effects.[21] Under physiological conditions, the concentration of NO fluctuates within the range of low values[22] and is produced mainly by nNOS and eNOS. NO derived from eNOS maintains the CNS microcirculation.[23] The nNOS seems to be the main enzyme target in the central nervous system. The NO generated by nNOS is involved in neuronal homeostasis by regulating CNS microcirculation, synaptogenesis, synaptic plasticity, and neurotransmitter release.[24, 25] Therefore, it might be able to prevent seizure.[26]

The purpose of this study was to evaluate the acute and sub-chronic anticonvulsant activity of edaravone on murine models of generalized tonic-clonic seizure induced by intraperitoneal (IP) or intravenous (IV) injections of pentylenetetrazole (PTZ) or electroshock (maximal electroshock seizure [MES]). We also aimed to investigate the probable effect of edaravone on NOS enzymes, particularly nNOS, and assessed its significance in the anti-seizure activity of edaravone.

Materials and Methods

Male albino mice (n=348), weighing 25-35 g, were obtained from the Laboratory Animal Breeding Center of Shiraz University of Medical Sciences (Shiraz, Iran). They were randomly assigned to 58 experimental groups (n=6 per group). The mice were housed in colony cages (5-6 mice in each cage) with free access to food and drinking water. They were kept under standard housing conditions (12-hour light:dark cycle, 25-35% humidity, and temperature 23±2 ˚C). The animals were acclimated at least three days before the onset of the study. The time for the experiments was between 10.00 AM to 2.00 PM. All the animal care and behavioral tests were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.[27] The study was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (code: IR.SUMS.REC.1394.S296).

Chemicals Preparation

Pentylenetetrazole, edaravone,

N(ω)-nitro-L-arginine methyl ester (L-NAME), and 7-nitroindazole (7-NI) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). The compounds were dissolved in physiological saline, and the solutions were freshly prepared on the day of the experiments.

Behavioral Seizure Evaluation Methods

Intraperitoneal PTZ-induced Seizure: The generalized tonic-clonic seizure in mice was induced by a single IP injection of PTZ (85 mg/Kg).[28, 29] The animals were monitored for the incidence of convulsion or mortality. Following PTZ injection, latency periods to the first myoclonic jerk and generalized clonic or tonic contractions were recorded. Myoclonic jerk is defined as a quick, shock-like contraction of one or more muscles that lasts for one to two seconds. Clonus is characterized by contractions and relaxations of all four limbs simultaneously, as well as a tail erection lasting longer than five seconds. Tonus is defined as the extension of the forelimb and/or hind limb.[30] Latency time is the interval between PTZ injection and the onset of myoclonic, clonic, or tonic seizures.

***Intravenous PTZ-induced seizure*:** In this method of inducing seizure, PTZ was administered intravenously into the mice tails using a syringe pump. The infusion was continued until the emergence of the first clonic seizure. The minimal dose of PTZ (mg/Kg of mouse weight) required to induce clonic seizure was considered as an index of seizure threshold.

***Electroshock model of seizure*:** In this method, an electroconvulsive apparatus (Model 7800, Ugo Basile, Camerino, Italy) was used, and an alternating electric current (50 Hz, 50 mA, and 0.2 s) was transmitted through the ears of mice via ear electrodes.[31] The ear electrodes were moistened with normal saline before being attached to the ears of the mice. Electroshock induces a range of motor convulsions that are dependent on the intensity of the electrical stimulation current.[32] Before conducting the experiments, it was determined what electrical current mice would require to induce hind-limbs extension in them. Data were expressed as tonic hind-limb extension duration (THE).

Treatment

Three methods, including IP and IV administrations of PTZ, and maximal electroshock seizure (MES) were administered to assess the anticonvulsant activity of edaravone in mice. There were two primary phases to the experiments. In the first phase of the experiment, acute and sub-chronic anticonvulsant effects of edaravone were assessed. In the second phase of the experiment, the role of NO in the anticonvulsant activity of edaravone was investigated. Edaravone was administered intraperitoneally to different groups of mice. In the acute series of the experiments, edaravone was injected 30 minutes before the administration of PTZ or inducing electroshock. In the sub-chronic series of the experiments, edaravone was injected intraperitoneally for eight days, and the last dose was injected 30 minutes before administering PTZ or inducing electroshock. In the second phase of the experiment, to evaluate the possible involvement of NO in the anticonvulsant effects of edaravone, L-NAME (5 mg/Kg), a nonspecific NOS inhibitor, or 7-NI (60 mg/Kg), a specific neuronal NOS inhibitor, was administered 30 minutes before the final dose of edaravone and 60 minutes before injecting PTZ or inducing electroshock.

By using the simple randomization method, 348 male albino mice were distributed into 58 groups (n=6 per group). The groups were categorized as follows:

1. Evaluation of the acute anti-seizure activity of edaravone

1A. Intraperitoneal PTZ-induced seizure model

Group 1: Vehicle

Groups 2, 3, and 4: Edaravone (7.5, 10, 12.5 mg/Kg)

1B. Intravenous PTZ-induced seizure model

Group 5: Vehicle

Groups 6, 7, and 8: Edaravone (5, 7.5, 10 mg/Kg)

1C. Maximal electroshock (MES)

Group 9: Vehicle

Groups 10 and 11: Edaravone (5, 10 mg/Kg)

2. Evaluation of the sub-chronic anti-seizure activity of edaravone

2A. Intraperitoneal PTZ-induced seizure model

Group 12: Vehicle

Groups 13, 14, and 15: Edaravone (5, 7.5, 10 mg/Kg)

2B. Intravenous PTZ-induced seizure model

Group 16: Vehicle

Groups 17, 18, and 19: Edaravone (5, 7.5, 10 mg/Kg)

2C. Maximal electroshock (MES)

Group 20: Vehicle

Groups 21 and 22: Edaravone (5, 10 mg/Kg)

3. Evaluation of NO involvement in the acute anti-seizure activity of edaravone

3A. Intraperitoneal PTZ-induced seizure model

Group 23: Vehicle

Group 24: Edaravone (12.5 mg/Kg)

Group 25: L-NAME (5 mg/Kg)

Group 26: 7-NI (60 mg/Kg)

Group 27: Edaravone (12.5 mg/Kg)+L-NAME (5 mg/Kg)

Group 28: Edaravone (12.5 mg/Kg)+7-NI (60 mg/Kg)

3B. Intravenous PTZ-induced seizure model

Group 29: Vehicle
Group 30: Edaravone (10 mg/Kg)
Group 31: L-NAME (5 mg/Kg)
Group 32: 7-NI (60 mg/Kg)
Group 33: Edaravone (10 mg/Kg)+L-NAME (5 mg/Kg)
Group 34: Edaravone (10 mg/Kg)+7-NI (60 mg/Kg)

3C. Maximal electroshock (MES)

Group 35: Vehicle
Group 36: Edaravone (5 mg/Kg)
Group 37: L-NAME (5 mg/Kg)
Group 38: 7-NI (60 mg/Kg)
Group 39: Edaravone (5 mg/Kg)+L-NAME (5 mg/Kg)
Group 40: Edaravone (5 mg/Kg)+7-NI (60 mg/Kg)

4. Evaluation of involvement of NO in the sub-chronic anti-seizure activity of edaravone

4A. Intraperitoneal PTZ-induced seizure model

Group 41: Vehicle
Group 42: Edaravone (10 mg/Kg)
Group 43: L-NAME (5 mg/Kg)
Group 44: 7-NI (60 mg/Kg)
Group 45: Edaravone (10 mg/Kg)+L-NAME (5 mg/Kg)
Group 46: Edaravone (10 mg/Kg)+7-NI (60 mg/Kg)

4B. Intravenous PTZ-induced seizure model

Group 47: Vehicle
Group 48: Edaravone (10 mg/Kg)
Group 49: L-NAME (5 mg/Kg)
Group 50: 7-NI (60 mg/Kg)
Group 51: Edaravone (10 mg/Kg)+L-NAME (5 mg/Kg)
Group 52: Edaravone (10 mg/Kg)+7-NI (60 mg/Kg)

4C. Maximal electroshock (MES)

Group 53: Vehicle
Group 54: Edaravone (5 mg/Kg)
Group 55: L-NAME (5 mg/Kg)
Group 56: 7-NI (60 mg/Kg)
Group 57: Edaravone (5 mg/Kg)+L-NAME (5 mg/Kg)
Group 58: Edaravone (5 mg/Kg)+7-NI (60 mg/Kg)

Statistical Analysis

Data were presented as means±SEM. Statistical analysis was performed using SPSS statistical software, version 18 (IBM, USA). To assess the dose-response data, one-way analyses of variance (ANOVA) followed by the Tukey *post hoc* test were used. To determine the protective effects of edaravone against tonic seizure and death, Chi square test was used. A P value of ≤0.05 was considered statistically significant.

Results

The Effects of Edaravone on Intraperitoneal PTZ-induced Seizure

The acute effects of edaravone (7.5, 10, and 12.5 mg/Kg) on latency times to myoclonic and clonic seizures are illustrated in figure 1A. According to data analysis, edaravone (12.5 mg/Kg) significantly prolonged latency times to myoclonic jerk and clonic seizure as compared to the vehicle group (P<0.001). When the acute effects of edaravone (7.5, 10, and 12.5 mg/Kg) were assessed for tonic seizure and mortality rate, it was revealed that edaravone (12.5 mg/Kg) provided more protection than the vehicle group against both the tonic seizure and the mortality rate (P=0.03 for both tonic seizure and mortality protection) (table 1). Figure 1B depicts the acute effects of nitric oxide synthase inhibitors (NOSIs) (L-NAME or 7-NI) and edaravone (12.5 mg/Kg) co-administration on latency times to myoclonic and clonic seizures. Data analysis revealed that NOSIs significantly reduced the anticonvulsant effect of edaravone (P<0.001). The results of acute treatments of mice with NOSIs (L-NAME or 7-NI) plus edaravone (12.5 mg/Kg) on tonic seizure and mortality rate are summarized in table 2. Analysis of data demonstrated that co-administrations of NOSIs with edaravone significantly decreased the effects of edaravone on tonic seizure and mortality rate (P=0.05).

The sub-chronic effects of edaravone (5, 7.5, and 10 mg/Kg) on latency times to myoclonic and clonic seizures are shown in figure 1C. Analysis of data showed that edaravone (5, 7.5, and 10 mg/Kg) increased latency times to myoclonic and clonic seizures significantly more than the vehicle group (P<0.001). The sub-chronic effects of edaravone (5, 7.5, and 10 mg/Kg) on tonic seizure and mortality rate are shown in table 3. Edaravone (7.5 and 10 mg/Kg) was found to be more effective than the vehicle group in preventing tonic seizure and mortality rate (P=0.05).

Figure 1D illustrates the effects of sub-chronic co-administration of NOSIs (L-NAME or 7-NI) and edaravone (10 mg/Kg) on myoclonic and clonic seizure latency times. The latency times to myoclonic and clonic seizures were observed to be significantly shorter when NOSIs (L-NAME and 7-NI) were administered in combination with edaravone (10 mg/Kg) than when edaravone (10 mg/Kg) was administered alone (P<0.001). The results of sub-chronic treatments of mice with co-administrations of NOSIs (L-NAME or 7-NI) and edaravone (10 mg/Kg) on tonic seizure and mortality rate are summarized in table 4.

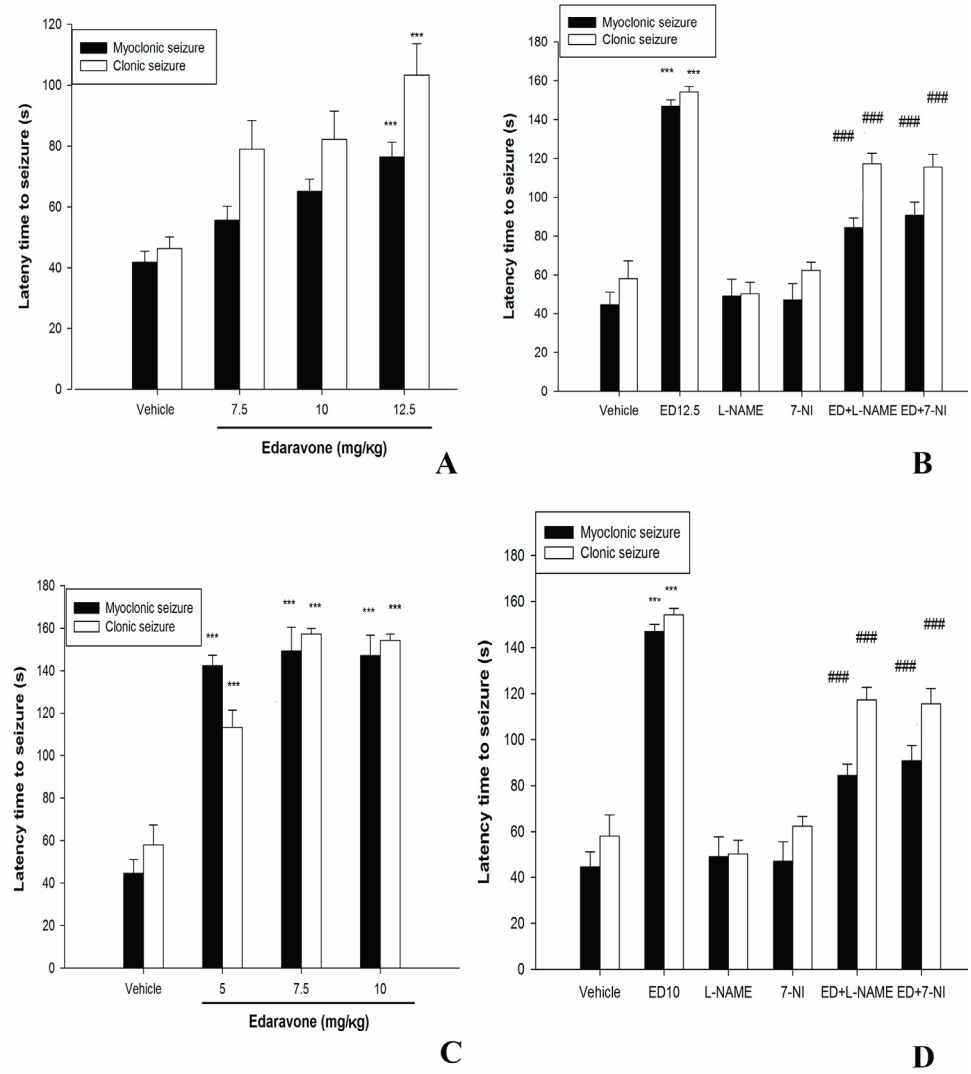

Figure 1: (A) Acute pretreatment of mice with edaravone (12.5 mg/Kg, IP) caused significant prolongation of latency times to the onsets of both myoclonic and clonic seizures induced by PTZ (85 mg/Kg, IP) in comparison to the vehicle group (P<0.001). (B) Acute pretreatment of mice with edaravone (12.5 mg/Kg, IP) plus L-NAME (5 mg/Kg, IP) or edaravone (12.5 mg/Kg, IP) plus 7-NI (60 mg/Kg, IP) significantly reduced the latency times to the onsets of both myoclonic and clonic seizures induced by PTZ (85 mg/Kg, IP) in comparison to those for edaravone (12.5 mg/Kg, IP) alone (P<0.001 for both co-administrations of LNAME plus edaravone or 7-NI plus edaravone). (C) Sub-chronic pretreatment of mice with edaravone (5, 7.5, 10 mg/Kg, IP) for eight days, resulted in significant prolongation of latency times to the onsets of both myoclonic and clonic seizures induced by PTZ (85 mg/Kg, IP) in comparison to the vehicle group (P<0.001). (D) Sub-chronic pretreatment of mice with edaravone (12.5 mg/Kg, IP) plus L-NAME (5 mg/Kg, IP) or edaravone (12.5 mg/Kg, IP) plus 7-NI (60 mg/Kg, IP) for eight days, reduced the latency times to both myoclonic and clonic seizures induced by PTZ (85 mg/Kg, IP) in comparison to the administation of edaravone alone (P<0.001 for both co-administrations of LNAME plus edaravone or 7-NI plus edaravone). ***P≤0.001 represents significant differences with the vehicle group, ###P≤0.001 represents significant differences with edaravone. ED: edaravone; L-NAME: N (ω)-nitro-L-arginine methyl ester; 7-NI: 7-nitroindazole

Table 1: Effects of acute pretreatment of mice with different doses of edaravone on the incidences of tonic seizures and mortality in intraperitoneal PTZ-induced model of seizure

Groups (n=6)	Tonic seizure protection (%)	P value	Mortality protection (%)	P value
Vehicle	44.40		44.40	
Edaravone (7.5 mg/Kg)	42.91	0.41[a]	42.91	0.41[a]
Edaravone (10 mg/Kg)	42.93	0.32[a]	42.93	0.32[a]
Edaravone (12.5 mg/Kg)	71.42*	0.03[a]	71.42*	0.03[a]

Percentages of the protections against the incidences of tonic seizure and mortality subsequent to intraperitoneal injections of PTZ (85 mg/Kg) were compared between the studied groups using the Chi square test. [a]The group was compared with the vehicle group. *P≤0.05 was considered significant.

Table 2: Effects of acute administrations of NOS inhibitors and edaravone on the incidences of tonic seizure and mortality in intraperitoneal PTZ-induced model of seizure

Groups (n=6)	Tonic seizure protection (%)	P value	Mortality protection (%)	P value
Vehicle	44.43		44.43	
Edaravone (12.5 mg/Kg)	71.43*	0.05[a]	71.43*	0.05[a]
L-NAME	50.01	0.13[a]	50.01	0.13[a]
7-NI	50.25	0.21[a]	50.25	0.21[a]
Edaravone (12.5 mg/Kg)+L-NAME	46.32*	0.05[b]	46.32*	0.05[b]
Edaravone (12.5 mg/Kg)+7-NI	40.51*	0.05[b]	40.51*	0.05[b]

Percentages of the protections against the incidences of tonic seizure and mortality subsequent to intraperitoneal injections of PTZ (85 mg/Kg) were compared between the studied groups using the Chi square test. [a]The group was compared with the vehicle group. [b]The group was compared with the edaravone group. *P≤0.05 was considered significant.

Table 3: Effects of sub-chronic treatment with different doses of edaravone on the incidences of tonic seizure and mortality in intraperitoneal PTZ-induced model of seizure

Groups (n=6)	Tonic seizure protection (%)	P value	Mortality protection (%)	P value
Vehicle	44.42		44.42	
Edaravone (5 mg/Kg)	48.11	0.15[a]	48.11	0.15[a]
Edaravone (7.5 mg/Kg)	85.71*	0.05[a]	85.71*	0.05[a]
Edaravone (10 mg/Kg)	85.73*	0.05[a]	85.73*	0.05[a]

Percentages of the protections against the incidences of tonic seizure and mortality subsequent to intraperitoneal injections of PTZ (85 mg/Kg) were compared between the studied groups using the Chi square test. [a]The group was compared with the vehicle group. *P≤0.05 was considered significant.

Table 4: Effects of sub-chronic administrations of NOS inhibitors and edaravone on the incidences of tonic seizure and mortality in intraperitoneal PTZ-induced model of seizure

Groups (n=6)	Tonic seizure protection (%)	P value	Mortality protection (%)	P value
Vehicle	40.02		40.02	
Edaravone (10 mg/Kg)	85.71*	0.03[a]	85.71*	0.03[a]
L-NAME	52.04	0.14[a]	50.04	0.14[a]
7-NI	55.33	0.23[a]	50.23	0.23[a]
Edaravone (10 mg/Kg)+L-NAME	48.02*	0.04[b]	48.02*	0.04[b]
Edaravone (10 mg/Kg)+7-NI	47.05*	0.04[b]	47.05*	0.04[b]

Percentages of the protections against the incidences of tonic seizure and mortality subsequent to intraperitoneal injections of PTZ (85 mg/Kg) were compared between the studied groups using the Chi square test. [a]The group was compared with the vehicle group. [b]The group was compared with the edaravone group. *P≤0.05 is considered significant.

When compared to edaravone (10 mg/Kg) alone, NOSIs were found to provide less protection against tonic seizure and mortality (P=0.04).

The Effects of Edaravone on IV PTZ-induced Seizure

The effects of acute and sub-chronic edaravone (5, 7.5, and 10 mg/Kg) administrations on IV PTZ-induced clonic seizure threshold are shown in figures 2A and 2B. When compared to the vehicle group, acute treatment of mice with edaravone (7.5 and 10 mg/Kg) and sub-chronic treatment with edaravone (10 mg/Kg) significantly increased seizure threshold (P<0.001).

Figures 2C and 2D show the effects of co-administration of NOSIs (L-NAME or 7-NI) with acute or sub-chronic doses of edaravone (10 mg/Kg) on IV PTZ-induced seizure threshold. In both series of acute and sub-chronic experiments, NOSIs significantly decreased the edaravone's (10 mg/Kg) anticonvulsant effects (P<0.001).

The Effects of Edaravone on Electroshock-induced Seizure

Figures 3A and 3B indicate the effects of acute and sub-chronic administrations of edaravone (5 and 10 mg/Kg) on the electroshock-induced seizure. Edaravone (5 and 10 mg/Kg) significantly decreased the length of THE induced by electroshock in both series of acute and sub-chronic experiments (P<0.001).

The effects of co-administration of NOSIs (L-NAME or 7-NI) and acute or sub-chronic doses of edaravone (5 mg/Kg) on electroshock-induced seizure threshold are shown in figures 3C and 3D, respectively. In both series of acute and sub-chronic experiments, NOSIs (L-NAME or 7-NI) significantly reduced the anticonvulsant effect of edaravone (5 mg/Kg, P<0.001).

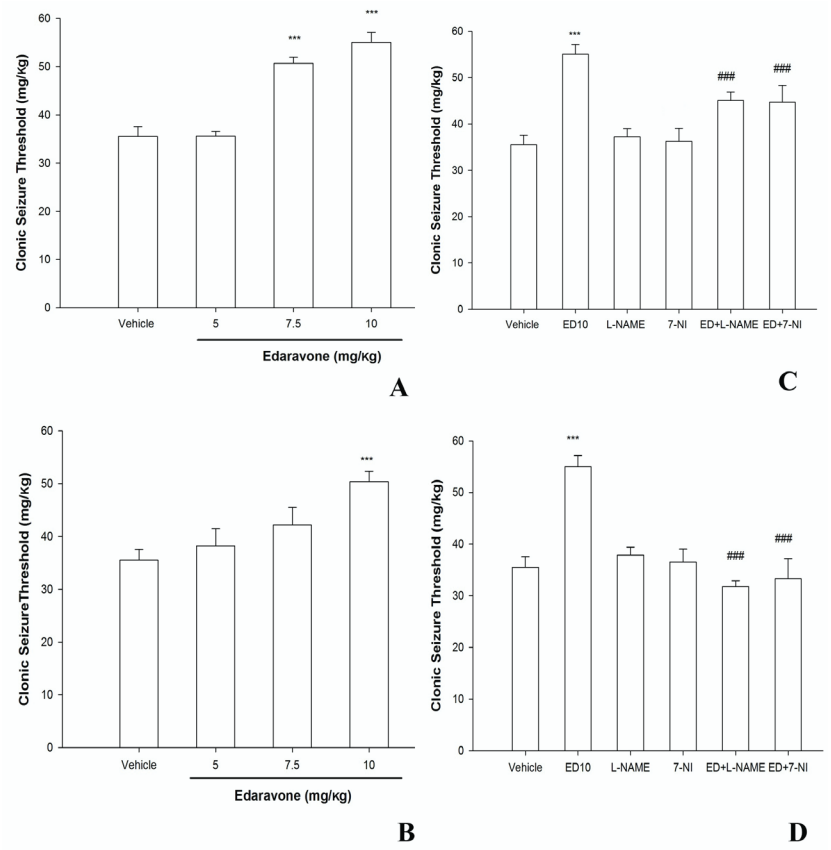

Figure 2: The graphs shows the mean±SEM of clonic seizure threshold induced by intravenous infusion of PTZ in mice pretreated with acute or sub-chronic intraperitoneal infusion of edaravone (A) Acute pretreatment of mice with edaravone (7.5, 10 mg/Kg, IP) increased the seizure threshold more than the vehicle group (P<0.001). (B) Acute pretreatment of mice with concomitant injections of edaravone (10 mg/Kg) plus L-NAME (5 mg/Kg, IP) or 7-NI (60 mg/Kg, IP) decreased the seizure threshold in comparison to the edaravone alone (P<0.001 for both co-administrations of L-NAME plus edaravone or 7-NI plus edaravone). (C) Sub-chronic pretreatment of mice with edaravone (10 mg/Kg, IP) for eight days, significantly increased the seizure threshold as compared to the vehicle group (P<0.001). (D) Sub-chronic pretreatment of mice with concomitant injections of edaravone (10 mg/Kg) plus L-NAME (5 mg/Kg, IP) or 7-NI (60 mg/Kg, IP) for eight days decreased the seizure threshold in comparison to edaravone alone (P≤0.001 for both co-administrations of L-NAME plus edaravone or 7-NI plus edaravone). ***P≤0.001 represents significant differences with the vehicle group, ### P≤0.001 represents significant differences with edaravone. ED: Edaravone; L-NAME: N (ω)-nitro-L-arginine methyl ester; 7-NI: 7-nitroindazole

Discussion

In this research, we assessed the ability of edaravone in acute and sub-chronic IP doses to prevent seizures induced by PTZ (IP and IV) or electroshock. In the IP PTZ model, pretreatment of mice with edaravone increased time latencies to the onsets of myoclonic and clonic seizures and protected against tonic seizures and mortality. In the IV PTZ model, edaravone increased the seizure threshold. In the MES model, edaravone decreased the duration of THE. Comparatively to mice pretreated with edaravone alone, the addition of L-NAME or 7-NI reduced time latencies to the seizures induced by IP PTZ, reduced seizure threshold in IV PTZ model, and increased THE duration in the MES model. These animal models are routinely used to screen the anti-seizure activity of various compounds, and each evaluates a distinct feature of epilepsy.[33] MES is a model for inducing an acute generalized tonic-clonic seizure in animals. Acute generalized absence-type seizures are produced using the PTZ -induced seizure model.[34] We can infer from the findings that edaravone may offer protection against generalized seizures of both absence and tonic-clonic types. It should be highlighted that the IV and IP PTZ models induced distinctive seizure patterns due to the difference in the rate and extent of PTZ delivery to the brain tissue. Indeed, depending on how PTZ is administered, multiple neuroanatomical regions are involved in the onset of seizures. A single seizure cluster was generated in the IV PTZ model, while a large number of small seizure clusters were produced in the IP PTZ model.[30,35] The mechanism of action of PTZ in inducing seizures is that it binds to the picrotoxin and benzodiazepine binding sites on $GABA_A$ receptors and blocks the normal current of chloride ion, resulting in depolarization of the neurons and induction of epileptic seizure.[36]

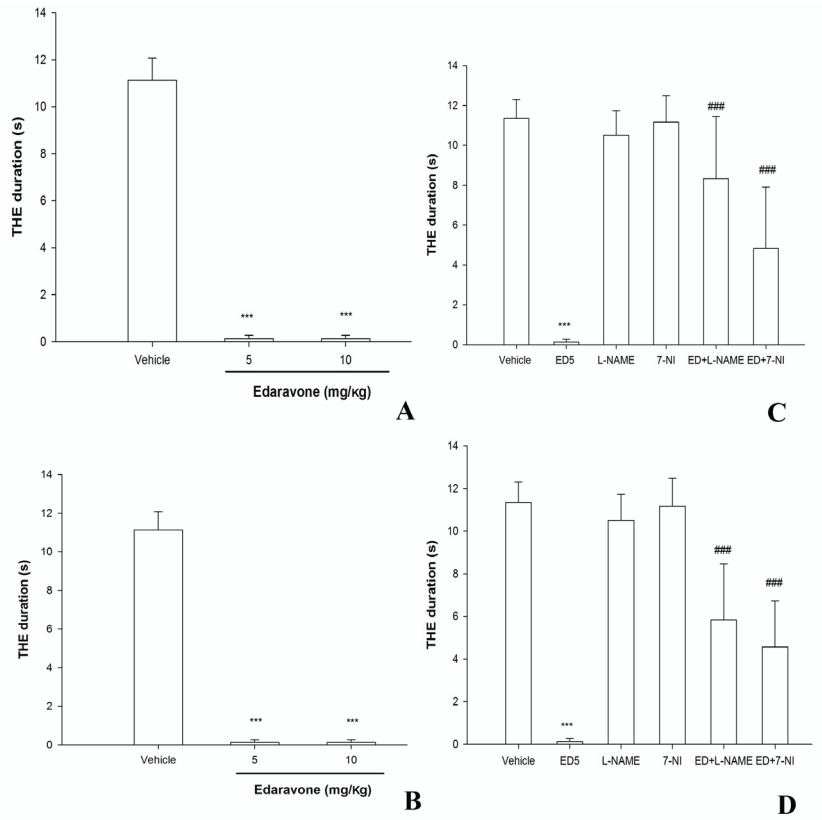

Figure 3: The graphs shows the mean±SEM of the duration of tonic hind-limb extension (THE) in mice pretreated with acute or sub-chronic intraperitoneal doses of edaravone: (A) Acute pretreatment of mice with edaravone (5, 10 mg/Kg, IP) decreased THE duration in comparison to the vehicle group (P<0.001). (B) Acute pretreatment of mice with concomitant injections of edaravone (5 mg/Kg, IP) plus L-NAME (5 mg/Kg, IP) or 7-NI (60 mg/Kg, IP) significantly increased THE duration in comparison to the administration of edaravone alone (P<0.001 for both co-administrations of LNAME plus edaravone or 7-NI plus edaravone). (C) Sub-chronic pretreatment of mice with edaravone (5, 10 mg/Kg, IP) for eight days, decreased THE duration as compared to the vehicle group (P<0.001). (D) Sub-chronic pretreatment of mice with concomitant injections of edaravone (5 mg/Kg, IP) plus L-NAME (5 mg/Kg, IP) or 7-NI (60 mg/Kg, IP) for eight days significantly increased THE duration in comparison to the administration of edaravone alone (P<0.001 for both co-administrations of LNAME plus edaravone or 7-NI plus edaravone). ***P≤0.001 represents significant differences with the vehicle group, ###P≤0.001 represents significant differences with edaravone. ED: Edaravone; L-NAME: N (ω)-nitro-L-arginine methyl ester; 7-NI: 7-nitroindazole

Therefore, it can be said that edaravone might activate $GABA_A$ receptors. On the other hand, the PTZ-induced seizure model was used to determine the onset or threshold of seizure, and the MES model evaluated the propagation of an induced seizure. Depending on the seizure severity and threshold, different models might produce different outcomes.[37, 38] Concerning the anti-seizure activity of edaravone in the MES model of seizure as well as the PTZ-induced seizure models, our findings demonstrated that edaravone not only prevented seizure propagation but also suppressed the impulse initiation and elevated the seizure threshold. Moreover, in several animal models of seizures, edaravone was shown to have anti-seizure activity.[39-41] Edaravone indicated anti-seizure activity in several murine models of seizure such as the pilocarpine-induced model,[39] penicillin-induced model,[40] and amygdala kindling.[41] All of these animal models, however, replicated the focal (partial) type of epilepsy. In a different research, Liu and others investigated the acute (but not sub-chronic) protective effects of edaravone in the IP PTZ seizure model.[42] To the best of our knowledge, the sub-chronic anti-seizure activity of edaravone in the generalized absence type of tonic-clonic seizures has never been studied, and this is the first study on this subject. In the second phase of this study, we investigated the involvement of NO in the mechanism of anti-seizure activity of edaravone. In the present study, NOS inhibitors were found to reduce the anti-seizure activity of edaravone in both acute and sub-chronic series of the experiment. These findings could be attributed to the role of NO in the mechanism of edaravone's anti-seizure mechanism. Therefore, we can infer that edaravone may induce the gene expressions or enzyme activities of eNOS or nNOS enzymes. Several other studies reported the interactions between edaravone and NOS enzymes. Following irradiation, edaravone reversed the decreased expression of eNOS mRNA and

protein in the rabbit ear artery.[43] Additionally, edaravone enhanced the expression of the eNOS gene in human umbilical vein endothelial cells in a rabbit model of spinal cord injury brought on by ischemia-reperfusion.[44] It was also shown that edaravone may reduce nNOS gene expression.[44, 45] At physiological conditions, the NO generated by nNOS, had a crucial role in the regulation of neuronal and vascular homeostasis.[26, 46] However, at higher concentrations, it might be involved in inflammatory pathways.[47] In the present study, 7-NI attenuated the protective effects of edaravone against seizure. This result might be attributed to the concentration-dependent nature of the effects of nitric oxide produced by nNOS. We concluded that 7-NI might enhance the edaravone-induced reduction in nNOS gene expression or enzyme activity. Therefore, the NO concentration might be reduced to the levels lower than the physiological level required to preserve neuronal homeostasis, and as a result, 7-nitroindazole diminished the protective effects of edaravone. However, this point requires further investigation to be confirmed. This study has a limitation. It lacks the measurement of mRNA or protein levels of NOS enzymes to confirm the important role of nitric oxide modulating activity of edaravone in producing anticonvulsant properties of this drug. However this work provides valuable results for future studies in this field.

Conclusion

The findings of the current study suggested that pretreatment with acute or sub-chronic doses of edaravone may have protective effects against absence and tonic-clonic types of acute generalized seizure. Further findings of this research showed that edaravone might exert its anti-seizure activity at least partially through NOS modulating pathways, particularly nNOS.

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Authors' Contribution

L.M: study conception, study design, analysis of data, drafting and revising the manuscript; E.S: study conception, analysis of data, drafting and revising the manuscript; M.D: study conception, proposal writing, drafting and revising the manuscript; F.P: study design, acquisition of data, drafting the manuscript; A.P: proposal preparation, analysis of data, drafting the manuscript; S.O: proposal preparation, acquisition of data, drafting the manuscript; All authors have read and approved the final manuscript and agree to be accountable to all aspects of the work to ensure that questions regarding the accuracy or integrity of each part of the work are properly reviewed and resolved.

Conflict of Interest: None declared.

References

1 Lee SK. Old versus New: Why Do We Need New Antiepileptic Drugs? J Epilepsy Res. 2014;4:39-44. doi: 10.14581/jer.14010. PubMed PMID: 25625087; PubMed Central PMCID: PMCPMC4295052.

2 Fattorusso A, Matricardi S, Mencaroni E, Dell'Isola GB, Di Cara G, Striano P, et al. The Pharmacoresistant Epilepsy: An Overview on Existant and New Emerging Therapies. Front Neurol. 2021;12:674483. doi: 10.3389/fneur.2021.674483. PubMed PMID: 34239494; PubMed Central PMCID: PMCPMC8258148.

3 Perucca E. The pharmacological treatment of epilepsy: recent advances and future perspectives. Acta Epileptologica. 2021;3:1-11. doi: 10.1186/s42494-021-00055-z.

4 Loscher W, Potschka H, Sisodiya SM, Vezzani A. Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options. Pharmacol Rev. 2020;72:606-38. doi: 10.1124/pr.120.019539. PubMed PMID: 32540959; PubMed Central PMCID: PMCPMC7300324.

5 Watanabe K, Tanaka M, Yuki S, Hirai M, Yamamoto Y. How is edaravone effective against acute ischemic stroke and amyotrophic lateral sclerosis? J Clin Biochem Nutr. 2018;62:20-38. doi: 10.3164/jcbn.17-62. PubMed PMID: 29371752; PubMed Central PMCID: PMCPMC5773834.

6 Kawasaki H, Ito Y, Kitabayashi C, Tanaka A, Nishioka R, Yamazato M, et al. Effects of Edaravone on Nitric Oxide, Hydroxyl Radicals and Neuronal Nitric Oxide Synthase During Cerebral Ischemia and Reperfusion in Mice. J Stroke Cerebrovasc Dis. 2020;29:104531. doi: 10.1016/j.jstrokecerebrovasdis.2019.104531. PubMed PMID: 31882337.

7 Yoshida H, Kwon AH, Kaibori M, Tsuji K,

Habara K, Yamada M, et al. Edaravone prevents iNOS expression by inhibiting its promoter transactivation and mRNA stability in cytokine-stimulated hepatocytes. Nitric Oxide. 2008;18:105-12. doi: 10.1016/j.niox.2007.11.003. PubMed PMID: 18078833.

8 Dzoljic E, Grbatinic I, Kostic V. Why is nitric oxide important for our brain? Funct Neurol. 2015;30:159-63. doi: 10.11138/fneur/2015.30.3.159. PubMed PMID: 26910176; PubMed Central PMCID: PMCPMC4610750.

9 Banach M, Piskorska B, Czuczwar SJ, Borowicz KK. Nitric oxide, epileptic seizures, and action of antiepileptic drugs. CNS Neurol Disord Drug Targets. 2011;10:808-19. doi: 10.2174/187152711798072347. PubMed PMID: 21999730.

10 Przegaliński E, Baran L, Siwanowicz J. The role of nitric oxide in chemically-and electrically-induced seizures in mice. Neuroscience letters. 1996;217:145-8. doi: 10.1016/0304-3940(96)13085-8.

11 Zhu X, Dong J, Han B, Huang R, Zhang A, Xia Z, et al. Neuronal Nitric Oxide Synthase Contributes to PTZ Kindling Epilepsy-Induced Hippocampal Endoplasmic Reticulum Stress and Oxidative Damage. Front Cell Neurosci. 2017;11:377. doi: 10.3389/fncel.2017.00377. PubMed PMID: 29234274; PubMed Central PMCID: PMCPMC5712337.

12 Del-Bel EA, Oliveira PR, Oliveira JA, Mishra PK, Jobe PC, Garcia-Cairasco N. Anticonvulsant and proconvulsant roles of nitric oxide in experimental epilepsy models. Braz J Med Biol Res. 1997;30:971-9. doi: 10.1590/s0100-879x1997000800010. PubMed PMID: 9361727.

13 Rasooli R, Pirsalami F, Moezi L. Posible involvement of nitric oxide in anticonvulsant effects of citicoline on pentylenetetrazole and electroshock induced seizures in mice. Heliyon. 2020;6:e03932. doi: 10.1016/j.heliyon.2020.e03932. PubMed PMID: 32462085; PubMed Central PMCID: PMCPMC7240119.

14 Kovacs R, Rabanus A, Otahal J, Patzak A, Kardos J, Albus K, et al. Endogenous nitric oxide is a key promoting factor for initiation of seizure-like events in hippocampal and entorhinal cortex slices. J Neurosci. 2009;29:8565-77. doi: 10.1523/JNEUROSCI.5698-08.2009. PubMed PMID: 19571147; PubMed Central PMCID: PMCPMC6665664.

15 Sharma S, Puttachary S, Thippeswamy T. Glial source of nitric oxide in epileptogenesis: A target for disease modification in epilepsy. J Neurosci Res. 2019;97:1363-77. doi: 10.1002/jnr.24205. PubMed PMID: 29230865; PubMed Central PMCID: PMCPMC6035106.

16 Hopper R, Lancaster B, Garthwaite J. On the regulation of NMDA receptors by nitric oxide. Eur J Neurosci. 2004;19:1675-82. doi: 10.1111/j.1460-9568.2004.03306.x. PubMed PMID: 15078541.

17 Paul V, Jayakumar AR. A role of nitric oxide as an inhibitor of gamma-aminobutyric acid transaminase in rat brain. Brain Res Bull. 2000;51:43-6. doi: 10.1016/s0361-9230(99)00206-3. PubMed PMID: 10654579.

18 Kourosh-Arami M, Hosseini N, Mohsenzadegan M, Komaki A, Joghataei MT. Neurophysiologic implications of neuronal nitric oxide synthase. Rev Neurosci. 2020;31:617-36. doi: 10.1515/revneuro-2019-0111. PubMed PMID: 32739909.

19 Picon-Pages P, Garcia-Buendia J, Munoz FJ. Functions and dysfunctions of nitric oxide in brain. Biochim Biophys Acta Mol Basis Dis. 2019;1865:1949-67. doi: 10.1016/j.bbadis.2018.11.007. PubMed PMID: 30500433.

20 Cinelli MA, Do HT, Miley GP, Silverman RB. Inducible nitric oxide synthase: Regulation, structure, and inhibition. Med Res Rev. 2020;40:158-89. doi: 10.1002/med.21599. PubMed PMID: 31192483; PubMed Central PMCID: PMCPMC6908786.

21 Lin TK, Chen SD, Lin KJ, Chuang YC. Seizure-Induced Oxidative Stress in Status Epilepticus: Is Antioxidant Beneficial? Antioxidants (Basel). 2020;9. doi: 10.3390/antiox9111029. PubMed PMID: 33105652; PubMed Central PMCID: PMCPMC7690410.

22 Tieu K, Ischiropoulos H, Przedborski S. Nitric oxide and reactive oxygen species in Parkinson's disease. IUBMB Life. 2003;55:329-35. doi: 10.1080/1521654032000114320. PubMed PMID: 12938735.

23 Toda N, Ayajiki K, Okamura T. Cerebral blood flow regulation by nitric oxide in neurological disorders. Can J Physiol Pharmacol. 2009;87:581-94. doi: 10.1139/y09-048. PubMed PMID: 19767882.

24 Schuman EM, Madison DV. Nitric oxide and synaptic function. Annu Rev Neurosci. 1994;17:153-83. doi: 10.1146/annurev.ne.17.030194.001101. PubMed PMID: 7516125.

25 Toda N, Okamura T. The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. Pharmacol Rev. 2003;55:271-324. doi: 10.1124/pr.55.2.3. PubMed PMID: 12773630.

26 Reis PA, de Albuquerque CFG, Gutierrez T,

Silva AR, de Castro Faria Neto HC. Role of nitric oxide synthase in the function of the central nervous system under normal and infectious conditions. Nitric Oxide Synthase–Simple Enzyme-Complex Roles London: InTech. 2017:55-70. doi: 10.5772/67816.
27 Ahmadi-Noorbakhsh S, Mirabzadeh Ardakani E, Sadighi J, Aldavood SJ, Farajli Abbasi M, Farzad-Mohajeri S, et al. Guideline for the Care and Use of Laboratory Animals in Iran. Lab Anim (NY). 2021;50:303-5. doi: 10.1038/s41684-021-00871-3. PubMed PMID: 34621075.
28 Koutroumanidou E, Kimbaris A, Kortsaris A, Bezirtzoglou E, Polissiou M, Charalabopoulos K, et al. Increased seizure latency and decreased severity of pentylenetetrazol-induced seizures in mice after essential oil administration. Epilepsy Res Treat. 2013;2013:532657. doi: 10.1155/2013/532657. PubMed PMID: 23819045; PubMed Central PMCID: PMCPMC3684096.
29 Pourgholami MH, Kamalinejad M, Javadi M, Majzoob S, Sayyah M. Evaluation of the anticonvulsant activity of the essential oil of Eugenia caryophyllata in male mice. J Ethnopharmacol. 1999;64:167-71. doi: 10.1016/s0378-8741(98)00121-4. PubMed PMID: 10197752.
30 Mandhane SN, Aavula K, Rajamannar T. Timed pentylenetetrazol infusion test: a comparative analysis with s.c.PTZ and MES models of anticonvulsant screening in mice. Seizure. 2007;16:636-44. doi: 10.1016/j.seizure.2007.05.005. PubMed PMID: 17570689.
31 Sattarinezhad E, Shafaroodi H, Sheikhnouri K, Mousavi Z, Moezi L. The effects of coenzyme Q10 on seizures in mice: the involvement of nitric oxide. Epilepsy Behav. 2014;37:36-42. doi: 10.1016/j.yebeh.2014.05.024. PubMed PMID: 24972157.
32 Castel-Branco MM, Alves GL, Figueiredo IV, Falcao AC, Caramona MM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol. 2009;31:101-6. doi: 10.1358/mf.2009.31.2.1338414. PubMed PMID: 19455265.
33 Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, et al. Animal models of epilepsy: use and limitations. Neuropsychiatr Dis Treat. 2014;10:1693-705. doi: 10.2147/NDT.S50371. PubMed PMID: 25228809; PubMed Central PMCID: PMCPMC4164293.
34 Loscher W, Honack D, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. Epilepsy Res. 1991;8:171-89. doi: 10.1016/0920-1211(91)90062-k. PubMed PMID: 1907909.
35 Keogh BP, Cordes D, Stanberry L, Figler BD, Robbins CA, Tempel BL, et al. BOLD-fMRI of PTZ-induced seizures in rats. Epilepsy Res. 2005;66:75-90. doi: 10.1016/j.eplepsyres.2005.07.008. PubMed PMID: 16144756.
36 Ramanjaneyulu R, Ticku MK. Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. Eur J Pharmacol. 1984;98:337-45. doi: 10.1016/0014-2999(84)90282-6. PubMed PMID: 6327331.
37 Giardina WJ, Gasior M. Acute seizure tests in epilepsy research: electroshock- and chemical-induced convulsions in the mouse. Curr Protoc Pharmacol. 2009;5:22. doi: 10.1002/0471141755.ph0522s45. PubMed PMID: 22294398.
38 Giordano C, Vinet J, Curia G, Biagini G. Repeated 6-Hz Corneal Stimulation Progressively Increases FosB/DeltaFosB Levels in the Lateral Amygdala and Induces Seizure Generalization to the Hippocampus. PLoS One. 2015;10:e0141221. doi: 10.1371/journal.pone.0141221. PubMed PMID: 26555229; PubMed Central PMCID: PMCPMC4640822.
39 Kamida T, Fujiki M, Ooba H, Anan M, Abe T, Kobayashi H. Neuroprotective effects of edaravone, a free radical scavenger, on the rat hippocampus after pilocarpine-induced status epilepticus. Seizure. 2009;18:71-5. doi: 10.1016/j.seizure.2008.06.012. PubMed PMID: 18672383.
40 Kurt S, Aygun H. Anticonvulsive effects of edaravone on penicillin-induced focal onset seizure model in the conscious rats. Fundam Clin Pharmacol. 2021;35:861-9. doi: 10.1111/fcp.12651. PubMed PMID: 33484001.
41 Kamida T, Abe E, Abe T, Ooba H, Fujiki M, Kobayashi H. Edaravone, a free radical scavenger, retards the development of amygdala kindling in rats. Neurosci Lett. 2009;461:298-301. doi: 10.1016/j.neulet.2009.06.039. PubMed PMID: 19545611.
42 Liu LM, Wang N, Lu Y, Wang WP. Edaravone acts as a potential therapeutic drug against pentylenetetrazole-induced epilepsy in male albino rats by downregulating cyclooxygenase-II. Brain Behav. 2019;9:e01156.

doi: 10.1002/brb3.1156. PubMed PMID: 30506635; PubMed Central PMCID: PMCPMC6346642.

43 Zhang XH, Matsuda N, Jesmin S, Sakuraya F, Gando S, Kemmotsu O, et al. Normalization by edaravone, a free radical scavenger, of irradiation-reduced endothelial nitric oxide synthase expression. Eur J Pharmacol. 2003;476:131-7. doi: 10.1016/s0014-2999(03)02151-4. PubMed PMID: 12969758.

44 Takahashi G, Sakurai M, Abe K, Itoyama Y, Tabayashi K. MCI-186 prevents spinal cord damage and affects enzyme levels of nitric oxide synthase and Cu/Zn superoxide dismutase after transient ischemia in rabbits. J Thorac Cardiovasc Surg. 2003;126:1461-6. doi: 10.1016/s0022-5223(03)00693-7. PubMed PMID: 14666020.

45 Yoshida H, Yanai H, Namiki Y, Fukatsu-Sasaki K, Furutani N, Tada N. Neuroprotective effects of edaravone: a novel free radical scavenger in cerebrovascular injury. CNS Drug Rev. 2006;12:9-20. doi: 10.1111/j.1527-3458.2006.00009.x. PubMed PMID: 16834755; PubMed Central PMCID: PMCPMC6741743.

46 Semenza GL. New insights into nNOS regulation of vascular homeostasis. J Clin Invest. 2005;115:2976-8. doi: 10.1172/JCI26792. PubMed PMID: 16276412; PubMed Central PMCID: PMCPMC1265879.

47 BAIG MS, Bakhshi F, Ye RD, Gao X, Malik AB, Minshall RD, et al. Role of nNOS in the progression of systemic inflammatory response induced by lipopolysaccharide. Wiley Online Library. 2012. doi: 10.1096/fasebj.26.1_supplement.lb546.