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Research Article

Valproic acid induced oxidative brain injury in rats: Protective effect of edaravone

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ABSTRACT

Valproic acid (2-propyl valeric acid, VPA) is the drug of choice for the treatment of migraine, bipolar disorder, and epileptic disorders in both children and adults. Although VPA has beneficial effects, long-term administration is reported to damage many tissues and organs. VPA administration increases free-radical production. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, EDA) is a potent antioxidant that protects against oxidative stress. The aim of our study was to investigate the protective effects of EDA against brain damage in VPA-treated rats. Male Sprague Dawley rats were used in our study. The rats were randomly divided into four groups: control, EDA, VPA, and VPA+EDA. EDA and VPA were administered intraperitoneally at doses of 30 mg/kg and 0.5 g/kg, respectively daily for 7 days. On day 8, all animals were sacrificed under anesthesia, and brain tissues were removed. VPA caused decreases in the levels of reduced glutathione (GSH, $P<0.05$), glutathione peroxidase (GPx, $P<0.001$), glutathione reductase (GR, $P<0.001$), and paraoxonase (PON, $P<0.0001$). On the other hand, VPA increased values of lipid peroxidation (LPO, $P<0.05$), catalase (CAT, $P<0.05$), superoxide dismutase (SOD, $P<0.0001$), glutathione-S-transferase (GST, $P<0.01$), xanthine oxidase (XO, $P<0.01$), adenosine deaminase (ADA, $P<0.001$), protein carbonyl (PC, $P<0.0001$), myeloperoxidase (MPO, $P<0.0001$), sialic acid (SA, $P<0.0001$), and acetylcholinesterase (AChE, $P<0.0001$) compared with the control group. EDA reversed all values. These results suggest that EDA administration potentially reduces VPA-induced brain injury.

Keywords: Antioxidant, Brain, Edaravone, Oxidative stress, Valproic acid.

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Introduction

Epilepsy is a chronic neurological disorder characterized by sudden and recurrent seizures. The condition is caused by excessive, irregular or synchronized electrical activity in the brain (34). The highest incidence of epilepsy occurs in childhood, particularly during the first 10 years of life (1, 9). Epilepsy can be effectively controlled with appropriate use of anticonvulsant drugs (36).

Valproic acid (VPA) is an anticonvulsant indicated as a first-line treatment for seizure disorders and epileptic syndromes (61). VPA is also used as a neuroprotective agent in brain disorders such as migraine (12) and bipolar disorder (15). Hepatotoxicity, pancreatitis, teratogenicity, hematologic disorders, weight gain, and cognitive impairment have been reported in individuals receiving the drug (62). Disruption of the balance between reactive oxygen species (ROS) and antioxidant mechanisms leads to physiological and biochemical dysfunction (23).

Antioxidants play an important role in disease prevention due to their reactive oxygen species scavenging activity (23). Edaravone (EDA) is a new, lipophilic, low-molecular-weight molecule with antioxidant properties. This substance acts by inhibiting non-enzymatic peroxidation and lipoxygenase activity (2, 54). EDA also exhibits anti-inflammatory, anti-apoptotic, and anti-necrotic effects (39). EDA has also been reported to be an effective neuroprotective agent for acute ischemic stroke (20) and amyotrophic lateral sclerosis (ALS) (66). Many studies have shown that EDA scavenges oxygen and nitrogen radicals, reduces oxidative stress-induced damage, exhibits neuroprotective effects (40), and aids in the management of oxidative stress-induced

neurodegenerative diseases such as Parkinson's disease (69), Alzheimer's disease (32), and multiple sclerosis (4).

The aim of our study was to evaluate the protective effects of EDA, whose antioxidant effects have been previously demonstrated against VPA-induced brain damage.

Materials and Methods

Experimental Animal Model: Thirty-eight Sprague Dawley rats (2.5-3 months old, males) were randomly divided into 4 groups. Animals were housed in standard cages under optimal temperature (20 ± 2°C) and a 12-h light/12-h dark cycle. Control group: Control animals administered physiological saline intraperitoneally once daily for 7 days (n=8). EDA group: Animals received 30 mg/kg EDA, dissolved in physiological saline, intraperitoneally, once daily for 7 days (n = 10). VPA group: Animals were administered 0.5 g/kg VPA, dissolved in physiological saline, by intraperitoneal injection once daily for 7 days (n = 10). VPA+EDA group: Animals were administered 0.5 g/kg VPA and 30 mg/kg EDA, both dissolved in physiological saline, via intraperitoneal injection once daily for 7 days (n = 10); the two drugs were given simultaneously. All animals were euthanized under anesthesia 16 hours after administration of VPA and EDA. On day 8, brain tissues were collected. Brain homogenates (10% w/v) were prepared in physiological saline (0.9% NaCl). To determine MPO activity, tissues were homogenized in hexadecyltrimethylammonium bromide (HETAB) solution.

Biochemical Analysis: Weighed brain tissue samples were homogenized in 0.9% saline using a glass homogenizer to prepare a 10% (w/v) brain tissue homogenate. The homogenate was centrifuged at 10,000 rpm for 20 minutes at +4 °C. The obtained brain tissue homogenates were placed in eppendorf tubes and stored at -76 °C until the day of the experiment. The supernatants were prepared for biochemical analyses and frozen until the experiments were performed. For this purpose, GSH and LPO values and CAT, SOD, GPx, GR, GST and XO activities were determined according to the methods of Beutler (11), Ledwozyw et al. (43), Aebi (3), Mylroie et al. (49) Wendel (79), Habig and Jakoby (25) and Corte and Stirpe (16), respectively. ADA was determined according to Karker's method (35), PON1 according to Furlong et al. (21), PC according to Levine et al. (45), MPO activity according to Wei and Frenkel (78). SA levels were determined according to Warren's method (77) and AChE activities were determined according to Ellman et al. (19). The protein content was determined according to the Lowry method using Bovine Serum Albumin (BSA) as a standard (46).

Statistical Analysis: Biochemical results were analyzed using GraphPad Prism 9.0 (GraphPad Software, San Diego, California, USA). Values are expressed as mean ± standard deviation (SD). For statistical analysis, a t-test and analysis of variance (ANOVA), followed by Tukey multiple comparison tests were used. $P<0.05$ was considered statistically significant.

Results

Brain Results: Significant decreases were observed in brain glutathione levels in the VPA group compared with the control group ($P<0.05$). GSH values decreased, but not significantly, in the EDA group compared with the control group and in the VPA group compared with the EDA group.

Significant increases in lipid peroxidation levels ($P<0.05$), catalase activity ($P<0.05$), and superoxide dismutase activity ($P<0.0001$) were detected in the VPA group compared with the control group (Figure 1, Table 1). In the EDA group, the LPO value decreased non-significantly, whereas CAT and SOD activities increased non-significantly compared with the control group. In the VPA group, the LPO value and the activities of CAT and SOD increased significantly compared to the EDA group ($P<0.05$, $P<0.01$). The VPA+EDA group reversed the changes in GSH ($P<0.01$), LPO ($P<0.05$), CAT, and SOD ($P<0.01$) in brain tissue compared with the VPA group. Brain GPx ($P<0.001$) and GR ($P<0.001$) activities were significantly decreased in the VPA group compared with the control group, while activities of GST ($P<0.01$) and XO ($P<0.01$) were increased. In the VPA+EDA group, GPx ($P<0.01$) and GR ($P<0.0001$) enzyme activities in brain tissue increased, whereas GST ($P<0.01$) and XO ($P<0.001$) enzyme activities decreased compared to the VPA group (Figure 2, Table 2). In the EDA group, GPx and GR activity values decreased non-significantly compared with the control group, while GST and XO activity values increased non-significantly. In the VPA group, compared with the EDA group, a significant decrease in GPx activity ($P<0.01$) and a significant increase in XO activity ($P<0.05$) were observed, whereas changes in GR (decrease) and GST (increase) were not significant.

The VPA group showed significant increases in brain ADA activity ($P<0.001$), MPO activity ($P<0.0001$), and PC levels ($P<0.0001$), and a significant decrease in PON activity ($P<0.0001$) compared to the control group. The VPA+EDA group reversed the changes in the parameters ADA ($P<0.001$), PON ($P<0.05$), MPO ($P<0.001$), and PC ($P< 0.001$) in brain tissue, compared with the VPA group (Figure 3, Table 3). PON activity was decreased significantly in the EDA group compared with the control group ($P<0.05$). Changes in ADA, MPO activities, and PC levels between the EDA and control groups were not significant. A significant decrease in PON activity

(P<0.001) and significant increases in ADA activity (P<0.01), MPO activity (P<0.001), and PC levels (P<0.0001) were observed in the VPA group compared with the EDA group. Brain SA levels (P<0.0001) and AChE activity levels (P<0.0001) were significantly increased in the VPA group compared with the control group. The VPA+EDA group had significantly decreased SA levels (P<0.0001) and AChE activity (P<0.0001) in brain tissue compared with the VPA group (Figure 4, Table 4). In the VPA group, significant increases in SA levels (P<0.001) and AChE activity (P<0.0001) were observed compared with the EDA group.

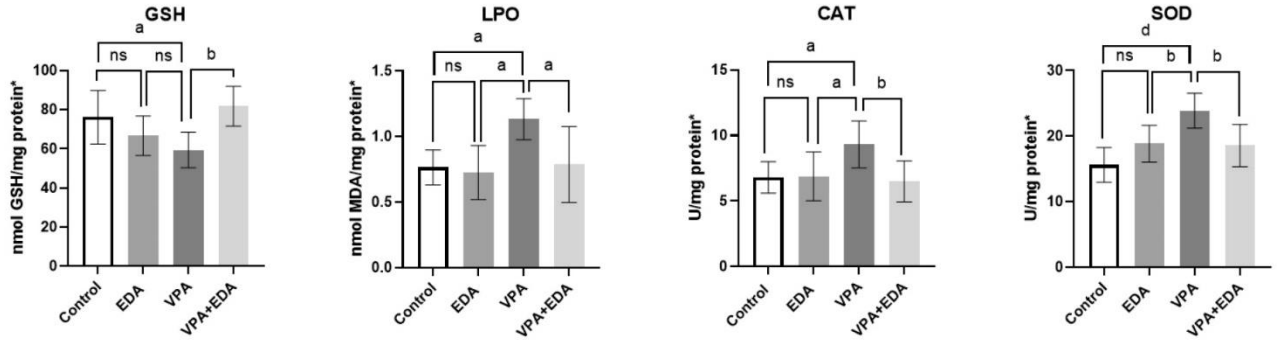


Figure 1. Brain GSH, LPO levels, and CAT, SOD activities

The columns represent mean ± SD. EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone, GSH: Glutathione, LPO: Lipid peroxidation, CAT: Catalase, SOD: Superoxide dismutase, [a]represent P<0.05, [b]represent P<0.01, [d]represent P<0.0001.

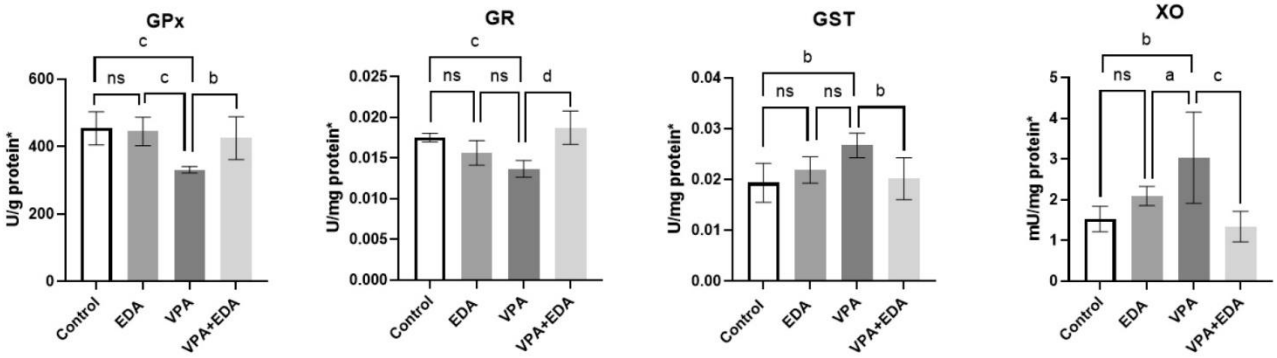


Figure 2. Brain GPx, GR, GST and XO activities

The columns represent mean ± SD. EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone, GP_x: Glutathione peroxidase, GR: Glutathione reductase, GST: Glutathione-S-transferase, XO: Xanthine oxidase. [b]represent P<0.01, [c]represent P<0.001, [d] represent P<0.0001.

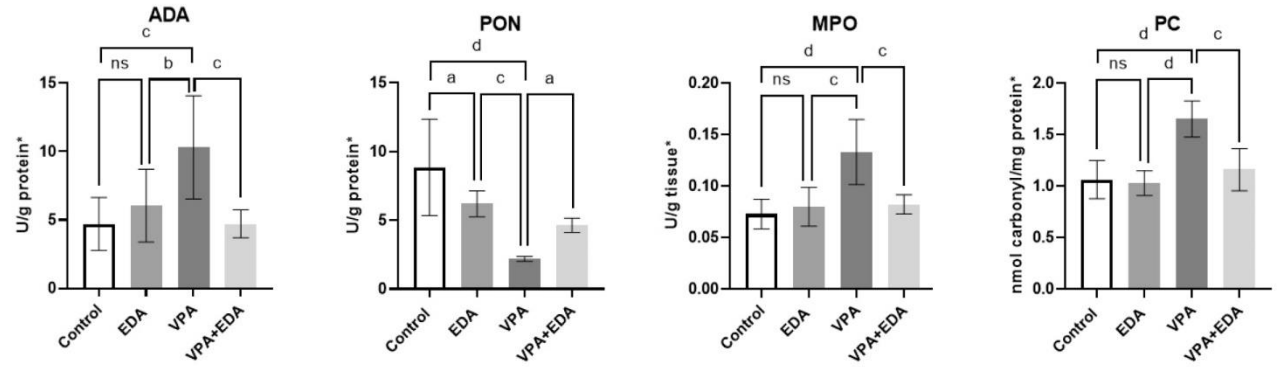


Figure 3. Brain ADA, PON, MPO activities and PC levels

The columns represent mean ± SD. EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone, ADA: Adenosine deaminase, PON: Paraoxonase, MPO: Myeloperoxidase, PC: Protein carbonyl. [a]represent P<0.05, [c]represent P<0.001, [d]represent P<0.0001.

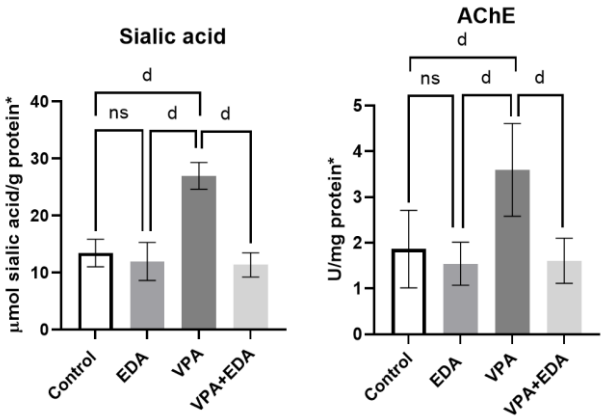


Figure 4. Brain sialic acid levels and AChE activities

The columns represent mean ± SD. EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone. AChE: Acetylcholine esterase. [d] represent P<0.0001.

Table 1. Brain GSH and LPO levels and CAT and SOD activities of all groups*.

Groups	GSH (nmol GSH/mg protein)	LPO (nmol MDA/mg protein)	CAT (U/g protein)	SOD (U/g protein)
Control	76.09 ± 13.71	0.76 ± 0.13	6.80 ± 1.19	15.61 ± 2.66
EDA	66.67 ± 10.11	0.72 ± 0.21	6.88 ± 1.86	18.84 ± 2.80
VPA	59.38 ± 9.12[a]	1.13 ± .16[a,d]	9.31 ± 1.79 [a,d]	23.87 ± 2.66[e,f]
VPA+EDA	81.73 ± 10.15[b]	0.79 ± 0.29[c]	6.49 ± 1.56[b]	18.56 ± 3.22[b]
PANOVA	0.01	0.05	0.01	0.0001

*Mean ± SD, EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone, GSH: Glutathione, LPO: Lipid peroxidation, CAT: Catalase, SOD: Superoxide dismutase, [a]P<0.05, [e]P<0.0001 vs control group; [b]P<0.01, [c]P<0.05 vs VPA group; [d]P<0.05, [f]P<0.01 vs EDA group.

Table 2. Brain GPx, GR, GST, and XO activities of all groups*.

	GPx (U/g protein)	GR (U/mg protein)	GST (U/mg protein)	XO (mU/mg protein)
Control	454.00 ± 49.30	0.018 ± 0.001	0.019 ± 0.004	1.53 ± 0.31
EDA	445.00 ± 42.03	0.016 ± 0.002	0022 ± 0.003	2.09 ± 0.24
VPA	331.30 ± 9.54[a,c]	0.014 ± 0.001[a]	0.027 ± 0.002[e]	3.03 ± 1.12[d,f]
VPA+EDA	425.00 ± 63.05[b]	0.019 ± 0.002[d]	0.020 ± 0.004[b]	1.34 ± 0.38[g]
PANOVA	0.0001	0.0001	0.01	0.0001

*Mean ± SD, EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone, GP_x; Glutathione peroxidase, GR: Glutathione reductase, GST: Glutathione-S-transferase, XO: Xanthine oxidase. [a]P<0.01, [e]P<0.01 vs control group; [b]P<0.01, [d]P<0.0001, [f]P<0.001 vs VPA group; [c]P<0.001, [g]P<0.05 vs EDA group.

Table 3. Brain ADA, PON, MPO activities and PC levels of all groups*.

Groups	ADA (U/g protein)	PON (U/g protein)	MPO (U/g tissue)	PC (nmol carbonyl/mg protein)
Control	4.71 ± 1.93	8.86 ± 3.50	0.073 ± 0.014	1.06 ± 0.19
EDA	6.05 ± 2.66	6.20 ± 0.95[f]	0.080 ± 0.019	1.03 ± 0.12
VPA	10.31 ± 3.77[a,c]	2.20 ± 0.18[d,g]	0.133 ± 0.032[d,g]	1.65 ± 0.18[d,h]
VPA+EDA	4.72 ± 1.02[b]	4.63 ± 0.52[e]	0.082 ± 0.009[b]	116 ± 0.20[b]
PANOVA	0.001	0.0001	0.0001	0.0001

*Mean ± SD, EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone. ADA: Adenosine deaminase, PON: Paraoxonase, MPO: Myeloperoxidase, PC: Protein carbonyl. [a]P<0.001, [d]P<0.0001, [f]P<0.05 vs control group; [b]P<0.001, [e]P<0.05 vs VPA group; [c]P<0.01, [g]P<0.001, [h]P<0.0001 vs EDA group.

Table 4. Brain sialic acid levels and AChE activities of all groups*.

Groups	Sialic acid (µmol sialic acid/g protein)	AChE (U/mg protein)
Control	13.44 ± 2.41	1.86 ± 0.85
EDA	11.95 ± 3.32	1.54 ± 0.48
VPA	26.97 ± 2. 36[a,c]	3.60 ± 1. 01[a,c]
VPA+EDA	11.35 ± 2.12[b]	1.61 ± 0.49[b]
P ANOVA	0.0001	0.0001

*Mean ± SD, EDA: Edaravone group, VPA: Valproic acid group, VPA+EDA: Valproic acid+Edaravone, AChE: Acetylcholine esterase.

Discussion and Conclusion

VPA is used to treat many seizures, but it is associated with systemic toxic effects. ROS formation is one of the causes of VPA toxicity. Jafarian and colleagues (31) noted that ROS formation in mitochondria increased following VPA administration. EDA's beneficial effects on mitochondria and its role in suppressing mitochondrial ROS formation have been previously reported (27, 28, 37, 55).

GSH is an important cell protective biomolecule against cytotoxic damage that is synthetically activated through electrophilic compounds and GST conjugation (59). In sodium valproate-induced autistic rats, GSH levels were decreased in the VPA group compared with the control group (47, 53). Another study examining VPA-induced autistic-like behaviors and brain oxidative stress markers reported reduced GSH levels following VPA administration (65). Amounts of GSH in the kidneys and liver of VPA-poisoned rats were reported to decrease compared with those in the control group (29,52). Chaudhary and colleagues reported VPA-induced kidney damage and increased GSH levels (13). In studies of VPA-induced brain damage, GSH levels were significantly lower in the VPA group than in the control group (72,74,75). In this study, we found that VPA decreased GSH levels compared with the control group. The decrease in GSH levels following VPA administration suggests an imbalance between oxidative damage and antioxidant defenses. The decrease in GSH levels in the VPA group suggests a reduced ability to neutralize ROS and protect cell components such as lipids, proteins, and nucleic acids from oxidative damage. A study on VPA-induced autism spectrum disorder reported that VPA+EDA caused an increase in GSH levels compared to the VPA group (47). EDA has been reported to increase GSH levels in rats with contrast agent-induced acute kidney injury (5). A study investigating the protective effect of EDA on cyclophosphamide-induced oxidative stress and neurotoxicity in rats reported that EDA increased GSH levels (62). In rats with VPA-induced kidney injury, EDA administration increased GSH levels compared to the VPA group (8). In our study, VPA administration decreased brain GSH levels, whereas VPA+EDA administration increased brain GSH levels. The increase in GSH levels is attributable to the therapeutic effect of EDA, a potent free-radical scavenger (41).

LPO is an important parameter measured to assess pathogenic processes resulting from oxidative stress (71). Measured oxidative stress markers, such as LPO, indicate significant damage to cell membranes. Studies have reported a significant increase in LPO levels in VPA-induced Fanconi syndrome (29) and in VPA-induced hepatotoxicity and kidney damage (8, 26, 52). Chaunhary and Parvez reported that VPA has neurotoxic effects, causing an increase in LPO levels (14). In VPA-induced toxicity, LPO levels in brain tissue were increased in the VPA group compared with the control group, and administration of chard extract to the VPA group decreased these levels (72). In this study, VPA caused an increase in LPO levels in brain tissue compared with the control group. In our study, a significant decrease in LPO levels was observed when EDA was co-administered with VPA. The reduction in LPO levels suggests that EDA exerts a protective effect on brain lipids, likely by directly scavenging free radicals and preventing lipid peroxidation. This antioxidant effect may help preserve cellular function and prevent damage caused by oxidative stress.

Oxidative-antioxidant balance is maintained by a system of endogenous antioxidant enzymes that protect cells against the harmful effects of ROS, which increase with VPA application in liver tissue. When this balance is disrupted, changes occur in the activities of these antioxidant enzymes (60). Chaundhary and Parvez reported that VPA causes neurotoxic effects and reduces antioxidant activities, such as CAT and SOD (14). It has been reported that antioxidant enzyme activities, such as CAT and GPx, decreased in pancreatic tissue with VPA-induced damage in the VPA group compared with the control group (56). Türkyılmaz et al. reported that CAT, SOD, GPx, GR, and GST activities increased following VPA administration in liver tissue (76). Bayrak and colleagues also reported increased levels of antioxidant

enzymes, such as CAT and SOD, in their studies of kidney damage caused by VPA (8). In this study, we found that VPA increased CAT, SOD, and GST activities compared with the control group. Because of decreased GSH levels, we can establish a relationship between increased activities of enzymes, such as CAT, SOD, and GST, and free radical production. However, we observed decreases in GPx and GR activities, which may be related to increased ROS levels and to depletion of GSH required for GR and GST activities. Administration of EDA to the VPA group reversed the findings because of EDA's antioxidant properties.

XO is one of the most important enzymatic sources of superoxide radicals and catalyzes the conversion of hypoxanthine and xanthine to uric acid. It is an important producer of ROS. In the presence of the XO enzyme in the tissue, free radical production is triggered by a large increase in the formation of its substrates, inosine and hypoxanthine (33). ADA plays an important role in acute immune-inflammatory conditions, and its serum level is an important biochemical marker of inflammation (64). The enzymes involved in purine metabolism are ADA and XO. ADA catalyzes the formation of inosine from adenosine. XO catalyzes the formation of uric acid from xanthine (22). In VPA-induced liver injury, XO and ADA activities increased (26). Türkyılmaz et al. reported that XO and ADA increased significantly in the VPA group compared to the control group (74, 75). In this study, we found that VPA increased the activities of XO and ADA compared with the control group. VPA is a potential source of free radicals. In parallel with the increase in ROS observed in this study, XO and ADA activities may also have increased in the VPA group. EDA regulated the antioxidant enzyme activities and decreased the XO and ADA activities by reducing ROS levels.

PON1 is another antioxidant enzyme associated with high-density lipoproteins. It acts by detoxifying lipid peroxides. It is a calcium-dependent esterase. It is also found in many organs such as liver, brain, lung, heart, kidneys and small intestines (6) Increased oxidative stress decreases PON1 synthesis *in vivo* and *in vitro* (51). A study investigating changes in the antioxidant system in adult epilepsy patients using anti-epileptic drugs reported that VPA use caused a decrease in PON activity (30). Different studies have shown that PON1 activity decreases significantly following VPA administration compared with the control group (73, 74, 75). PON activity has been reported to decrease in various metabolic disorders (67,70). In our study, we also found a decrease in PON activity in the VPA group. This decrease may be related to the elevated levels of free radicals induced by VPA. Administration of EDA to the VPA group increased the activity of this enzyme. This increase in PON activity may have enabled EDA to restore and stabilize antioxidant enzymes and GSH levels.

PC levels are among the most widely used markers of protein oxidation (68). There is growing evidence that toxic carbonyls cause many human diseases (10). Our study demonstrates that VPA significantly increases PC levels. The increase in PC production is due to increased oxidative stress induced by the substance administered to the experimental group. EDA prevented increases in PC levels and effectively restored brain cells to their normal physiological state.

MPO is released by neutrophils in response to various stimuli, including substances that damage tissue (38). In a study investigating valproate-induced liver oxidative stress and cellular imbalance, increased MPO activity was reported (26). MPO overactivity results from high levels of ROS during inflammation, necrosis, and other toxic insults (7). Furthermore, MPO causes collapse of the central nervous system. It has been reported that MPO activity brain tissue increases with VPA administration (42). In this study, MPO activity in the brain increased with VPA administration. Treatment with EDA decreased brain tissue MPO activity, as suggested by Lehmann et al. (44), probably because it exerts both antioxidant and restorative effects on the blood-brain barrier.

SA levels have been reported to increase in inflammatory conditions, possibly due to increased levels of acute-phase glycoproteins, hypertriglyceridemia, and atherosclerosis (17, 18). Many studies have reported that SA is a marker for inflammatory diseases. Natori et al. reported that VPA caused sialic acid disruption in neurons and brain tissue (50). SA levels increased in valproic acid-induced toxicity (74). The increase in SA levels in the VPA group was consistent with other studies. EDA administration has been reported to cause a significant decrease in SA levels related to pancreatic function compared with VPA animals (57). Our study also found increased brain SA levels in VPA-treated rats, whereas EDA administration decreased SA levels. EDA treatment reversed these levels, likely through preservation of membrane stability. Acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh), plays an important role in neurodegenerative diseases and is found at neuromuscular junctions and cholinergic brain synapses (58). A study in adult patients with epilepsy who were receiving antiepileptic drugs reported that VPA decreased AChE activity (30). A study investigating stereotypic repetitive behaviors in sodium valproate-induced autism reported increased AChE levels (53). In this study, we found that VPA increased AChE activity compared to the control group. This increase in AChE activity may be due to elevated levels of neurotoxic compounds. When EDA was administered to rats in the

experimental group, the elevated AChE activity was reduced due to the antioxidant effect of this EDA.

As a conclusion VPA is a widely used antiepileptic agent. Although it has beneficial effects, it also has serious side effects that can be observed in brain tissue. For this purpose, EDA was chosen as a protective agent because its effects have been demonstrated in previous studies, and it is a potent antioxidant. The biochemical results obtained from this study support the protective effects of EDA on brain tissue exposed to VPA.

Ethical Statement

This study was approved by İstanbul University Local Ethics Committee for Animal Experiments (2010/54).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

NHC and RY contributed equally to all aspects of the study. All authors have read and approved the final manuscript.

Data Availability Statement

The data are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Aaberg KM, Gunnes N, Bakken IJ, et al** (2017): *Incidence and prevalence of childhood epilepsy: a nationwide cohort study.* Pediatrics, **139**, e20163908.
2. **Abe K, Yuki S, Kogure K** (1988): *Strong attenuation of ischemic and postischemic brain edema in rats by a novel free radical scavenger*. Stroke, **19**, 480–5.
3. **Aebi H** (1984): *Catalase in vitro*. Methods Enzymol, **105**, 121–126.
4. **Agresti C, Mechelli R, Olla S, et al** (2020): *Oxidative status in multiple sclerosis and off-targets of antioxidants: the case of edaravone.* Curr Med Chem, **27**, 2095–2105.
5. **Alshogran OY, Aseel YAT, Shreen DN** (2022): *Exploring the effects of edaravone in rats with contrast-induced acute kidney injury*. Life Sci, **309**, 121006.
6. **Aviram M, Hardak E, Vaya J, et al** (2000): *Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON 1 esterase and peroxidase-like activities.* Circulation, **101**, 2510–2517.
7. **Baldissera MD, Souza CF, Santos RCV, et al** (2018): *Blood–brain barrier breakdown and myeloperoxidase activity in silver catfish experimentally infected with Pseudomonas aeruginosa*. J Fish Dis, **41**, 209-213.
8. **Bayrak BB, Sancar S, Hacihasanoglu Cakmak N, et al** (2025): *Ameliorative effects of edaravone against valproic acid-induced kidney damage*. J Mol Histol, **56,** 4.
9. **Beghi E** (2020): *The epidemiology of epilepsy.* Neuroepidemiology, **54**, 185–191.
10. **Berlett BS, Stadtman ER** (1997): *Protein oxidation in aging, disease, and oxidative stress.* J Biol Chem, **272**, 20313-20316.
11. **Beutler E** (1975): Reduced Gutathione (GSH). 112–114. In: Bergmeyer HV (ed) Red Blood Cell Metabolism. A Manual Of Biochemical Methods, 2nd edn. Grune and Stratton, New York.
12. **Brown BL, Craycraft LK, Justice SB** (2020): *Valproic acid in the treatment of migraines.* Adv Emerg Nurs J, **42**, 243–253.
13. **Chaudhary S, Ganjoo P, Raiusddin S, et al** (2015): *Nephroprotective activities of quercetin with potential relevance to oxidative stress induced by valproic acid*. Protoplasma, **252**, 209–217.
14. **Chaudhary S, Parvez S** (2012): *An in vitro approach to assess the neurotoxicity of valproic acid-induced oxidative stress in cerebellum and cerebral cortex of young rats,* Neuroscience, **225**, 258-268.
15. **Chen YCB, Liang CS, Wang LJ, et al** (2022): *Comparative effectiveness of valproic acid in different serum concentrations for maintenance treatment of bipolar disorder: A retrospective cohort study using target trial emulation framework.* EClinMed, **54,**101678.
16. **Corte ED, Stirpe F** (1968): *Regulation of xanthine oxidase in rat liver: modifications of the enzyme activity of rat liver supernatant on storage at 20 degrees*. Biochem J, **108**, 349–351.
17. **Crook M, Tutt P** (1992): *Serum sialic acid concentration in patients with hypertriglyceridaemia showing the Frederickson's IIB phenotype.* Clin Sci, **83**, 593–595.
18. **Crook M, Haq M, Haq S, et al** (1994): *Plasma sialic acid and acute-phase proteins in patients with myocardial infarction*. Angiology, **45**, 709–715.
19. **Ellman GL, Courtney KD, Andres V, et al** (1961): *A new and rapid colorimetric determination of acetylcholinesterase activity.* Biochem Pharmacol, **7**, 88-95.
20. **Feng S, Yang Q, Liu M, et al** (2011): *Edaravone for acute ischaemic stroke.* Cochrane Database of Systematic Reviews, **12**, CD007230.
21. **Furlong CE, Richter RJ, Seidel SJ** (1988): *Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon*. Am J Hum Gen, **43**, 230–238.
22. **Gomes CV, Kaser MP, Tomé AR, et al** (2011): *Adenosine receptors and brain diseases: neuroprotection and neurodegeneration.* Biochim Biophys Acta, **1808**, 1380-1399.
23. **Graf W, Oleinik O, Glauser T, et al** (1998): *Altered antioxidant enzyme activities in children with a serious adverse experience related to valproic acid therapy.* Neuropediatrics, **29**, 195–201.
24. **Gunata M, Parlakpinar H, Acet HA** (2020): *Melatonin: A review of its potential functions and effects on neurological diseases.* Rev Neurol, **176**, 148–65.
25. **Habig WH, Jakoby WB** (1981): *Assays for differentation of glutathione-S-transferases.* Methods Enzymol, **77**, 398-405.

26. **Hacihasanoglu Cakmak N, Yanardag R** (2015): *Edaravone, a free radical scavenger, protects liver against valproic acid induced toxicity.* J Serb Chem Soc, **80**, 627–637.
27. **Hassan MQ, Akhtar MS, Akhtar M, et al** (2015): *Edaravone protects rats against oxidative stress and apoptosis in experimentally induced myocardial infarction: Biochemical and ultrastructural evidence.* Redox Rep, **20**, 275–81.
28. **Hassan MQ, Akhtar MS, Afzal O, et al** (2020): *Edaravone and benidipine protect myocardial damage by regulating mitochondrial stress, apoptosis signalling and cardiac biomarkers against doxorubicin-induced cardiotoxicity*. Clin Exp Hypertens, **42**, 381-392.
29. **Heidari R, Jafari F, Khodaei F, et al** (2018): *Mechanism of valproic acid-induced Fanconi syndrome involves mitochondrial dysfunction and oxidative stress in rat kidney*. Nephrol, **23**, 351–361.
30. **Işık M, Demir Y, Kırıcı M, et al** (2015): *Changes in the anti-oxidant system in adult epilepsy patients receiving anti-epileptic drugs.* Arch Physiol Biochem, **121**, 97-102.
31. **Jafarian I, Eskandari MR, Mashayekhi V, et al** (2013): *Toxicity of valproic acid in isolated rat liver mitochondria*. Toxicol Mech Methods, **23**, 617-623.
32. **Jiao SS, Yao XQ, Liu YH, et al** (2015): *Edaravone alleviates Alzheimer's diseasetype pathologies and cognitive deficits.* Proc Natl Acad Sci U S A, **112**, 5225–5230.
33. **Jin DQ, Li G, Kim JS, et al** (2004): *Preventive effects of laminaria japonica aqueous extract on the oxidative stress and xanthine oxidase activity in streptozotocin-induced diabetic rat liver*. Biol Pharm Bull, **27**,1037–1040.
34. **Johnson EL** (2019): *Seizures and epilepsy*. Med Clin North Am, **103**, 309–324.
35. **Karker H** (1964): *Method for estimation of serum adenosine deaminase.* Scand J Clin Lab Invest, **16**, 570-574.
36. **Katyayan A, Diaz-Medina G (2021):** *Epilepsy: Epileptic syndromes and treatment.* Neurol Clin, **39**, 779–795.
37. **Kawasaki T, Kitao T, Nakagawa K, et al** (2007): *Nitric oxide-induced apoptosis incultured rat astrocytes: protection by edaravone, a radical scavenger*. Glia, **55**, 1325-1333.
38. **Kettle AJ, Winterbourn CC** (1997): *Myeloperoxidase: a key regulator of neutrophil oxidant production*. Redox Rep, **3,** 3-15.
39. **Kikuchi K, Tancharoen S, Takeshige N, et al** (2013): *The efficacy of edaravone (radicut), a free radical scavenger, for cardiovascular disease.* Int J Mol Sci, **14**,13909–13930.
40. **Kikuchi K, Uchikado H, Miyagi N, et al** (2011): *Beyond neurological disease: new targets for edaravone.* Int J Mol Med, **28**, 899–906.
41. **Kikuchi K, Takeshige N, Miura N, et al** (2012): *Beyond free radical scavenging: beneficial effects of edaravone (Radicut) in various diseases (review).* Exp Ther Med, **3**, 3–8.
42. **Kumar H, Sharma B** (2016): *Memantine ameliorates autistic behavior, biochemistry & blood brain barrier impairments in rats.* Brain Res Bull, **124**, 27-39.
43. **Ledwozyw A, Michalak J, Stephien A, et al** (1986): *The relationship plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis*. Clin Chim Acta, **155**, 275–284.
44. **Lehmann M, Regland B, Blennow K, et al** (2003): *Vitamin B12-B6-folate treatment improves blood-brain barrier function in patients with hyperhomocysteinaemia and mild cognitive impairment.* Dement Geriatr Cogn Disord, **16**, 145-150.
45. **Levine RL, Garland D, Oliver CN, et al** (1990): *Determination of carbonyl content in oxidatively modified proteins*. Methods Enzymol, **186**, 464-478.
46. **Lowry OH, Rosebrough NJ, Farr AL, et al** (1951): *Protein measurement with the Folin phenol reagent.* J Biol Chem, **193**, 265– 275.
47. **Lu XY, Meng-Qing L, Yong-Tao L, et al** (2024): *Oral edaravone ameliorates behavioral deficits and pathologies in a valproic acid-induced rat model of autism spectrum disorder*. Neuropharmacology, **258**, 110089.
48. **Meister A** (1983): *Selective modification of glutathione metabolism*. Science, **220**, 472–477.
49. **Mylroie AA, Collins H, Umbles C, et al** (1986): *Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate*. Toxicol Appl Pharmacol, **82**, 512–520.
50. **Natori T, Kodaira F, Hirasawa T, et al** (2008): *Augmentation of polysialic acid by valproic acid in early postnatal mouse hippocampus and primary cultured hippocampal neurons*. J Biosci Bioeng, **105**, 164-167.
51. **Navab M, Hama-Levy S, Van Lenten BJ, et al** (1997): *Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio*. J Clin Invest, **99**, 2005–2019.
52. **Nematollah A, Maloos N, Abdolali N, et al** (2017): *Zinc deficiency and oxidative stress involved in valproic acid induced hepatotoxicity: Protection by zinc and selenium supplementation*. Biol Trace Elem Res, **179**, 102–109.
53. **Nermin E, Sheikh A, Petrilla J, et al** (2020): *The dual-active histamine h3 receptor antagonist and acetylcholine esterase ınhibitor e100 alleviates autistic-like behaviors and oxidative stress in valproic acid induced autism in mice*. Int J Mol Sci, **21**, 3996.
54. **Nishi H, Watanabe T, Sakurai H, et al** (1989): *Effect of MCI-186 on brain edema in rats.* Stroke, **20**, 1236–40.
55. **Okatani Y, Wakatsuki A, Enzan H, et al** (2003): *Edaravone protects against ischemia/reperfusion-induced oxidative damage to mitochondria in rat liver*. Eur J Pharmacol, **465**, 163-170.
56. **Oktay NŞ, Alev Tüzüner B, Tunalı S, et al** (2024): *Potential protective role of radicut in valproic acid-induced oxidative stress in rat spleen.* Igusabder, **22**, 308-319.
57. **Oktay S, Alev-Tüzüner B, Tunalı S, et al** (2017): *Investigation of the effects of edaravone on valproic acid induced tissue damage in pancreas.* Marmara Pharm J, **21**, 570–577.
58. **Phyu MP, Tangpong J** (2014): *Neuroprotective effects of xanthone derivative of Garcinia mangostana against lead-induced acetylcholinesterase dysfunction and cognitive impairment.* Food Chem Toxicol, **70**, 151-156.
59. **Reed DJ** (1990): *Glutathione: Toxicological implications*. Annu Rev Pharmacol Toxicol, **30**, 603–31.
60. **Rodriguez C, Mayo JC, Sainz RM, et al** (2004): *Regulation of antioxidant enzymes: A significant role for melatonin*. J Pineal Res, **36**,1–9.
61. **Romoli M, Mazzocchetti P, D'Alonzo R, et al** (2019): *Valproic acid and epilepsy: from molecular mechanisms to clinical evidences*. Curr Neuropharmacol, **17**, 926–946.

62. **Safdar A, Ismail F** (2023): *A comprehensive review on pharmacological applications and drug-induced toxicity of valproic acid.* Saudi Pharm J, **31**, 265–278.
63. **Sanjiv S, Abhishek K** (2019): *Protective effect of edaravone on cyclophosphamide ınduced oxidative stress and neurotoxicity in rats*. Curr Drug Saf, **14**, 209-216.
64. **Sari RA, Tays S, Yilmaz O, et al** (2003): *Correlation of serum levels of adenosine deaminase activity and its isoenzymes with disease activity in rheumatoid arthritis.* Clin Exp Rheumatol, **21,** 87-90.
65. **Seyedinia SA, Tarahomi P, Abbarin D, et al** (2023): *Saffron and crocin ameliorate prenatal valproic acid-induced autistic-like behaviors and brain oxidative stress in the male offspring rats.* Metab Brain Dis, **38**, 2231–2241.
66. **Shen Y, Zhang J, Xu Y, et al** (2023): *Ultrasound-enhanced brain delivery of edaravone provides additive amelioration on disease progression in an ALS mouse model.* Brain Stimul, **16**, 628–641.
67. **Sokmen BB, Tunali S, Yanardag R** (2012): *Effects of vitamin U (S-methyl methionine sulphonium chloride) on valproic acid induced liver injury in rats.* Food Chem Toxicol, **50**, 3562-3566.
68. **Stadtman ER, Levine RL** (2000): *Protein oxidation*. Ann N Y Acad Sci, **899,** 191-208.
69. **Tarazi FI, Sahli ZT, Wolny M, et al** (2014): *Emerging therapies for Parkinson's disease: from bench to bedside.* Pharmacol Ther, **144**, 123–133.
70. **Taş S, Sarandöl E, Dirican M** (2014): *Vitamin B6 supplementation ımproves oxidative stress and enhances serum paraoxonase/arylesterase activities in streptozotocin-ınduced diabetic rats.* Sci World J, 351598.
71. **Thimmulappa RK, Chattopadhyay I, Rajasekaran S** (2019): Oxidative Stress Mechanism In The Pathogenesis Of Environmental Lung Diseases. 103-137. Oxidative Stress in Lung Diseases. Springer Nature. Singapore.
72. **Tunali S, Cimen ES, Yanardag R** (2020): *The effects of chard on brain damage in valproic acid-induced toxicity*. J Food Biochem, **44**, e13382.
73. **Tunali S, Kahraman S, Yanardag R** (2015): *Vitamin U, a novel free radical scavenger, prevents lens injury in rats administered with valproic acid*. Hum Exp Toxicol, **34**, 904-910.
74. **Turkyilmaz B, Altas N, Arisan I, et al** (2021): *Effect of vitamin B6 on brain damage in valproic acid induced toxicity*. J Biochem Mol Toxicol, **35**, e22855.
75. **Turkyilmaz IB, Sokmen BB, Yanardag R** (2020): *Alpha-lipoic acid prevents brain injury in rats administered with valproic acid*. J Biochem Mol Toxicol, **34**, e22580.
76. **Türkyılmaz IB, Karatuğ Kaçar A, Bolkent Ş, et al** (2023): *Damage to liver tissue caused by valproic acid used for treating epilepsy: Protective effects of vitamin B6*. Arch Epilepsy, **29**, 9-15.
77. **Warren L** (1959): *The thiobarbituric acid assay of sialic acids*. J Biol Chem, **234**, 1971-1975.
78. **Wei H, Frenkel K** (1991): *In vivo formation of oxidized DNA bases in tumor promoter-treated mouse skin*. Cancer Res, **51**, 4443-4449.
79. **Wendel A** (1981): *Glutathione peroxidase*. Methods Enzymol, **77**, 325.

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