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The effects of edaravone, a free-radical scavenger in lung injury induced by valproic acid demonstrated via different biochemical parameters

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Abstract

In this study, we aimed to evaluate whether edaravone (EDA) has a protective role against valproic acid (VPA)-induced lung damage via its antioxidative activity. Male Sprague–Dawley rats were split into four groups. Control ($n = 8$) rats; rats given EDA (30 mg kg^{-1} day^{-1}; $n = 10$); rats given only (VPA, 500 mg kg^{-1} day^{-1}; $n = 10$); rats given VPA + EDA (in the same dose and time) for 7 days. EDA and VPA were applied intraperitoneally. After 8 days, lung tissues were immediately taken from the rats. In lung homogenates, reduced glutathione, total antioxidant status levels, and superoxide dismutase, glutathione peroxidase, sodium/potassium ATPase, paraoxonase1, and carbonic anhydrase activities significantly abated, whereas catalase, glutathione reductase, glutathione-S-transferase activities insignificantly decreased in the VPA-treated group. In contrast, lipid peroxidation, reactive oxygen species, and total oxidant status levels, glycoprotein and protein carbonyl contents, nitric oxide, hydroxyproline levels, and xanthine oxidase, lactate dehydrogenase, arginase, and prolidase activities significantly increased in the VPA-given group. Administration of EDA caused the reverse effects. As a consequence, EDA prevented oxidative stress-mediated lung injury via its robust antioxidant effects.

KEYWORDS
n-dipropyl acetic acid, lung damage, oxidative stress, synthetic antioxidant

1 | INTRODUCTION

Valproic acid (n-dipropyl acetic acid, VPA), branched short-chain fatty acid, is a derivative of pentanoic acid. VPA is mainly prescribed in the treatment of epilepsy, generalized and partial seizures, and for use as a mood stabilizer. Also, VPA is applied in migraines, bipolar, anxiety, mental illness, and Parkinson's disease.[1] Moreover, the drug is commonly used in pediatric patients with epilepsy.[2] Although VPA has been applied in the medication of epilepsy and related disorders for more than 50 years, the presence of accumulated evidence demonstrates that VPA has several side/adverse effects on several tissues such as the brain,[3] liver,[4] pancreas,[5] gastrointestinal system,[6] testis,[7] and kidney.[8] In fact, it has been stated that VPA monotherapy in migraine treatment in women and girls of childbearing age causes multiple teratogenic effects.[9,10] In addition, the use of VPA in certain types of seizures may cause unwanted effects on the hematological system such as platelet dysfunction and thrombocytopenia, which then rarely induce diffuse alveolar hemorrhage in humans.[11] Therapeutic doses of VPA in bipolar disorder can also cause pericardial and pleural effusion.[12] A recent study shows that VPA-mediated oxidative stress can give rise to structural distortion and fibrotic alterations in the lung of rats. This situation is sourced by nuclear factor-erythroid-2-related factor (Nrf2) expression insufficiency which leads to pulmonary oxidant/antioxidant imbalance.[13] Due to being critical for the regulation of antioxidants and phase II detoxification enzymes, the Nrf2,

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and the antioxidant response element signaling pathway are important defensive pathways.[14]

As free radicals are reactive and unstable molecules that have unpaired electrons in their outer orbit, they easily attack other molecules such as proteins, carbohydrates, lipids, and nucleic acids. These free radicals are continuously produced in cells, they are called reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well.[15] The production and elimination of ROS and RNS are strictly well-balanced by the complex antioxidant system, which includes reduction-oxidation enzymatic systems, nonenzymatic scavengers, and dietary components.[16] The decline in the levels of antioxidant molecules and/or the elevation of oxidant (ROS and RNS) levels causes disruption of the balance mentioned above.[17] However, this imbalance usually results in oxidative stress in cells, which adversely affects many tissues such as the lungs.[18] In contrast, excessive oxidative stress may be associated with amplification of inflammatory response, thereby causing alveolar cell apoptosis in chronic obstructive pulmonary disease (COPD). Besides this, in patients with COPD, Nrf2 expression/activation is diminished. This is due to anti-inflammatory effect of Nrf2 whose ability is related to inhibit the expression of proinflammatory cytokines, like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).[19] Investigation of chemically induced lung injury, as well as the potential protective role of both natural products and synthetic molecules, has been of great interest to researchers. [20–22]

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, EDA; Radicut®) is a highly efficient, powerful, and effective radical scavenger. After being synthesized in Japan about 20 years ago, it has been a clinically prescribed drug that is used in treatment for amyotrophic lateral sclerosis and cerebral infarction and shown to prevent oxidative stress in many tissues.[23] The reason for its potent role against oxidative stress is the neutralization of free radicals such as hydroxyl radicals and peroxynitrite radicals.[24] However, it is frequently used in cardiovascular, cerebrovascular, gastrointestinal, endocrine, and respiratory diseases.[25] Also, it has been shown that EDA possesses not only antinecrotic, antiapoptotic, anti-inflammatory activities but also protection against oxidative stress-mediated by various agents in the lungs.[26] Anti-inflammatory effects of EDA have been attributed to its ability to suppress nuclear factor-kappa B (NF-κB) activation and cyclo-oxygenase-2 (COX-2) expression.[27] Likewise, EDA has been shown to attenuate experimental asthma in mice through induction of hemeoxygenase-1 (HO-1) and kelch-like ECH-associated protein-1 (Keap1)/Nrf2 pathway.[14] Additionally, it has been reported to reduce cyclophosphamide-induced oxidative stress by upregulating Nrf2 expression in rats.[28]

On the basis of the information mentioned above, this study was planned to examine whether EDA had a potential protective role against VPA-induced oxidative lung injury.

2 | MATERIALS AND METHODS

2.1 | Chemicals

The VPA and EDA were purchased for Merck (catalog numbers 8.14439 and 8.07080, respectively). All chemicals were of the purest grade commercially available.

2.2 | Animals and experimental design

As described in Figure 1, male Sprague–Dawley rats, 2.5–3 months old and clinically healthy, were chosen for this study. All the experimental procedures were approved by the Istanbul University Local Ethics Committee on Animal Research (no. 2010/54). Animals were randomly split into four groups as follows: Group I ($n = 8$) was control rats. Group II ($n = 10$) was control animals applied only EDA (30 mg kg^{-1} day^{-1})[29] for 7 days. Group III ($n = 10$) was injected only VPA (500 mg kg^{-1} day^{-1})[30] for 7 days and lastly, Group IV ($n = 10$) was applied EDA 2 h after the administration of VPA for 7 days (at the same time and dose). EDA and VPA were injected intraperitoneally. After 8 days of the experiment, lung tissues were taken for biochemical investigations. Lung tissues were immediately washed with cold 0.9% NaCl and frozen at −86°C for further assessment on the day of the experiments.

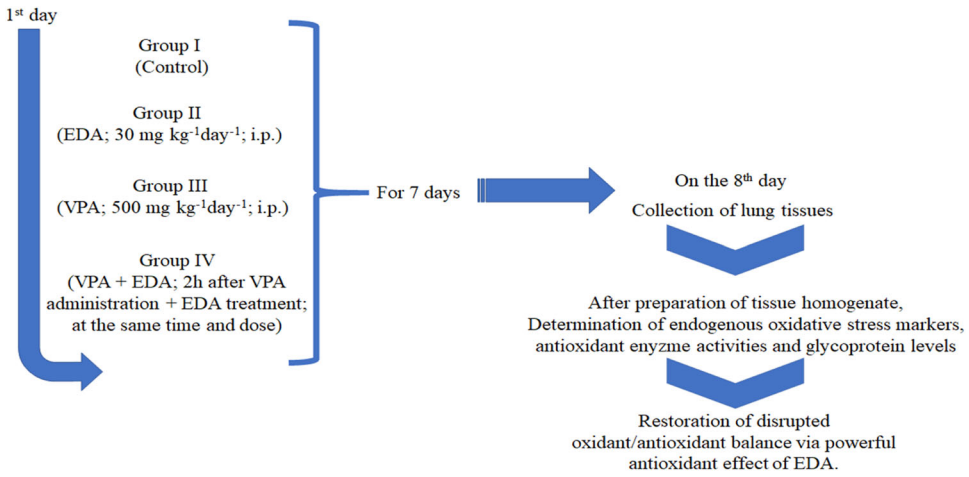


FIGURE 1 Schematic representation of the experimental design. EDA, edaravone; VPA, valproic acid

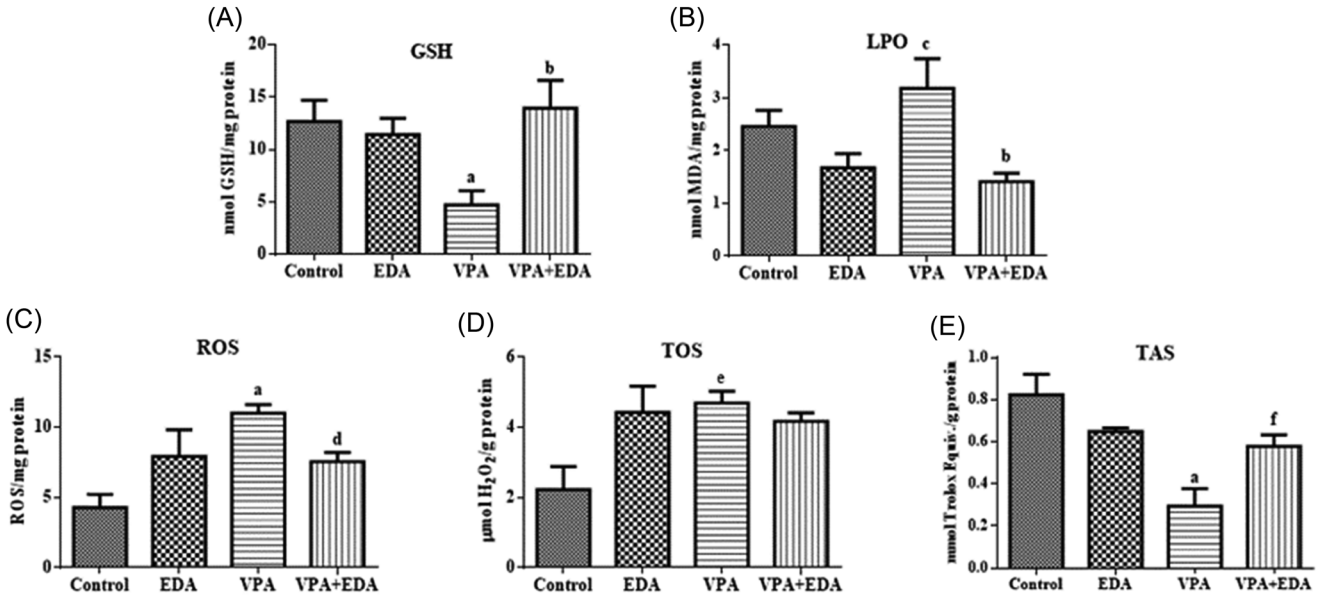


FIGURE 2 Effect of edaravone on lung tissue reduced glutathione, lipid peroxidation, reactive oxygen species, total oxidant, and total antioxidant status levels of control and experimental groups of rats. (A) GSH, (B) LPO, (C) ROS; (D) TOS; (E) TAS. All data are presented as mean ± SD. Control animals (n = 8); EDA (n = 10) rats injected only EDA (30 mg kg^{-1} day^{-1}) for 7 days; VPA (n = 10) rats injected only VPA (500 mg kg^{-1} day^{-1}) for 7 days; VPA + EDA (n = 10) rats applied EDA 2 h after the administration of VPA for 7 days (at the same time and dose). EDA, edaravone; GSH, reduced glutathione; LPO, lipid peroxidation; MDA, malondialdehyde; ROS, reactive oxygen species, TAS, total antioxidant status; TOS, total oxidant status; VPA, valproic acid. $^{a}p < 0.0001$, $^{c}p < 0.05$, and $^{e}p < 0.01$ versus control; $^{b}p < 0.0001$, $^{d}p < 0.05$, and $^{f}p < 0.001$ versus VPA

2.3 | Biochemical analyses

Lung tissues were homogenized in cold 0.9% NaCl with glass equipment to obtain 10% (w/v) homogenate. These homogenates were centrifuged at 10,000*g* at 4°C for 10 min and clear supernatants were used for biochemical assays. Lung tissues reduced glutathione (GSH) and lipid peroxidation (LPO) levels were determined by the methods published previously by Beutler[31] using Ellman's reagent and Ledwozyw et al.,[32] respectively. Reactive oxygen species (ROS), total oxidant status (TOS), and total antioxidant status (TAS) levels were estimated by the methods published previously by Zhang et al.[33] and Erel,[34,35] respectively. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) activities were done according to Aebi,[36] Mylroie et al.,[37] and Paglia and Valentine,[38] and modified by Wendel,[39] Beutler,[40] and Habig and Jakoby,[41] respectively. Sodium/potassium ATPase (Na^+/K^+-ATPase), xanthine oxidase (XO), lactate dehydrogenase (LDH), paraoxonase1 (PON1), and carbonic anhydrase (CA) activities and protein carbonyl (PC) levels were evaluated according to methods published previously by Ridderstap and Bonting,[42] Corte and Stirpe,[43] Wroblewski,[44] Furlong et al.,[45] Verpoorte et al.,[46] and Levine et al.,[47] respectively. Winzler's method[48] was used to assess hexosamine and hexose levels. Fucose levels were determined according to the method reported by Zacharias and Shettles.[49] Nitric oxide (NO), hydroxyproline (Hyp) levels, arginase, and prolidase activities were estimated according to Miranda et al.,[50] Reddy and Enwemeka,[51] Geyer and Dabich,[52] and Chinard,[53] respectively. Protein content in the supernatants was determined using Lowry's method[54] with bovine serum albumin as standard.

2.4 | Statistical analyses

All data were expressed as mean ± SD. The results were assessed using an unpaired *t-test* and analysis of variance (ANOVA) followed by Tukey's multiple comparisons posthoc tests using GraphPad Prism 6.0 statistics software. Values of $p < 0.05$ were regarded as significant.

3 | RESULTS

3.1 | Effects of EDA on lung tissue oxidative stress markers

Lung tissue GSH, LPO, ROS, TOS, and TAS levels are depicted in Figure 2. In the VPA group, GSH levels were remarkably lower than that of control rats ($p < 0.0001$). In contrast, LPO levels in the VPA-treated group were remarkably elevated as compared with the control group ($p < 0.05$). Application of EDA to the VPA group significantly reversed both GSH and LPO levels, respectively ($p < 0.0001$) (Figures 2A and 2B). A notable increase in the levels of both ROS ($p < 0.0001$) and TOS ($p < 0.01$) in the VPA-given group as compared with control rats was detected. A considerable decline in ROS levels ($p < 0.05$), and an insignificant decrease in TOS levels were observed when VPA-given rats were treated with EDA. In contrast, an obvious decline in TAS levels was seen in VPA-given rats when compared with control rats ($p < 0.0001$). EDA administration to VPA rats brought about a notable increase in TAS levels ($p < 0.001$) (Figure 2C–E).

3.2 | Effects of EDA on endogenous antioxidant enzymes

Lung tissue CAT, SOD, GPx, GR, and GST activities are presented in Table 1. CAT and GR activities insignificantly abated in VPA-given rats as compared with control rats. Although SOD and GPx activities notably declined when compared with control rats ($p < 0.001$ and $p < 0.0001$, respectively), GST activities were not altered in the VPA group. Inversely, EDA treated to VPA group gave rise to an obvious elevation of the CAT, SOD, GPx, GR, and GST activities ($p < 0.0001$ and $p < 0.05$, respectively) (Table 1).

3.3 | Effects of EDA on lung tissue Na^+/K^+-ATPase, XO, LDH, PON1, and CA activities and PC levels

Lung tissues Na^+/K^+-ATPase, XO, LDH, PON1, and CA activities and PC levels are shown in Table 2. As seen in Table 2, it is detected that Na^+/K^+-ATPase, PON1, and CA activities remarkably declined in VPA-treated rats as compared with control rats ($p < 0.001$ and $p < 0.01$, respectively). In contrast, XO and LDH activities and PC levels in the VPA group were notably elevated when compared to the control group ($p < 0.01$ and $p < 0.0001$, respectively). Treatment with EDA to the VPA group caused recovery of these alterations ($p < 0.0001$, $p < 0.01$, and $p < 0.05$, respectively) (Table 2).

3.4 | Effects of EDA on lung tissue glycoprotein levels

Hexosamine, hexose, and fucose levels (glycoprotein components) in lung tissues are presented in Figure 3. As seen in the VPA-given group, there was a statistically significant increase in hexosamine ($p < 0.01$), hexose ($p < 0.001$), and fucose ($p < 0.05$) levels in lung tissues than that of control rats. Conversely, application of EDA with VPA reverted these alterations of hexosamine ($p < 0.05$), hexose ($p < 0.05$), and fucose levels ($p < 0.01$) in lung tissues, respectively (Figure 3A–C).

3.5 | Effects of EDA on lung tissue NO and Hyp levels as well as arginase and prolidase activities

Levels of NO and Hyp and activities of arginase and prolidase are presented in Figure 4. In the VPA-given rats, there was a remarkable elevation of both NO and Hyp levels compared with the control group ($p < 0.0001$) (Figure 4A, B). Besides this, activities of arginase and prolidase in the VPA group were remarkably elevated as compared with control rats ($p < 0.0001$ and $p < 0.001$) (Figure 4C, D). Treatment of the VPA group with EDA resulted not only in an obvious decline in NO and Hyp levels ($p < 0.0001$) (Figure 4A, B) but also a decline in arginase and prolidase activities ($p < 0.001$) (Figure 4C, D).

4 | DISCUSSION

Due to the antiepileptic effect, VPA has been used for the treatment of psychiatric conditions such as epilepsy and bipolar disorder since 1975.[55] Although VPA is well tolerated and mainly eliminated by the liver, there have been considerable studies (both clinically and experimentally), showing that it has side effects on various tissues, especially on the liver[56] and kidneys.[8,57] Nevertheless, limited studies on the unwanted effects of VPA-induced lung injury exist.[11,12] In contrast, Oztay et al.[13] put forward that VPA injection at a dose of 500 mg kg^{-1} day^{-1} caused lung toxicity via alterations of both biochemical and inflammatory markers. EDA is as effective an antioxidant as natural ones such as glutathione.[58] Due to the effective antioxidant and possible protective role of EDA, we aimed to investigate whether EDA has protective and ameliorative effects on VPA-induced oxidative lung injury in rats.

The reduced GSH, an intracellular free sulfhydryl containing tripeptide, scavenges oxygen-centered free radicals (known as ROS)

TABLE 1 Lung tissue CAT, SOD, GPx, GR, and GST activities of control and experimental groups of rats

Groups	CAT (U/mg protein)	SOD (U/g protein)	GPx (U/g protein)	GR (U/g protein)	GST (U/g protein)
Control	77.40 ± 4.97	7.59 ± 1.76	142.60 ± 24.84	3.98 ± 0.36	17.64 ± 5.94
Control + EDA	70.60 ± 8.52	5.93 ± 1.44	122.50 ± 12.71	2.82 ± 0.86	19.33 ± 2.26
VPA	71.41 ± 4.34	3.62 ± 0.51*	67.44 ± 6.91**	3.03 ± 0.11	17.63 ± 5.01
VPA + EDA	102.70 ± 4.22***	5.75 ± 0.56****	139.90 ± 6.89***	5.68 ± 0.89***	48.39 ± 3.98***
p_{ANOVA}	0.0001	0.0004	0.0001	0.0001	0.0001

Note: All data are presented as mean ± SD. Control animals ($n = 8$); EDA ($n = 10$), rats injected only EDA (30 mg kg^{-1} day^{-1}) for 7 days; VPA ($n = 10$), rats injected only VPA (500 mg kg^{-1} day^{-1}) for 7 days; VPA + EDA ($n = 10$), rats applied EDA 2 h after the administration of VPA for 7 days (at the same time and dose).

Abbreviation: ANOVA, analysis of variance; CAT, catalase; EDA, edaravone; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase; SOD, superoxide dismutase; VPA, valproic acid.

*$p < 0.001$; **$p < 0.0001$ versus control; ***$p < 0.0001$; ****$p < 0.05$ versus VPA.

TABLE 2 Lung tissue Na^+/K^+-ATPase, XO, LDH, PON1, and CA activities and PC levels of control and experimental groups of rats

Groups	Na^+/K^+-ATPase (nmol P_i/mg protein/h)	XO (U/g protein)	LDH (U/mg protein)	PON1 (U/g protein)	CA (U/mg protein)	PC (nmol/mg protein)
Control	6.95 ± 0.80	1.38 ± 0.28	1.50 ± 0.0.37	30.29 ± 4.85	97.63 ± 11.56	1.47 ± 0.34
Control + EDA	7.32 ± 1.48	1.42 ± 0.82	0.62 ± 0.24	18.23 ± 2.73	62.74 ± 14.21	1.01 ± 0.31
VPA	2.52 ± 0.65*	3.22 ± 0.82**	11.99 ± 1.40***	17.43 ± 1.56**	61.22 ± 7.35*	3.22 ± 0.78***
VPA + EDA	9.87 ± 1.40****	1.16 ± 0.59*****	1.08 ± 0.24****	26.05 ± 4.01******	106.60 ± 3.71****	0.64 ± 0.18****
p_{ANOVA}	0.0001	0.0043	0.0001	0.0003	0.0001	0.0001

Note: All data are presented as mean ± SD. Control animals ($n = 8$); EDA ($n = 10$), rats injected only EDA (30 mg kg^{-1} day^{-1}) for 7 days; VPA ($n = 10$), rats injected only VPA (500 mg kg^{-1} day^{-1}) for 7 days; VPA + EDA ($n = 10$), rats applied EDA 2 h after the administration of VPA for 7 days (at the same time and dose).

Abbreviations: ANOVA, analysis of variance; CA, carbonic anhydrase; EDA, edaravone; LDH, lactate dehydrogenase; Na^+/K^+-ATPase, sodium/potassium ATPase; PC, protein carbonyl; PON1, paraoxonase1; VPA, valproic acid; XO, xanthine oxidase.

*$p < 0.001$; **$p < 0.01$; ***$p < 0.0001$ versus control; ****$p < 0.0001$; *****$p < 0.01$; ******$p < 0.05$ versus VPA.

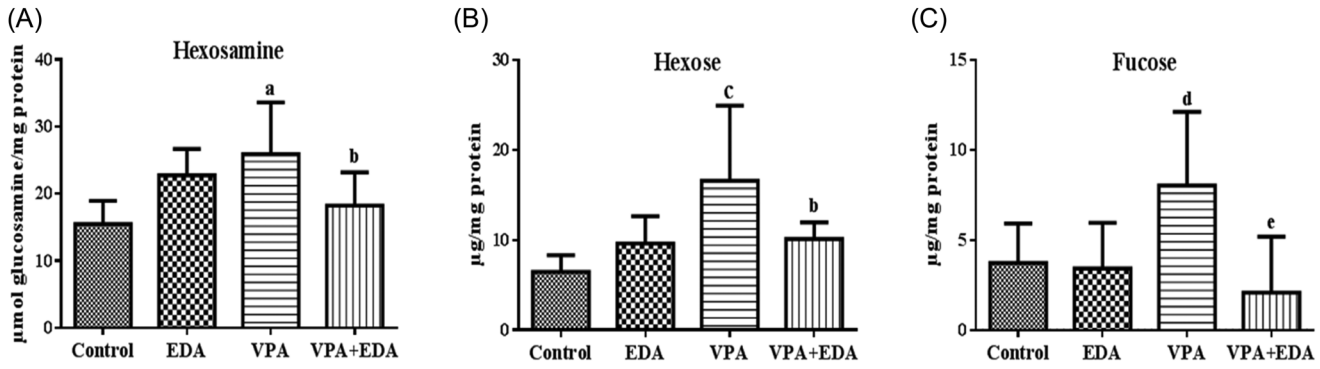


FIGURE 3 Effect of edaravone on lung tissue glycoprotein levels of control and experimental groups of rats. (A) Hexosamine, (B) hexose, and (C) fucose. All data are presented as mean ± SD. Control animals ($n = 8$); EDA (n = 10), rats injected only EDA (30 mg kg^{-1} day^{-1}) for 7 days; VPA ($n = 10$), rats injected only VPA (500 mg kg^{-1} day^{-1}) for 7 days; VPA + EDA ($n = 10$), rats applied EDA 2 h after the administration of VPA for 7 days (at the same time and dose). EDA, edaravone; VPA, valproic acid. $^a p < 0.01$, $^c p < 0.001$, and $^d p < 0.05$ versus control; $^b p < 0.05$, and $^e p < 0.01$ versus VPA

including hydroxyl, hypochlorous, and lipid peroxyl radicals, which is also essential for GSH-related enzymatic antioxidant defenses. Malondialdehyde (MDA), as an index of LPO, is a parameter that is often measured in pathogenic processes associated with oxidative stress.[59] Therefore, we determined both GSH and LPO levels in homogenates of VPA-induced oxidative lung damage. In this study, GSH levels obviously decreased, while LPO levels were notably elevated in VPA-given rats when compared with control rats. The diminishment of GSH and an increase in LPO levels may be due to the toxic mechanism of VPA metabolism, which resulted in an imbalance of antioxidant defense and ROS generation.[60] Similar results have been obtained by other studies, which indicated that VPA causes oxidative stress on various tissues.[55] In the present study, administration of EDA to VPA groups reversed the alterations of both GSH and LPO levels. Restoration of GSH levels may be associated with the preventive effect of EDA. It is reported that the therapeutic administration of EDA on acute pancreatitis-associated lung injury caused a notable decrease in LPO levels.[61] EDA probably showed a protective role through its free-radical scavenging effects.[24] This effect of EDA may be due to protecting cells from oxidative stress by regulating the antioxidant GSH pathway.[62]

To determine the degree of cellular and/or tissue damage caused by oxidative stress, it is necessary to measure the antioxidant response to both biological fluids and tissue. However, besides measuring the free radicals and other parameters in the antioxidant defense system, determination of the total oxidant and antioxidant status has been important to express the cumulative effect of oxidative stress in the organism.[34,63] An elevation of the formation of ROS and RNS in the lungs is effective in modulating redox signals. Therefore, they cause irreversible damage to highly important biomolecules such as enzymes, lipids, carbohydrates, and DNA, and initiate various pathogenic cellular processes as well.[59] For these reasons, we focused on determining ROS, TOS, and TAS levels in lung tissue. In our study, both ROS and TOS levels were notably elevated, whereas TAS levels remarkably decreased in the VPA group. Reports indicate that TOS and TAS levels are similarly altered in pediatric seizure patients taking VPA monotherapy for 60 days.[64] Treatment with EDA restored ROS, TOS, and TAS levels by regulating the

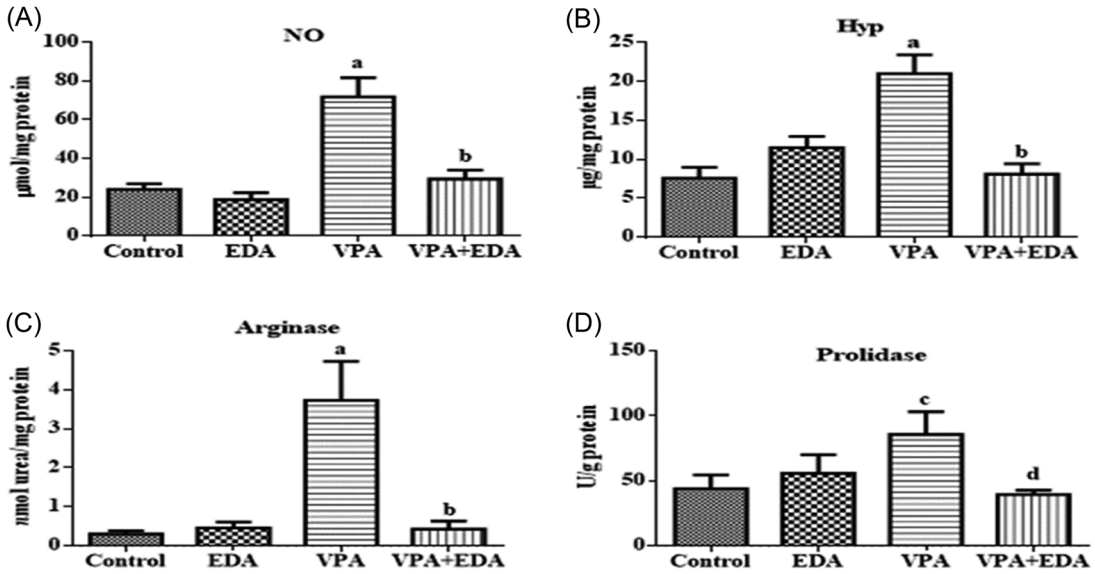


FIGURE 4 Effect of edaravone on lung tissue nitric oxide and hydroxyproline levels and arginase and prolidase activities of control and experimental groups of rats. (A) NO, (B) Hyp, (C) arginase; (D) prolidase. All data are presented as mean ± SD. Control animals (n = 8); EDA (n = 10) rats injected only EDA (30 mg kg^{-1} day^{-1}) for 7 days; VPA (n = 10) rats injected only VPA (500 mg kg^{-1} day^{-1}) for 7 days; VPA + EDA (n = 10) rats applied EDA 2 h after the administration of VPA for 7 days (at the same time and dose). EDA, edaravone; Hyp, hydroxyproline; NO, nitric oxide; VPA, valproic acid. $^{a}p < 0.0001$ and $^{c}p < 0.001$ versus control; $^{b}p < 0.0001$, and $^{d}p < 0.001$ versus VPA

oxidant-antioxidant status of lung tissue, which is a result of EDA's strong antioxidant effect.[65]

Redox status of cells is kept in balance by the complex intracellular antioxidant defense system, including endogenous antioxidant enzymes (i.e., CAT, SOD, GPx, GR, and GST), which protect the cells from the deleterious effects of free radicals. When the oxidant-antioxidant balance is disturbed in favor of free radicals, alterations of the activities of these antioxidant enzymes occur.[16] In the present study, except for GST, all of the other endogenous enzyme activities declined in VPA-induced oxidative lung injury. Decreased activities of CAT, SOD, and GPx enzymes were reported by Vidya and Subramanian[66] who conducted research on the effects of valproate-sodium salt (SVP) on the liver and kidney. In another study, Oktay et al.[67] revealed that VPA-induced pancreatic damage causes a decline in the activities of the same enzymes mentioned above. El-Shenawy and Hamza[68] reported that activities of CAT, SOD, glutathione-related enzymes declined when rats were treated with different doses of SVP. Our findings are in agreement with those Oztay et al.[69] who noted that VPA led to a decrease in antioxidant enzyme activities by inducing Nrf2 expression in rat lungs. In the present study, EDA treatment to VPA-given rats resulted in reversal effects. The reason for the restoration of CAT and SOD activities may be due to the decrease in the number of free radicals as a result of the antioxidant potential of EDA.[70] The recovered activities of GPx and GR may also be associated with the increment of GSH levels after EDA treatment, which may have retarded the excessive formation of ROS. In a study conducted on paraquat-induced lung injury, it was shown that EDA treatment caused inhibition of oxidative stress through decreasing intracellular ROS and LPO status, as well as elevation of SOD activities in human type II alveolar epithelial cells.[71] In another study on lung injury and pulmonary fibrosis induced by paraquat, Zhi et al.[72] reported that EDA prevented paraquat-induced lung fibrosis by decreasing MDA levels and increased SOD and GPx levels. Taking together the improvement of endogenous antioxidants as well as enzyme activities, this effect may be due to suppressive and robust free-radical scavenger effects of EDA. It may also have contributed to the positive regulation of glutathione-related enzymes and antioxidant genes, including oxygenase, by increasing the expression of redox-sensitive Nrf2 that activates endogenous antioxidant mechanisms.[13,73]

Sodium/potassium ATPase is a multifactorial transmembrane protein complex that functions in cell adhesion and motility, and is responsible for cellular electrochemical maintenance and triggering of intracellular signaling.[74] PON1 is an enzyme that is a Ca^{2+}-dependent hydrolytic glycoprotein. Alterations of its activity are linked to various types of disease. Low PON1 activity in serum and/or tissue is related to cellular damage and tissue injury.[75] Above neutral pH, CA catalyzes the reversible hydration of gaseous CO_2 to carbonic acid, which dissociates to give hydrogen carbonate.[76] In the present study, Na^+/K^+-ATPase, PON1, and CA activities in the VPA group were lower than that of the control group. In contrast, the application of EDA to VPA-given rats recovered these alterations. The present findings were in parallel with previously published research on VPA toxicity on different tissues.[29,77,78] XO is an enzyme that has both dehydrogenase and oxidoreductase activities. In the organism, free radicals are continuously produced in normal cellular function. XO is responsible for the generation of free radicals such as superoxide anion and H_2O_2.[79] LDH is a well-known enzyme that

catalyzes the conversion of pyruvic acid to lactic acid with concomitant conversion of NADH to NAD, which occurs under hypoxic conditions.[80] PC is known as oxidative modification of carbonyl groups of L-arginine, L-lysine, L-proline, and L-threonine residues of proteins, which can cause serial disease such as lung fibrosis. Therefore, the number of carbonyl residues in the polypeptide chain may be strongly associated with oxidative tissue damage.[81] In our present study, XO and LDH activities and PC levels were remarkably elevated as a result of VPA-induced oxidative stress when compared to control rats. An increased XO activity and elevated PC levels may be the result of the toxic mechanism of VPA triggering oxidative stress in the lungs.[60,82] This may be due to oxidative stress, which could provoke activation of NF-κB that is a major inducer for classically activated proinflammatory macrophages, and COX-2 which regulates proinflammatory eicosanoids formation by promoting lung inflammation.[25] These results were consistent with previously published articles.[29,77,78] In the present study, a significant decline in both XO and LDH activities, as well as PC levels, was detected when EDA was administered to VPA-given rats. This is because EDA has a powerful scavenging effect on free radicals.[83]

Hexosamine, hexose, and fucose are important glycoproteins found in all living organisms. These glycoproteins that include carbohydrate (as sugar) moiety covalently connected to their amide part found in the extracellular matrix play an important role in tissue stabilization.[84] More so, they exhibit a crucial function in the cell membrane and surface constituents, cell-cell recognition, and cellular differentiation. Due to involvement in important membrane functions, glycoproteins are very susceptible to hazardous effects of free radicals resulted in disruption of membrane integrity.[85] In several complications of disease caused especially by oxidative stress, alterations of glycoprotein levels are frequently observed in plasma and tissues. Any alterations in the levels of glycoprotein components may be the indication of several diseases such as hepatotoxicity,[84] diabetes-associated complications,[86] pulmonary tuberculosis,[87] and lung fibrosis.[88] In the present study, glycoprotein levels were notably higher in VPA-induced oxidative stress than that of control rats. VPA administration has long been reported to cause oxidative stress.[4] An increase in glycoprotein levels in lung tissues may be associated with the increment of LPO levels as well as the decrement of antioxidant enzyme activities. Thus, causing disruption of membrane integrity. In contrast, treatment of VPA-given rats with EDA reverted the increased glycoprotein components toward near-normal levels. The decline of glycoprotein levels means that EDA may positively regulate glycoprotein metabolism which is elevated in VPA-induced oxidative stress.

Nitric oxide, a small gaseous molecule with unpaired electrons, may be called a "double-edged sword," which acts both as physiological and pathological processes. This molecule is a very reactive free radical that the arrangement of one atom of nitrogen and one atom of oxygen leaves an unpaired electron.[89] NO is also produced by isoforms of NO synthase enzymes such as inducible NOS (iNOS) in the lung airway and distal lung constriction. Under conditions of low L-arginine concentrations, it has been put forward that NO levels may indirectly be related to arginase activity in macrophages. Under the circumstances mentioned above, iNOS synthesizes both NO and superoxide radical anion to form peroxynitrite.[90] Measurement of the Hyp levels is used as a biomarker to determine the effect of free-radical-induced pathophysiological processes and several diseases.[91] Arginase catalyzes the conversion of L-arginine into L-ornithine and urea, which is mainly found in the liver and some tissues such as kidneys, brain, and lung. Moreover, it has been revealed that arginase plays a key function in asthma and COPD.[92] Prolidase, a manganese-dependent metalloenzyme, releases proline or hydroxyproline residue from the carboxyl terminus from iminodipeptides. It plays a crucial role in cell growth and the recycling of proline in collagen synthesis. Prolidase activity is easily affected by oxidative stress conditions.[93] Also, it is reported that alteration of prolidase activity in serum is used as an index of biomarkers for oxidative stress.[94] In this study, NO and Hyp levels were significantly elevated, while arginase and prolidase activities were significantly elevated in the VPA-treated group. An acceptable explanation of elevation of NO levels and arginase activities in lung tissue may be associated with a decrease in L-arginine availability as a result of upregulation of iNOS that generates both NO and superoxide radicals.[92] Current outcomes are in parallel with those of Labib et al.[20] who noted that bleomycin-mediated pulmonary inflammation led to an increase in NO levels and SOD enzyme activities in addition to expressions of cytokines such as TNF-α and IL-6 in mice. Besides this, an increase in arginase activities may be due to a response to oxidative stress and inflammatory process in the lungs.[95] In contrast, it has been reported that nitrite and Hyp levels were found to be elevated in arsenic-induced oxidative lung damage in mice.[96] Sacan et al.[97] revealed that both prolidase activity and Hyp levels increased as a result of diabetes-induced oxidative lung injury. In the present study, administration of EDA to the VPA group restored not only NO and Hyp levels, but also arginase and prolidase activities to normal levels. This protective effect may be attributed to the capability of EDA that leads to positive regulation of Nrf2 and iNOS expressions.[27] Our findings are consistent with research in which EDA treatment causes a decrease in Hyp levels in animals with lung injury and pulmonary fibrosis.[98,99]

5 | CONCLUSION

In conclusion, VPA caused a decline in the activity of antioxidant defense enzymes, extracellular matrix proteins (e.g., collagen-related enzymes), and integral membrane proteins (such as Na^+/K^+-ATPase) as a result of VPA-induced oxidative lung damage. Also, VPA administration triggered an increase in NO and Hyp levels, as well as LPO, PC, and glycoprotein levels in the lung. This study highlights the fact that EDA may play a restorative role against VPA-induced oxidative lung damage. The efficacy of EDA is associated with its strong antioxidant effect.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

The authors declare that all data were generated in-house and that no paper mill was used. Bertan B. Bayrak, Neziha Hacihasanoglu Cakmak, and Refiye Yanardag conceived and designed the study. Bertan B. Bayrak and Sebahat Yilmaz performed experiments. Bertan B. Bayrak, Sebahat Yilmaz, Neziha Hacihasanoglu Cakmak, and Refiye Yanardag analyzed data. Bertan B. Bayrak, Sebahat Yilmaz, and Refiye Yanardag wrote the manuscript. Bertan B. Bayrak, Sebahat Yilmaz, Neziha Hacihasanoglu Cakmak, and Refiye Yanardag read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The authors declare that (the/all other) data supporting the findings of this study are available within the article.

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