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Edaravone, a free radical scavenger, protects liver against valproic acid induced toxicity

NEZIHA HACIHASANOGLU CAKMAK and REFIYE YANARDAG*

Department of Chemistry Faculty of Engineering, Istanbul University, 34320, Avcilar-Istanbul, Turkey

Abstract: Valproic acid (VPA) is a well-established anticonvulsant drug that has been increasingly used in the treatment of many forms of generalized epilepsy. Edaravone (EDA, 3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent free radical scavenger. In this study, the aim was to investigate the effects of EDA on VPA-induced hepatic damage. Male Sprague Dawley rats were divided into four groups. Group I was control animals. Group II was control rats given valproic acid (500 mg kg^{-1} day^{-1}) for seven days. Group III was given only EDA (30 mg kg^{-1} day^{-1}) for seven days. Group IV was given VPA+EDA (at the same dose and in the same time). EDA and VPA were administered intraperitoneally. On the 8th day of the experiment, blood samples and liver tissue were taken. Serum aspartate and alanine aminotransferase, alkaline phosphatase and bilirubin levels, liver myeloperoxidase, xanthine oxidase, adenosine deaminase, Na$^+$/K$^+$ATPase, sorbitol dehydrogenase, glutamate dehydrogenase, DT-diaphorase, arginase and thromboplastic activities, lipid peroxidation, protein carbonyl levels were increased whereas paraoxonase and biotinidase activities and glutathione levels were decreased in the VPA group. Application of EDA with VPA protected against VPA-induced effects. These results demonstrated that administration of EDA is potentially beneficial to reduce hepatic damage in VPA-induced hepatotoxicity, probably by decreasing oxidative stress.

Keywords: edaravone; hepatotoxicity; hepatic enzymes; rat; valproic acid; free radicals.

INTRODUCTION

Valproic acid (2-propylpentanoic acid, VPA) is one of the most widely prescribed antiepileptic drugs (AEDs) worldwide, and is also used against migraine, cluster headaches and bipolar psychiatric disorders.[1] In addition, VPA has a wide range of therapeutic applications in many cancer types, including breast

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cancer, glioma, acute myelogenous leukemia, thyroid cancer, endometrial carcinoma and neuroblastoma[2] and human immune deficiency virus infection.[3] VPA shows important potential adverse effects, including weight gain, hepatotoxicity, pancreatitis, skin rash, hair loss, antifolate activity, teratogenicity, bone marrow suppression and hyperammononemic encephalopathy.[4–7] One of the most important clinical adverse effects ascribed to VPA therapy is hepatic failure.[6] Various studies showed protection against VPA-induced hepatotoxicity by antioxidants, including α-tocopherol, N,N'-diphenylphenylenediamine[8] and vitamin U.[9]

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186; EDA) is a newly developed free radical scavenger for clinical use that can quench free radical reaction by trapping a variety of free radical species.[10] It can act as a hydroxyl radical scavenger[11,12] and has antioxidant effects against both the hydroxyl radical and iron-dependent lipid peroxidation.[13,14] Several experimental studies reported that EDA may have noteworthy effects on hepatic ischemia – reperfusion injury.[15,16]

The purpose of the present study was to evaluate the ability of EDA to protect hepatic tissue against VPA-induced toxicity.

EXPERIMENTAL

Animals and experimental design

Male Sprague Dawley rats were used in this study. The animals were 2.5–3 months old and clinically healthy. All the experimental procedures were approved by the Istanbul University Local Ethics Committee on Animal Research. The rats were randomly divided into four groups. Group I ($n = 8$) was intact control animals. Group II ($n = 10$) was given only VPA (500 mg kg^{-1} day^{-1}) for seven days. Group III ($n = 10$) was given only EDA (30 mg kg^{-1} day^{-1}) for seven days and lastly, Group IV ($n = 10$) was given VPA+EDA (at the same dose and in the same time). EDA and VPA were given by intraperitoneally. On the 8th day of the experiment, all of the animals were fasted overnight and then sacrificed under anesthesia. Blood and liver tissue were taken from the animals.

Biochemical assays

In this study, biochemical investigations were made on serum and liver tissues from all groups. Liver tissue samples were washed with 0.9 % saline and kept frozen until the day of the experiments. Serum aspartate (AST) and alanine aminotransferase (ALT) activities were estimated by the Reitman and Frankel methods.[17] Serum alkaline phosphatase (ALP) activity was assayed by two points methods.[18] Serum total and direct bilirubin levels were determined by the method of Jendrassik and Grof.[19] Liver tissues were taken from animals, homogenized in 0.9 % saline to make a 10 % (w/v) homogenate. The homogenates were centrifuged and used for determination of the protein, glutathione (GSH), lipid peroxidation (LPO), protein carbonyl (PC) levels and enzyme activities. GSH levels were determined according to the Beutler method using Ellman's reagent.[20] LPO levels were estimated by the Ledwozyw method.[21] PC levels were measured by the method of Levine.[22] The myeloperoxidase (MPO) activity was determined according to Wei and Frenkel,[23] paraoxonase (PON1) according to Furlong *et al.*,[24] xanthine oxidase (XO) according to Corte and Stirpe,[25] adenosine deaminase (ADA) according to the Karker method,[26] Na$^+$/K$^+$ATPase according to Ridderstap and Bont-

ing,[27] biotinidase according to Wolf *et al.*,[28] sorbitol dehydrogenase (SDH) according to Berg-meyer *et al.*,[29] glutamate dehydrogenase (GLDH) according to Ellis and Goldberg,[30] DT--diaphorase (DTD) according to Ernster *et al.*[31] and arginase according to Geyer and Dabich.[32] The thromboplastic activity (TF) was determined by the method of Ingram and Hills.[33] The protein content in the supernatants was estimated by the Lowry method using bovine serum albumin as standard.[34]

Statistical analysis

The biochemical results were analyzed by one-way ANOVA followed by the Duncan/Newman-Keuls multiple comparison test. The values are expressed as mean ± *SD*. Values of *p* less than 0.05 were considered significant.

RESULTS

The serum AST, ALT and ALP activities are given in Table I. The serum AST, ALT and ALP activities were significantly increased in the VPA group compared to the control group ([a]$p < 0.05$, [c]$p < 0.005$, Table I). Administration of EDA significantly decreased the AST, ALT and ALP activities in the VPA group ([b]$p < 0.005$, [d]$p < 0.05$, Table I). The serum total and direct bilirubin were analyzed to evaluate hepatic injury. The serum total and direct bilirubin were significantly increased in the VPA group as compared to the control group ([c]$p < 0.005$, Table I). EDA given to the VPA group lowered the serum total and direct bilirubin levels in a significant manner when compared to the VPA group ([b]$p < 0.005$, [e]$p < 0.005$, Table I).

TABLE I. Serum aspartate aminotransferase (AST / U L^{-1}), alanine aminotransferase (ALT / U L^{-1}) and alkaline phosphatase (ALP / U L^{-1}) activities and serum total and direct bilirubin levels for all groups; mean±*SD*; [a]$p < 0.05$ *vs.* control group, [b]$p < 0.005$ *vs.* VPA group; [c]$p < 0.005$ *vs.* control group; [d]$p < 0.05$ *vs.* VPA group; [e]$p < 0.005$ *vs.* VPA group

Group	AST	ALT	ALP	Total bilirubin µmol L^{-1}	Direct bilirubin µmol L^{-1}
Control	144.77±38.72	53.49±17.89	104.28±27.74	5.81±1.48	1.40±0.42
Control+EDA	171.28±36.38	54.12±12.11	125.96±15.61	5.32±1.39	1.35±0.53
VPA	207.57±41.16[a]	87.62±19.89[c]	177.44±45.11[c]	11.27±3.48[c]	2.49±0.61[c]
VPA+EDA	148.57±27.81[b]	59.54±9.47[b]	108.77±42.63[d]	5.64±1.93[b]	1.33±0.62[e]
p_{ANOVA}	0.004	0.001	0.002	0.0001	0.001

Hepatic glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PC) levels are presented in Table II. The GSH levels were significantly decreased in the VPA group ([a]$p < 0.05$). Administration of EDA significantly protected against the reduction in GSH levels in the EDA-treated VPA (VPA+EDA) group in comparison to the VPA group ([b]$p < 0.05$, Table II). Hepatic LPO and PC levels were significantly increased in the VPA group compared to control group ([a]$p < 0.05$). Administration of EDA showed significant protection against oxidation in the LPO and PC levels in the EDA-treated VPA group compared to the VPA group ([b]$p < 0.05$, Table II). The MPO activity in the hepatic tissue was

significantly elevated in the VPA group. This can demonstrate enhanced infiltration of neutrophils to the hepatic tissue ([c]$p < 0.0005$). Administration of EDA significantly decreased the hepatic MPO levels ([d]$p < 0.0005$) in the VPA group. The hepatic PON activity was significantly decreased in the VPA group ([e]$p < 0.005$). EDA inhibited these decreases significantly ([b]$p < 0.05$, Table II).

TABLE II. Liver glutathione (GSH / nmol mg^{-1} prot.), lipid peroxidation (LPO nmol MDA mg^{-1} prot.) and protein carbonyl (PC / nmol mg^{-1} prot.) levels and myeloperoxidase (MPO / U g^{-1} tissue) and paraoxonase (PON / mU mg^{-1} prot.) activities for all groups; mean±SD; [a]$p < 0.05$ *vs.* control group, [b]$p < 0.05$ *vs.* VPA group; [c]$p < 0.0005$ *vs.* control group; [d]$p < 0.0005$ *vs.* VPA group; [e]$p < 0.005$ *vs.* VPA group

Group	GSH	LPO	PC	MPO	PON
Control	33.31±7.6	0.75±0.26	1.91±1.08	0.67±0.085	9.03±0.50
Control+EDA	36.61±8.77	0.86±0.51	2.50±1.34	0.62±0.0067	10.18±3.13
VPA	24.14±4.88[a]	2.38±1.26[a]	4.96±2.51[a]	0.83±0.0051[c]	6.54±1.48[e]
VPA+EDA	33.60±6.94[b]	0.86±0.54[b]	1.88±0.92[b]	0.65±0.10[d]	12.31±4.66[b]
p_{ANOVA}	0.017	0.001	0.003	0.0001	0.029

The hepatic XO and ADA activities were significantly increased in the VPA group ([a]$p < 0.005$, [c]$p < 0.05$, Table III). Administration of EDA significantly decreased the hepatic XO and ADA activities ([b]$p < 0.05$, Table III). The activity of Na$^+$/K$^+$ATPase, indicating the functional transport capacity of the hepatic cells, was increased in the VPA group compared with control group ([c]$p < 0.05$). However the EDA-treated VPA group had significantly decreased hepatic Na$^+$/K$^+$ATPase activity ([d]$p < 0.005$, Table III). Hepatic biotinidase activity was significantly decreased in the VPA group as compared to the control group ([a]$p < 0.005$), whereas biotinidase activity in the hepatic tissue was significantly increased in the EDA-treated VPA group as compared with VPA group ([b]$p < 0.05$, Table III). Hepatic SDH was significantly increased in the VPA groups as compared to the controls ([a]$p < 0.005$). On the other hand, EDA administration to the VPA group resulted in a significant decrease in SDH activity in comparison to the VPA group ([e]$p < 0.005$, Table III).

TABLE III. Liver xanthine oxidase (XO / mU mg^{-1} prot.), adenosine deaminase (ADA / mU mg^{-1} prot.), sodium potassium ATPase (Na$^+$/K$^+$ATPase, µmol P mg^{-1} prot., 10 min), biotinidase (nmol min mg^{-1} prot.) and sorbitol dehydrogenase (SDH / mU mg^{-1} prot.) activities for all groups; mean±SD; [a]$p < 0.005$ *vs.* control group, [b]$p < 0.05$ *vs.* VPA group; [c]$p < 0.05$ *vs.* control group; [d]$p < 0.005$ *vs.* VPA group; [e]$p < 0.005$ *vs.* VPA group

Group	XO	ADA	Na$^+$/K$^+$ATPase	Biotinidase	SDH
Control	1.81±0.51	2.31±0.66	0.159±0.052	0.035±0.006	0.938±0.311
Control+EDA	3.16±1.91	10.18±1.13	0.129±0.041	0.031±0.011	0.960±0.158
VPA	6.71±3.75[a]	5.09±3.03[c]	0.298±0.160[c]	0.022±0.006[a]	3.86±2.11[a]
VPA+EDA	3.20±2.43[b]	2.40±0.88[b]	0.126±0.053[d]	0.034±0.012[b]	1.30±0.73[e]
p_{ANOVA}	0.001	0.003	0.001	0.003	0.0001

The hepatic GLDH, DTD and arginase activities were significantly increased in VPA group compared to control group ([a]$p < 0.05$, [c]$p < 0.005$). EDA significantly decreased the GLDH, DTD and arginase activities in the VPA groups as compared with the VPA group ([b]$p < 0.05$, [d]$p < 0.005$, Table IV). The TF activity was increased in the VPA group ([a]$p < 0.05$) but was significantly decreased by EDA administration ([b]$p < 0.05$, Table IV).

TABLE IV. Liver glutamate dehydrogenase (GLDH/mU mg^{-1} prot.), DT-diaphorase (nmol min mg^{-1} prot.), arginase (µmol urea mg^{-1} prot.) and thromboplastic activities (TF/s) for all groups; mean±SD; [a]$p < 0.05$ vs. control group, [b]$p < 0.05$ vs. VPA group; [c]$p < 0.005$ vs. control group; [d]$p < 0.005$ vs. VPA group

Group	GLDH	DT-Diaphorase	Arginase	TF
Control	0.25 ± 0.09	5.55 ± 1.76	0.42 ± 0.14	209.63 ± 91.19
Control+EDA	0.31 ± 0.014	6.41 ± 2.21	0.47 ± 0.20	196.10 ± 51.74
VPA	1.085 ± 0.748[a]	11.89 ± 2.99[c]	0.87 ± 0.43[a]	121.40 ± 27.52[a]
VPA+EDA	0.483 ± 0.323[b]	7.19 ± 2.91[d]	0.53 ± 0.24[b]	172.10 ± 51.80[b]
p_{ANOVA}	0.0001	0.0001	0.012	0.012

DISCUSSION

One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as AST, ALT and ALP. The increase in the activities of these enzymes in serum during VPA administration, is thus indicative of hepatocellular damage.[35] In this study, treatment with EDA significantly reversed the alterations in the status of these enzymes to normal levels, probably due to its highly effective free radical scavenger activity,[36] thus, maintaining the hepatocellular membrane integrity.

Serum bilirubin is a potential marker for hepatic dysfunction and any abnormal increase in the levels of bilirubin in serum denotes hepatobiliary disease and hepatocellular dysfunction.[37] In the present study, the elevated levels of bilirubin observed in the VPA group corresponded to extensive hepatic damage. Its accumulation is a measure of binding, conjugation and excretory capacity of hepatocytes.[38] Recent reports showed that EDA exhibits excellent efficacy in both hepatic ischemia–reperfusion and acute hepatic injury in rat models.[39] Accordingly, in this study, EDA prevented the increases of the total and direct bilirubin levels.

It is well known that GSH is involved in the protection of normal cellular structure and function by maintaining redox homeostasis, quenching free radicals, and participating in detoxification reactions. Several studies reported the implication of an increased generation of free radicals and oxidative stress in the toxic mechanism of VPA.[40,41] The present study showed significantly decreased levels of GSH in the liver of VPA treated rats, as compared to the control. This decrease in GSH level may be due to increased utilization of GSH to scavenge

toxic intermediates. Administration of EDA significantly increased the hepatic GSH levels in the group given VPA, showing a protective effect.

LPO results from excessive reactive oxygen species (ROS) and it was shown that VPA induced LPO production in rat hepatocyte cultures.[8,42] Tong *et al*.[40] reported that hepatic LPO levels were elevated on day 14 in VPA therapy. Another study demonstrated that a single dose of VPA in rats leads to an elevation in plasma and liver of the endogenous lipid peroxidation marker.[43] According to the present study, VPA increased LPO levels of liver compared with the control group. Similar to this result, Sokmen *et al*. reported significantly increased hepatic LPO levels in VPA treated rats.[9] However administration of EDA prevented these changes.

PC levels are the most commonly used markers of protein oxidation.[44] There is increasing evidence for the involvement of toxic carbonyls in numerous human diseases.[45] The present study showed the PC levels were significantly increased. The increased production of PC indexed the enhanced oxidative stress caused by VPA. EDA prevented the enhancement of the PC levels and restored the liver cells to their normal physiological state.

When neutrophils are stimulated by various stimulants, MPO, as well as other tissue-damaging substances, is released from these cells.[46] In the present study, VPA treatment caused significant increases in hepatic MPO activity. These results suggested that VPA induced neutrophil-dependent oxidative damage. EDA inhibited oxidative injury and decreased MPO activities. The protective effect of EDA on hepatic injury was mediated in part by blocking tissue neutrophil infiltration.

PON1 is another antioxidant enzyme closely associated with high-density lipoproteins. It is a calcium-dependent esterase that detoxifies lipid peroxides, and is widely distributed in many tissues, including liver, brain, lung, heart, kidneys and small intestines.[47] Increased oxidative stress was shown to reduce PON1 synthesis *in vivo* and *in vitro*.[48] Varoglu *et al*.[49] demonstrated that serum PON1 activity was decreased in epileptic patients rather than in the control subjects. In another study, the administration of vitamin U significantly increased hepatic PON1 activity in rats treated with VPA.[9] In the present study, PON1 activity was significantly lower in the VPA group. The decrease in PON1 activity is directly related to the degree of hepatic damage. Administration of EDA significantly increased hepatic PON1 activity in rats treated with VPA.

XO is one of the most important enzyme sources of the superoxide radical and catalyses the conversion of hypoxanthine and xanthine to uric acid. It is a major potential source of ROS. Free radical generation by mediation of XO enzyme in tissue is triggered by a large increase in the formation of its substrates inosine and hypoxanthine.[50] The present study showed that VPA administration increases XO activity in rat liver. This increase in XO activity on VPA treatment

may enhance the generation of ROS and may partly be responsible for the increased lipid peroxidation and oxidative injury. However, EDA treatment may prevent hepatotoxicity by decreasing XO activity.

ADA catalyzes the irreversible deamination of adenosine and deoxyinosine to inosine and deoxyinosine respectively.[51] It was reported that ADA has an important role in acute immune inflammatory reactions, and its serum level has been used as a biochemical marker for inflammation and disease.[52] A recent study showed that VPA administration increased ADA activity in rat liver.[9] In the VPA group, in the present study, elevated hepatic ADA activity indicated hepatic damage. Additionally, the reduced ADA activity in the VPA plus EDA group showed significant improvement in the hepatic tissue.

ATPases are lipid-dependent enzymes involved in active transport processes and have been implicated in the pathogenesis of hepatic cell injury.[53] Enhanced susceptibility of membranes to oxidative stress can lead to loss of protein thiol, thereby changing the functions of membrane.[54] In the present study, increased hepatic Na^+/K^+ATPase activity was observed in VPA-supplemented rats. It was originally postulated that the increase in Na^+/K^+ATPase in the liver after VPA administration was due to increased oxidative phosphorylation and oxygen consumption to supply ATP for ATPase activity. On administering EDA, the Na^+/K^+ATPase activity was significantly decreased in hepatic tissue.

Biotinidase is synthesized mainly by the liver and then secreted into the blood[55] and is decreased in the plasma of both humans and experimental animals with hepatic cirrhosis. Pispa[56] noted a 50 % decrease in biotinidase activity, and a 30 % decrease in serum activity of partially hepatectomized rats. Nagamine *et al*.[57] implicated biotinidase deficiency in chronic hepatic disease. It was reported in several studies that chronic VPA use could alter the biotinidase enzyme activity in humans[58] and rats.[59] The decrease in the liver biotinidase activity may be due to the inhibition of the biosynthetic and secretory capacity of the liver after administration of a toxic dose of VPA. The present study showed that VPA administration decreased the biotinase activity in rat liver. In our study, administration of EDA significantly increased liver biotinidase activity in rats treated with VPA, showing again a restoration in hepatic tissue.

SDH catalyzes the conversion of sorbitol to fructose in the presence of NAD. SDH is a specific indicator of hepatocellular injury in rodents and has a reported value in humans.[60] In the present study, hepatic SDH activity was increased due to liver injury. The increased activities of SDH are a sensitive index of hepatic injury as demonstrated in rats intoxicated with VPA. However, EDA treatment significantly decreased the SDH activity. A decrease of liver SDH activity indicates improvement of hepatic injury.

GLDH, a key enzyme in amino acid oxidation and urea production, has several features that make it attractive as a potential biomarker of drug-induced

hepatocellular toxicity.[61] Cotariu *et al.*[35] showed that VPA induces dose-dependent changes in the hepatic GLDH activity. In this study, the GLDH activity was increased depending on the liver injury induced by VPA. Elevations in GLDH activity could be the result of enzyme release from cellular membranes due to hepatocellular necrosis. EDA treatment significantly decreased the enzymatic activity and thus prevented the hepatic injury.

DTD is a cytosolic enzyme that is localized mainly in the liver, kidney and gastrointestinal tract.[31] DTD, which is a flavoprotein, catalyzes the two-electron reduction and detoxification of quinones and their derivatives.[62] DTD protein forms homodimers and reduces quinones to hydroquinones in a way that prevents the one electron reduction of quinones, which results in the production of ROS. An increase in the activity of DTD may also correspond to an increase in oxidative stress. In the present study, VPA increased the activity of DTD, suggesting a response to increased oxidative stress. EDA treatment normalized the DTD activity.

Arginase is highly liver specific making it a candidate biomarker that shows higher specificity compared to the hepatic enzymes.[63] Arginase activity was reported to be increased in chronic hepatic damage by thioacetamide.[64] In the present study, there was an increase in the activity of arginase in the liver of the VPA group. Administration of EDA restored the arginase activity to a normal level in the hepatic tissue, indicating an improving effect in hepatic injury.

TF is an important coagulation factor that initiates extrinsic blood coagulation with FVII. It is a cell membrane component, and its activity was measured by the prothrombin time test.[65] Shortened clot formation time indicates increased TF activity that could easily be changed by alterations in the membrane composition or lipid peroxidation composition of the membrane due to oxidative stress.[66–68] In the present study, VPA-induced injury increased the TF activity in the hepatic tissue. This increase could be attributed to inflammation of the hepatic membrane. Anti-inflammatory effects of EDA were previously reported.[69,70] EDA treatment reversed the increased TF activity in the liver of rat receiving VPA therapy, due to the anti-inflammatory effect of EDA.

EDA has potent hydroxyl radical scavenging activity. In various experimental models, EDA was reported to protect several organs, such as the brain, heart[71] and liver[72] from free radical-mediated injuries.

This is the first study that evaluates the protective effects of EDA against VPA-induced hepatic damage in experimental animals. In conclusion, the results indicate that VPA-induced hepatotoxicity is associated with increased oxidative stress. EDA shows protection against this damage. The protective effect of EDA observed in the present study is due to the scavenging of free radicals. Therefore, it is suggested that EDA could be potentially useful for the prevention of hepatic toxicity during epileptic disease.

И З В О Д

ЕДАРАВОН ШТИТИ ЈЕТРУ ОД ТОКСИЧНОГ ДЕЈСТВА ВАЛПРОИНСКЕ КИСЕЛИНЕ
УКЛАЊАЈУЋИ СЛОБОДНЕ РАДИКАЛЕ

NEZIHA HACIHASANOGLU CAKMAK и REFIYE YANARDAG

Department of Chemistry, Faculty of Engineering, Avcilar-Istanbul University, Turkey

Валпроинска киселина (VPA) је добро познати антиконвулзивни лек који се ко-
ристи за терапију епилепсије, а едаравон (EDA, 1-фенил-3-метил-2-пиразолин-5-он) је
потентан хватач слободних радикала. У овом раду је испитиван ефекат EDA на јетру
оштећену применом VPA. Пацови мушког пола су подељени у четири групе. Групу I су
чиниле контролне животиње. Пацовима у групи II је давана VPA (500 mg kg^{-1} по дану)
током седам дана. Животињама у групи III је даван само EDA (30 mg kg^{-1} по дану) током
седам дана. Пацовима у групи IV је комбиновано давано VPA+EDA (у дозама и вре-
менском периоду као и осталима). EDA и VPA су уношени интраперитонеално. Осмог
дана од почетка експеримента, животињама су вађени узорци крви и јетре. Концен-
трације или активности следећих параметара су биле повећане у групи II: а) серумска
аспартат- и аланин-аминотрансфераза, алкална фосфатаза и билирубин, као и б) јет-
рена мијелопероксидаза, ксантин-оксидаза, аденозин-деаминаза, Na$^+$/K$^+$АТРаза, сорби-
тол-дехидрогеназа, глутамат-дехидрогеназа, ДТ-дијафораза, аргиназа, тромбопластична
активност, липидни пероксиди и протеински карбонили. Истовремено су смањене
активности параоксоназе и биотинидазе, као и концентрација глутатиона. Применом
EDA омогућена је заштита од ефеката VPA. Ови резултати указују да је коришћењем
EDA могуће умањити оштећење јетре индуковано применом VPA, вероватно преко сма-
њења оксидативног стреса.

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