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Edaravone's Hepatoprotective Effects Against Oxidative Stress in Valproic Acid–induced Rat Model

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Abstract: In this experimental study, the effect of edaravone (EDA) on liver damage caused by valproic acid (VPA) was investigated. The antioxidant, oxidative stress, and inflammation indicators such as glutathione (GSH), total lipid (TL), sialic acid (SA), aspartate (AST) and alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) were examined. Male Sprague Dawley rats were used in the experiment and randomly divided into 4 groups. The experiment lasted for 7 days. Group I: control group rats; Group II: rats receiving 0.5 g/kg VPA intraperitoneally daily. Group III: rats receiving 30 mg/kg EDA intraperitoneally daily. Group IV: rats receiving 0.5 g/kg VPA and 30 mg/kg EDA intraperitoneally daily (at the same time). On day 8, all animals were sacrificed under anesthesia, and liver tissues were removed. VPA caused the decreases in GSH, CAT, SOD, GPx, GR, and GST values and the increases in AST, ALT, ALP, GGT, sialic acid, and total lipid values. EDA reversed the in all values. These results suggest that EDA administration potentially reduces liver injury in VPA-induced hepatotoxicity.

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

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1. INTRODUCTION

Epilepsy is one of the most common neurological problems, occurring in 1-2% of people worldwide, although it is particularly prevalent among young people (1). Anti-epileptic drugs (AEDs) are primarily used to treat epilepsy. AEDs are widely used as long-term adjunctive therapy or as monotherapy for other indications. AEDs include drugs that are highly susceptible to interactions (2,3). Valproic acid (2-propylpentanoic acid, VPA) is one of the oldest and most frequently prescribed drugs for epilepsy, bipolar disorder, migraine prophylaxis, schizoaffective disorders, addiction diseases and neuropathic pain (4,5). VPA activity is mediated by an increase in the synthesis and release of γ-aminobutyric acid and blockade of voltage-sensitive sodium channels (6,7). VPA is used as a neuroprotector in cases of Alzheimer's disease (8), migraine (9), and bipolar disorders for multiple tumors, neurodegenerative diseases such as Huntington's disease, Parkinson's disease, Duchenne progressive dystrophy, etc., and human immunodeficiency syndrome (10,11). Epidemiological studies suggest that VPA can cause hepatotoxicity (12), pancreatitis (13), and teratogenicity (5). The mechanism that causes liver damage has not been fully elucidated; hepatotoxicity may be due to reasons such as the development of oxidative stress, increased apoptosis, and microvesicular liver steatosis (14,15).

An increase in the amount of reactive oxygen characterizes oxidative stress. Disruption of the balance between reactive oxygen species (ROS) and antioxidant mechanisms leads to physiologic and biochemical dysfunctions (16,17). Antioxidants play an important role in disease prevention due to their reactive oxygen species scavenging activity (18). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a novel free radical scavenger with potent antioxidant properties and is used in patients with acute brain infarction. Several studies have shown that it prevents cell damage caused by oxidative

stress by capturing hydroxyl radicals and scavenging ROS (19,20). EDA is a lipophilic molecule, and mainly non-enzymatic peroxidation is a new antioxidant moving by inhibiting lipoxygenase activity *in vitro* (21-23). In addition to edaravone's antioxidizing activity, it has anti-inflammatory, anti-apoptotic, and anti-necrotic effects (24).

This study aimed to investigate the potential protective effect of EDA, which has antioxidant properties, against VPA-induced liver injury**.**

2. EXPERIMENTAL SECTION

2.1. Chemicals

VPA and EDA were obtained from Merck (Darmstadt, Germany). All other chemicals used in the experiments were of analytical purity and were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), and Fluka (Buchs, Switzerland).

2.2. Laboratory Animals and Experimental Design

All the experimental procedures were approved by the Istanbul University Local Ethics Committee on Animal Research (2010/54-the ethic committee number).

Thirty-eight Sprague Dawley rats (2.5-3 months old, male) were randomly divided into 4 groups. The animals were housed in the standard cage with optimal temperature (20 °C±2) and light/dark (12 h light/12 h dark) conditions. Group I: control rats (n=8). Group II: rats receiving intraperitoneal 0.5 g/kg VPA daily for 7 days (n=10). Group III: rats receiving 30 mg/kg EDA intraperitoneally daily for 7 days (n=10). Group IV: rats receiving 0.5 g/kg VPA, intraperitoneally 30 mg/kg EDA administration daily for 7 days (n=10) (at the same time). All rats were sacrificed under anesthesia 16 hours after VPA and EDA administration. On day 8, liver tissues were taken. Liver homogenates (10% w/v) were prepared in physiological saline (NaCl, 0.9%).

2.3. Biochemical Analysis

Biochemical analyses were performed on blood, serum, and liver homogenates according to the methods specified below.

2.3.1. Estimation of Glutathione (mg % GSH)

Glutathione (GSH) levels were determined by the method using metaphosphoric acid and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) (25).

2.3.2. Estimation of Total Lipid (mg % Lipid)

The sulfophosphovanillin method was used for the determination of total lipids in serum. This method is based on the principle of pink coloration of lipids with vanillin in sulfuric and phosphoric acid medium. The color intensity was determined in a spectrophotometer at 532 nm (26).

2.3.3. Estimation of Sialic Acid (mmol sialic acid/L)

Sodium periodate was used to oxidize sialic acid (SA) in concentrated phosphoric acid. Next, TBA was combined with the product of periodate oxidation. A pink chromophore was obtained, which was then extracted into cyclohexanone (27).

2.3.4. Estimation of Aspartate Transaminase (U/ g protein)

Aspartate transaminase (AST) activities were measured by converting L-glutamic acid to oxaloacetic acid, and the color was given by 2,4 dinitrophenyl hydrazine in the medium. The color obtained was measured with a spectrophotometer at 546 nm (28).

2.3.5. Estimation of Alanine Transaminase (U/ g protein)

Alanine transaminase (ALT) activities were measured by converting L-alanine to pyruvic acid and the color given by 2,4 dinitrophenyl hydrazine in the medium. The color obtained was measured with a spectrophotometer at 546 nm (28).

2.3.6. Estimation of Alkaline Phosphatase (U/ g protein)

Alkaline phosphatase (ALP) activities were determined at 405 nm according to the two-point method (29).

2.3.7. Estimation of Gamma-glutamyl Transferase (U/ g protein)

Gamma-glutamyl transferase (GGT) activity is based on the determination of the amount of p-nitroaniline formed as a result of the reaction by reading it in a spectrophotometer (30).

2.3.8. Estimation of Catalase (U /mg protein)

Catalase (CAT) activities were determined based on the reduction of hydrogen peroxide (H_2O_2) to water (H_2O) (31). The decrease in absorbance was measured spectrophotometrically at 240 nm.

2.3.9. Estimation of Superoxide Dismutase (U /g protein)

Superoxide dismutase (SOD) activities were measured as the ability to increase the rate of photooxidation of riboflavin-sensitised o-dianisidine (32).

2.3.10. Estimation of Glutathione Peroxidase (U /g protein)

Glutathione peroxidase (GPx) activities were determined according to the Wendel method, in which the conversion of GSH to GSSG was measured (33).

2.3.11. Estimation of Glutathione Reductase (U /g protein)

Glutathione reductase (GR) activity is based on calculating of the proportion of NADPH oxidized during the reduction of oxidized glutathione (GSSG) by GR at 340 nm (33).

2.3.12. Estimation of Glutathione-S-Transferase (U / mg tissue)

Glutathione-S-transferase (GST) activity was assayed by determining the amount of product obtained by conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) (34).

2.3.13. Estimation of Proteins

Lowry et al., developed the method to determine the amount of protein in liver tissue (35).

2.4. Statistics

Statistical analysis of biochemical results was calculated with GraphPad Prism 9.0 (GraphPad Software, San Diego, California, USA). Values are shown as mean ± standard deviation (SD). Unpaired t-test and analysis of variance (ANOVA) followed by Tukey multiple comparison analyses were used for the results. A value of P<0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

In this study, VPA administration caused a decrease in GSH levels (p<0.01) and an increase in total lipid (p<0.0001) and SA (p<0.01) levels compared to the control group. In addition, GSH levels (p<0.001) increased and total lipid (p<0.0001) and sialic acid levels (p<0.01) decreased in the VPA+ EDA treated group compared to the VPA (Figure 1).

In our study, there was a significant increase in AST (p<0.0001), ALT (p<0.0001), ALP (p<0.0001) and GGT (p<0.001) activities in the VPA group compared to the control group. However, there was a significant decrease in AST (p<0.0001), ALT (p<0.0001), ALP (p<0.0001) and GGT (p<0.01) activities in the VPA+EDA group compared to the VPA group (Figure 2).

The present study showed that the administration of VPA was associated with a decrease in the activities of CAT (p<0.001), SOD (p<0.01), GPx (p<0.05), GR (p<0.001), GST (p<0.0001) compared to the control group. VPA+EDA group caused a significant increase in CAT (p<0.01), SOD (p<0.01), GPx (p<0.001), GR (p<0.01) and GST (p<0.05) activities compared to VPA group (Figure 3).

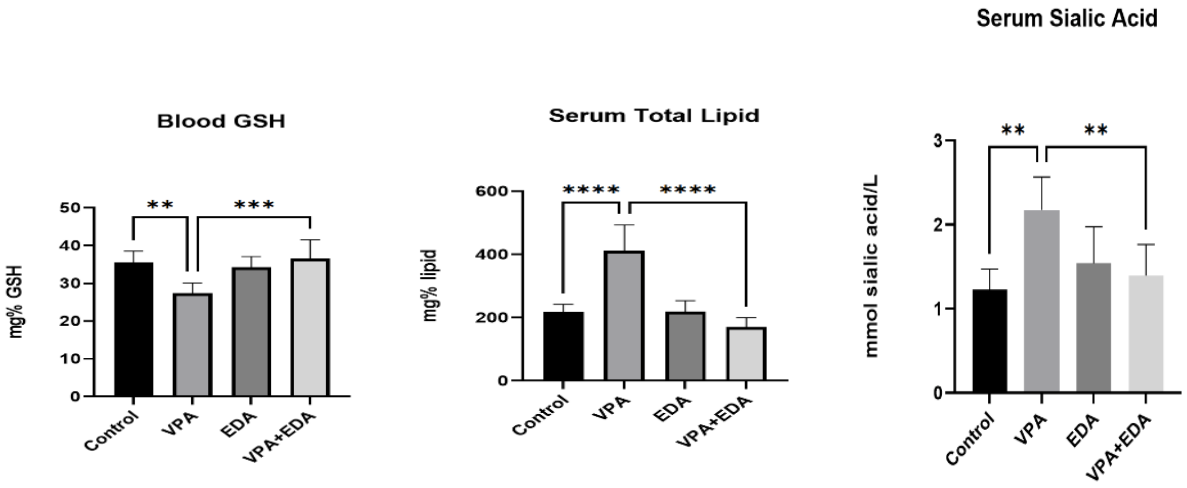

Figure 1: Blood GSH, serum total lipid and serum sialic acid levels.
The columns represent mean ± SD. VPA: Valproic acid group, EDA: Edaravone group, VPA+EDA: Valproic acid+Edaravone, GSH: Glutathione.
** represent p <0.01, ***represent p <0.001, **** represent p <0.0001

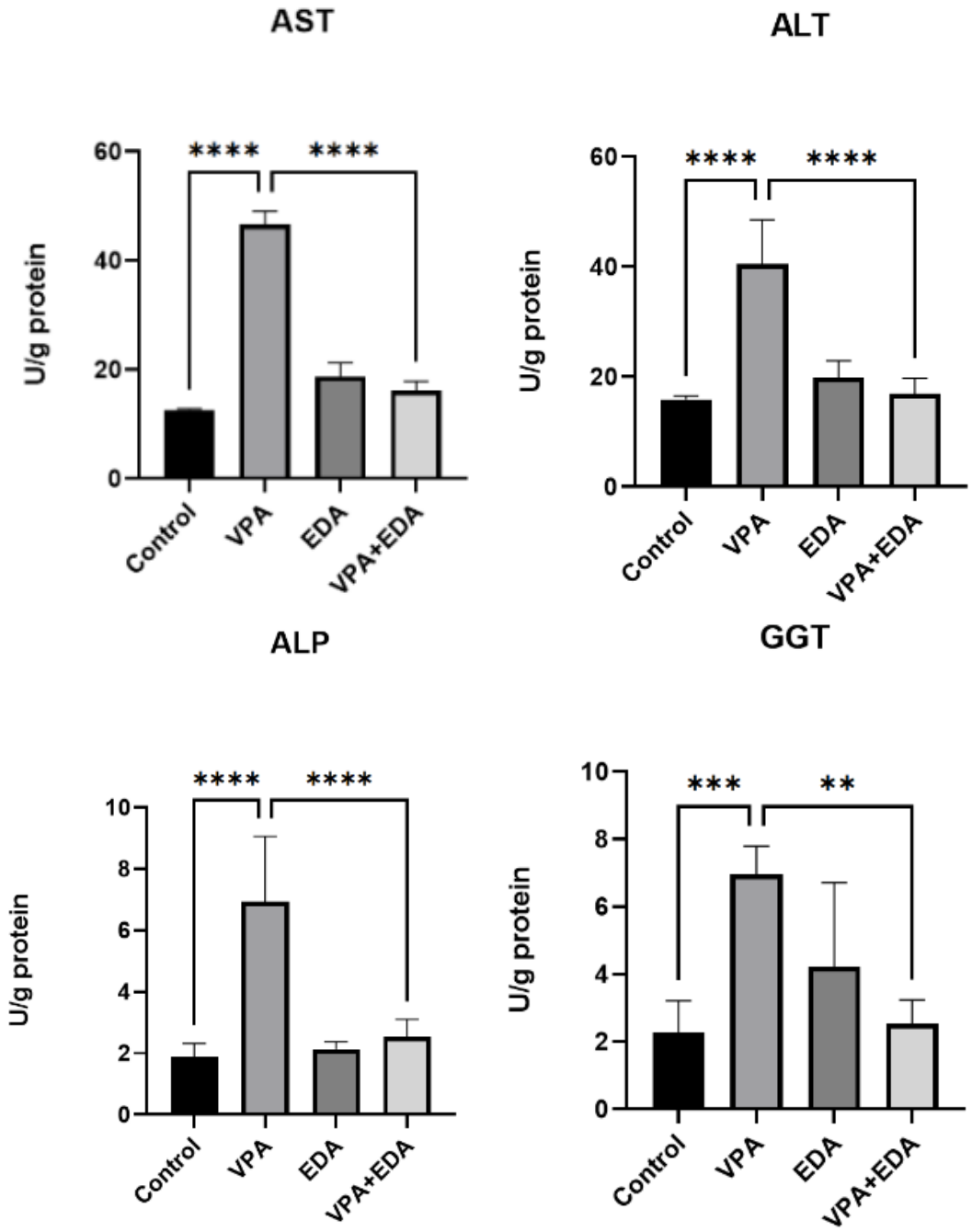

Figure 2: Liver AST, ALT, ALP and GGT activities of the groups.
The columns represent mean ± SD. VPA: Valproic acid group, EDA: Edaravone group, VPA+EDA: Valproic acid+Edaravone, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase.
** represent p <0.01, ***represent p <0.001, **** represent p <0.0001

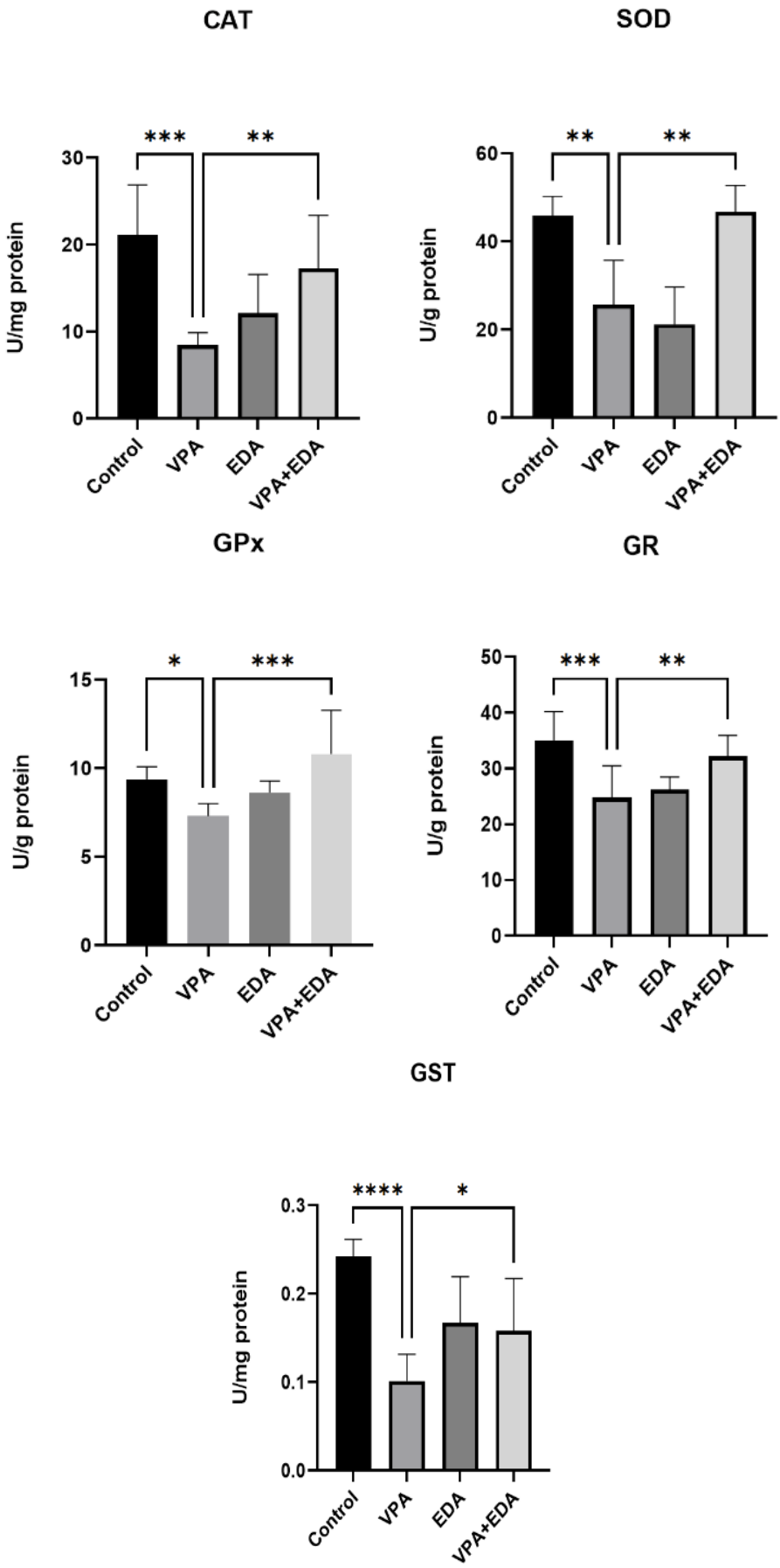

Figure 3: Liver CAT, SOD, GPx, GR and GST activities of the groups
The columns represent mean ± SD. VPA: Valproic acid group, EDA: Edaravone group, VPA+EDA: Valproic acid+Edaravone, CAT: Catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GR: Glutathione reductase, GST: Glutathione-S-transferase.
*represent p <0.05, ** represent p <0.01, ***represent p <0.001, **** represent p <0.0001

VPA is prescribed as a first-line antiepileptic drug due to its high efficacy and low cost and is one of the most common causes of acute liver failure (36,37). Therefore, VPA has been used since the 1960s to treat seizures and mood disorders and to treat many diseases, such as migraine (38). Hepatocyte damage is a common effect after VPA administration and can sometimes lead to irreversible, fatal liver failure. However, oxidative stress is generally considered critical for hepatocyte damage (39). Superoxide radicals, hydroxyl radicals, and hydrogen peroxide radicals cause an increase in ROS in the body, and this increase in ROS disrupts the antioxidant and oxidant balance in the body. Many organs and tissues in the body are adversely affected by this (17, 40). Free radicals impair cell functions and can cause cell death by destroying membrane lipids and proteins (41).

Antioxidants play an important role in disease prevention due to their reactive oxygen species scavenging activity (18). The production of reactive metabolites and ROS can affect GSH balance (42). GSH is an important cell protective biomolecule against synthetic activated cytotoxicity by electrophilic compounds and through glutathione-S-transferase (GST) conjugation (43). GSH is also an important antioxidant agent capable of immediate enzymatic (glutathione peroxidase, GPx) -mediated formation of ROS hydrogen peroxide and lipid hydroperoxides (44). ROS are also are removed by antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and reduced GSH. Antioxidants play an important role in disease prevention due to their ROS scavenging activity (45,46).

Edaravone is a free radical scavenger previously approved in Japan for the treatment of patients with acute ischemic stroke, and EDA has also been approved for the treatment of amyotrophic lateral sclerosis due to its neuroprotective effect (23,47). The radical scavenging activity of EDA is mediated by an electron-donating mechanism on a wide range of radical species (48-51). However, the antioxidant mechanisms of EDA are not fully understood. Accordingly, it is hypothesized that EDA may manage oxidative stress by regulating ROS-NOX signaling pathways.

It has been reported that GSH concentration in liver tissue was significantly reduced in VPA group compared to control group (52-54). In another study, it was reported that the amount of GSH decreased in the VPA group compared to the control group (55). In addition, Oktay et al. reported that there was no significant change in GSH level in the VPA group compared to the control group (56). In our study, we found that GSH levels decreased with VPA compared to the control group. Alzoubi et al. reported an increase in GSH levels using EDA in the treatment of memory impairment caused by chronic L-methionine administration (57). In the study investigating the protective effect of EDA on cyclophosphamide-induced oxidative stress and neurotoxicity in rats, it was reported that the amount of GSH increased with EDA (58). EDA increased GSH levels in a study on oxidative stress and allergic airway inflammation (59). In our study, GSH levels were significantly increased in the VPA + EDA group compared to the VPA group.

Dyslipidemia is implicated in the development of cardiovascular diseases. In particular, high total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels as well as low high-density lipoprotein cholesterol (HDL-C) are associated with cardiovascular mortality. VPA is well known to cause weight gain and insulin resistance and to increase triglyceride levels (60). Different effects of VPA on total cholesterol have been described in previous studies. Nikolaos et al. reported that VPA decreased total cholesterol levels, while Erminio et al. reported that VPA increased cholesterol levels (61,62). According to another study, a decrease in total cholesterol and low-density lipoprotein levels was observed in pediatric groups given VPA (63). In our study, we found that total lipid levels in serum increased with VPA compared to the control group. Experimental studies show that antioxidants have protective effects on atherosclerosis and endothelial damage. Dietary antioxidants have been reported to protect endothelial function (64,65) and prevent atherosclerosis in cholesterol-fed rabbits (66). Xi et al. have reported that mice given high doses of cholesterol in their diet and given EDA at the same time for 4 weeks had smaller atherosclerosis lesions (67). In our study, total lipid levels in serum decreased with EDA administration to the VPA group.

SA levels have been reported to increase during inflammatory processes, probably due to increased levels of acute phase glycoproteins, hypertriglyceridemia, and atherosclerosis (68-72). Various studies have reported that SA is a marker for inflammatory diseases. Increased SA levels reflect the body's self-protection (68-72). In our study, VPA administered to rats caused a significant increase in sialic acid levels in serum. Oktay et al. reported that administration of VPA+EDA group SA levels decreased when compared to the VPA group (56). EDA administration has been reported to cause a significant decrease in SA levels in pancreatic functions compared to VPA animals (73). In our study, it was observed that SA levels, which were increased by EDA administration to the VPA group, decreased.

Abdelkader et al., (2020) reported that VPA administration caused a significant increase in ALT, AST, ALP, and GGT activities in serum, which are considered to be an indicator of hepatocellular damage (53). Various studies have shown that VPA administration causes liver damage and significantly increases serum ALT, AST, and ALP levels compared to the control group (54,74). Koroglu et al. showed that administration of VPA group serum ALT levels significantly decreased when compared to the control group. There was no significant difference among the groups in terms of serum AST and GGT levels (75). In our study, VPA caused a significant increase in AST, ALT, ALP, and GGT activities in liver tissue. Hassanein et al. reported that the administration of VPA+EDA group AST, ALT, and ALP levels decreased

when compared to the VPA group (76). In our study, VPA+EDA group AST, ALT, ALP and GGT values decreased compared to VPA group.

Reactive oxygen species formed in the body are also removed by antioxidant enzymes. Antioxidants play an important role in the prevention of diseases due to their ROS scavenging activities (45,46). VPA administration has been reported to significantly decrease CAT in the autistic groups compared with the healthy groups (77). It has been reported that the activities of SOD were decreased in the liver of VPA-treated rats compared to the control group (75). In our study, CAT and SOD enzyme activities were lower in the VPA group compared to the control group. Sheng-Rui Fan et al. showed that administration of EDA group CAT and SOD levels increased when compared to the VPA group (78). EDA administration has been reported to cause a significant increase in SOD levels in heart functions compared to VPA animals (79). All these studies (78,79) support our findings that CAT and SOD values increased in rats administered VPA+ EDA.

There are conflicting results in GPx activities. CE reported decreased GPx activity in erythrocytes of patients treated with VPA, and Cotariu et al. reported decreased GPx activity in rats treated with intraperitoneal VPA (80,81). In contrast to these results, Hamed et al., Cengiz et al., and Kurekci et al. found an increase in GPx activity in VPA-treated patients (82-84). In our study, a decrease in GPx activity was observed in the VPA group. The decrease in GPx levels may indicate that the antioxidant capacity, which is effective in preventing various damages caused by VPA metabolism and its side effects, has decreased. It has been reported that EDA administration caused a significant increase in GPx activity in the VPA group (85). In our study, a decrease in GPx activity and GSH levels was observed in the VPA group and a significant increase in GSH and GPx activities in the VPA + EDA group.

GR is one of the antioxidant enzymes (86) and Oztaylan et al. investigated the effect of VPA on the lens and reported that the amount of GR increased with VPA administration (87). Turkyilmaz et al. reported that GR activity decreased in VPA-induced brain injury (55). In another study, it was reported that GR activity decreased in the VPA group compared to the control group (88). In this study, we found that GR activity in liver tissue was significantly decreased in the VPA group. Hassan et al. reported an increase in GR, one of the antioxidant enzymes, in heart tissue when EDA was given for protection against isoproterenol (ISO) (89). Bayrak et al. reported that glutathione reductase activities decreased insignificantly in the VPA group in lung tissue, whereas GR activity increased significantly in the EDA group (85). In our study, VPA+EDA group GR values increased compared to the VPA group.

Tong et al. found an increase in α-GST levels, a marker of hepatocyte damage, in serum 4 days after VPA treatment (90), while Chaudhary et al. found a decrease in GST activity in the cerebellum and cerebral cortex (91). In another study, in valproic acid-induced brain injury, GST decreased in the VPA group compared to the control group (55). It was reported that a decrease in GST levels in a rat model study of VPA-induced autism spectrum disorder (92). We found a significant decrease in GST activity in liver tissue in the VPA group compared to the control group. EDA administration has been reported to cause a significant increase in GST levels in heart functions compared to VPA animals (79). In another study, GST activity increased in the VPA+EDA group compared to the VPA group (89). Lu et al. reported an increase in GST levels in the VPA+EDA group compared to the EDA group (92). We found a significant decrease in GST activity in liver tissue VPA+ EDA group compared to the VPA group.

4. CONCLUSION

VPA is a widely used anti-antiepileptic. Although it has beneficial effects, there are many systems and organs that are affected due to its serious side effects. The liver is the organ most exposed to and affected by toxicity and free radical species. Protecting this tissue is a vital goal for all research. For this purpose, EDA was chosen as a preservative because its protective effects have been shown in previous studies and it is a good antioxidant. The biochemical results obtained from this study support the protective effects of EDA on liver tissue exposed to VPA.

5. CONFLICT OF INTEREST

There are no conflicts to declare.

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