Potential Protective Role of Radicut in Valproic Acid-Induced Oxidative Stress in Rat Spleen

Nihal Şehkar OKTAY[*], Burçin ALEV TÜZÜNER[**], Sevim TUNALI[***], Ebru EMEKLİ ALTURFAN[****], Tuğba TUNALI AKBAY[*****], Refiye YANARDAĞ[******], Ayşen YARAT[*******]

Abstract

Aim: The study aims to evaluate the potential protective role of Radicut (RAD) in Valproic acid (VPA)-induced oxidative stress in splenic tissues of rats.

Method: Rats were divided into groups as follows: Group 1: Controls (n=8), Group II: R: RAD-given group (30 mg/kg/day, n=8), Group III: V: VPA-given group (0.5 g/kg/day, n=10), Group IV: V+R: VPA+RAD-given group (30 mg/kg/day, n=11). VPA, RAD, and VPA+RAD were given to the animals for 7 days (i.p). Biochemical parameters related to oxidative stress were determined in spleen homogenates.

Results: VPA elevated oxidative stress by increasing lipid peroxidation and sialic acid levels, increasing alkaline phosphatase activity, and decreasing superoxide dismutase, glutathione-S- transferase, and glutathione peroxidase activities. Administration of RAD to VPA-given group decreased LPO, SA levels, and acid phosphatase levels, and increased tissue factor, SOD, GST, and GPx activities.

Conclusion: RAD reversed the biochemical results in the V group, by clarifying its protective effect. RAD has the potential to prevent oxidative stress during VPA treatment, which could be beneficial.

Keywords: Radicut, valproic acid, oxidative stress, antioxidants, spleen, rat.

Özgün Araştırma Makalesi (Original Research Article)
Geliş / Received: 22.08.2023 & **Kabul / Accepted:** 19.03.2024
DOI: https://doi.org/10.38079/igusabder.1348285
[*] (Corresponding Author) Assoc. Prof. Dr., Department of Basic Medical Science, Faculty of Dentistry, Marmara University, Istanbul, Türkiye. E-mail: nihal.oktay@marmara.edu.tr ; nsehkar@yahoo.com
ORCID https://orcid.org/0000-0002-2878-288X
[**] Asst. Prof., Istanbul Gelisim University, Faculty of Dentistry, Department of Basic Medical Sciences, Biochemistry, Istanbul, Türkiye ; Asst. Prof., Istanbul Gelisim University, Life Sciences and Biomedical Engineering Application and Research Centre, Istanbul, Türkiye. E-mail: btuzuner@gelisim.edu.tr ORCID https://orcid.org/0000-0001-5122-4977
[***] Prof. Dr., Istanbul University-Cerrahpasa, Department of Chemistry, Faculty of Engineering, Istanbul, Türkiye. E-mail: stunali@istanbul.edu.tr ORCID https://orcid.org/0000-0003-3363-1290
[****] Prof. Dr., Department of Basic Medical Science, Faculty of Dentistry, Marmara University, Istanbul, Türkiye. E-mail: ebruemekli@yahoo.com ORCID https://orcid.org/0000-0003-2419-8587
[*****] Prof. Dr., Department of Basic Medical Science, Faculty of Dentistry, Marmara University, Istanbul, Türkiye. E-mail: ttunali@marmara.edu.tr ORCID https://orcid.org/0000-0002-2091-9298
[******] Prof. Dr., Istanbul University-Cerrahpasa, Department of Chemistry, Faculty of Engineering, Istanbul, Türkiye. E-mail: refiyeyanardag@yahoo.com ORCID https://orcid.org/0000-0003-4185-4363
[*******] Prof. Dr., Department of Basic Medical Science, Faculty of Dentistry, Marmara University, Istanbul, Türkiye. E-mail: ayarat@marmara.edu.tr ORCID https://orcid.org/0000-0002-8258-6118

ETHICAL STATEMENT: All experimental protocols were approved by the Marmara University Animal Care and Use Committee (134.2013mar, Date: 20.02.2014).

Radicut'ın Sıçan Dalağındaki Valproik Asite Bağlı Oksidatif Streste Potansiyel Koruyucu Rolü

Öz

Amaç: Çalışma, sıçanların dalak dokularında Valproik asitin (VPA) neden olduğu oksidatif streste Radikutun (RAD) potansiyel koruyucu rolünü değerlendirmeyi amaçlamaktadır.

Yöntem: Sıçanlar belirtildiği gibi gruplara ayrılmıştır: Grup 1: Kontrol (n=8), Grup II: R: RAD verilen grup (30 mg/kg/gün, n=8), Grup III: V: VPA verilen grup (0,5 g/kg/gün, n=10), Grup IV: V+R: VPA+ RAD verilen grup (30 mg/kg/gün, n=11). Hayvanlara 7 gün süreyle (i.p) VPA, RAD ve VPA+RAD verildi. Oksidatif stresle ilgili biyokimyasal parametreler dalak homojenatlarında tayin edildi.

Bulgular: VPA, dalakta lipid peroksidasyon ve siyalik asit seviyelerini yükselterek, alkalen fosfataz aktivitesini artırarak ve süperoksit dismutaz, glutatyon s transferaz ve glutatyon peroxidaz aktivitelerini azaltarak oksidatif stresi yükseltmiştir. VPA verilen gruba RAD uygulanması, LPO, SA ve asit fosfataz düzeylerini azaltırken, doku faktörü, SOD, GST ve GPx aktivitelerini artırdı.

Sonuç: RAD, koruyucu etkisini göstererek VPA verilen gruptaki biyokimyasal sonuçları normale çevirdi. RAD, VPA tedavisi sırasında yararlı olabilecek oksidatif stresi önleme potansiyeline sahiptir.

Anahtar Sözcükler: Radikut, valproik asit, oksidatif stres, antioksidanlar, dalak, sıçan.

Introduction

Valproic acid (2-Propyl-Pentanoic Acid, VPA) is a widely preferred antiepileptic drug in the world. In addition, it is used in controlling mood disorders, bipolar and schizophrenia, several kinds of seizures, and in the treatment of migraine[1]. The use of VPA may cause serious complications, although the mechanism of which has not yet been determined. These complications are thought to be caused by oxidative stress. Depending on the use of VPA, hepatotoxicity, teratogenicity, pancreatitis, and even coma or death may develop. VPA can inhibit various functions of both the innate and the adaptive immune cells[2].

Radicut (RAD, 3-methyl-1-phenyl-2-pyrazoline-5-one, Edaravone) was the first neuroprotective drug to be introduced worldwide. RAD acts as a radical scavenger and performs anti-oxidant ability by inhibiting lipid peroxidation[3]. RAD can diffuse into many disease-affected organs and protect many tissues. As well as being a free radical scavenger, RAD has anti-cytokine, anti-apoptotic, and anti-necrotic effects in several diseases[4].

The spleen, being the biggest peripheral lymphatic organ, stores approximately one-fourth of the body's lymphocytes and is critical in launching an immune response[5]. It

combines the innate and adaptive immune systems and is also responsible for making substances that have a key role in inflammation and healing[6]. Thus, dysfunction of the spleen as a result of VPA-induced toxicity or damage may also lead to impaired immune response. Soria-Castro et al have reported an increased bacterial load of 24 and 48 hpi in the spleen of VPA-treated mice[2]. Espandiari et al. have revealed that VPA toxicity tends to affect the spleen significantly and appears to be the most susceptible in the young[7].

Based on the suppression of the immune system functions by VPA, the study aims to evaluate the potential protective role of RAD in VPA-induced oxidative stress in splenic tissues of rats.

Material and Methods

Subjects

All experimental protocols were approved by the Marmara University Animal Care and Use Committee (134.2013mar, Date:20.02.2014). Thirty-seven female Sprague Dawley rats were included in the study. The animals were fed with a standard pellet provided by Varnalı Feed Mill in Silivri, Istanbul. (Turkey) and they access water ad libitum.

Experimental Design

Rats were divided into four groups: Group 1: Controls (n=8), Group II: R: RAD-given group (30 mg/kg/day, n=8), Group III: V: VPA-given group (0.5 g/kg/day, n=10), Group IV: V+P: VPA+RAD-given group (30 mg/kg/day, n=11). VPA (Merck, Germany), RAD (Fluka, Switzerland), and VPA+RAD were dissolved in saline and given to the animals for 7 days (i.p). On the 8th day, rats were sacrificed under anesthesia, tissues were taken, and homogenized, and 10% (w/v) spleen homogenates were prepared.

Parameters

Biochemical parameters; total protein[8], lipid peroxidation (LPO)[9], sialic acid (SA)[10], glutathione (GSH)[11], acid phosphatase (ACP)[12], alkaline phosphatase (ALP)[12], tissue factor (TF)[13], superoxide dismutase (SOD)[14], glutathione-S-transferase (GST)[15], glutathione peroxidase (GPx)[16], catalase (CAT)[17] were determined in spleen homogenates.

Statistics

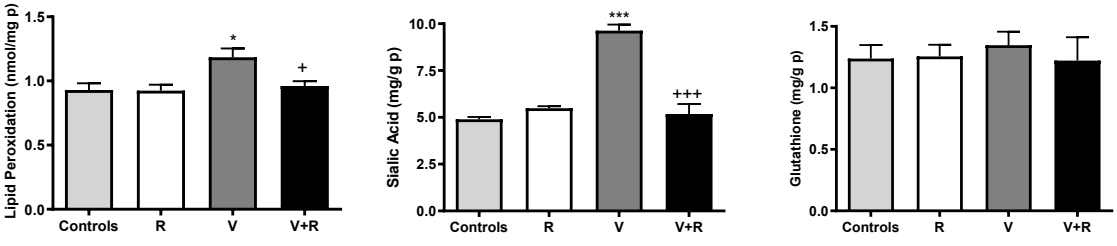
Statistical analysis of the study was performed by using Kruskal Wallis and Dunn's multiple comparison tests of the GraphPad Prism 6.0 (San Diego, USA). Values are given as mean±standard error and a p-value less than 0.05 was regarded as significant.

Results

Levels of LPO, SA and GSH

Figure 1 shows the LPO, SA, and GSH levels. Significant increases in spleen LPO and SA levels were found in the V group compared to the controls ($p<0.05$, $p<0.001$, respectively), and a significant decrease in LPO and SA levels were detected in the V+R group compared to the V group ($p<0.05$, $p<0.001$, respectively). GSH activity was increased in the V group compared to the controls and also administration of VPA decreased GSH activity in the V+R group compared to the V group, but these results were statistically insignificant.

Figure 1. Levels of LPO, SA, and GSH in spleen tissue.



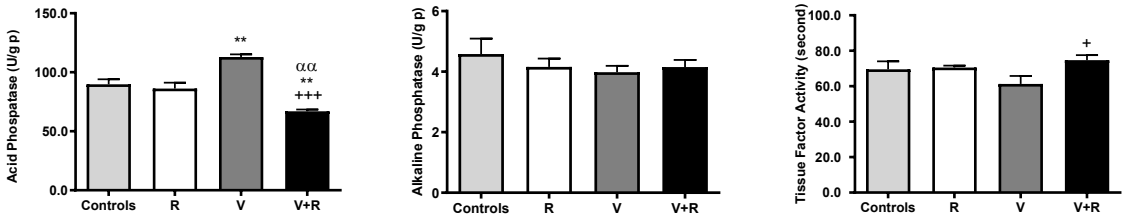
R: Radicut-given group; V: Valproic acid-given group; V+R: Valproic acid and Radicut-given group. *$p<0.05$, ***$p<0.001$ significantly different from Controls; +$p<0.05$, +++$p<0.001$ significantly different from group V.

Activities of ACP, ALP, and TF

Activities of ACP, ALP, and TF are shown in Figure 2. ACP level was significantly increased in the V group compared to the controls ($p<0.01$) and administration of RAD decreased ACP level significantly in the V+R group compared to controls, R group, and the V group ($p<0.01$, $p<0.01$, $p<0.001$, respectively). A slight increase was found in the ALP activity of the V group compared to the controls, but the result was statistically insignificant. TF activity increased in the V group compared to the controls, but the result

was statistically insignificant. Besides, administration of RAD significantly decreased TF activity in the V+R group compared to the V group ($p<0.05$).

Figure 2. Activity of ACP, ALP, and TF in spleen tissue.

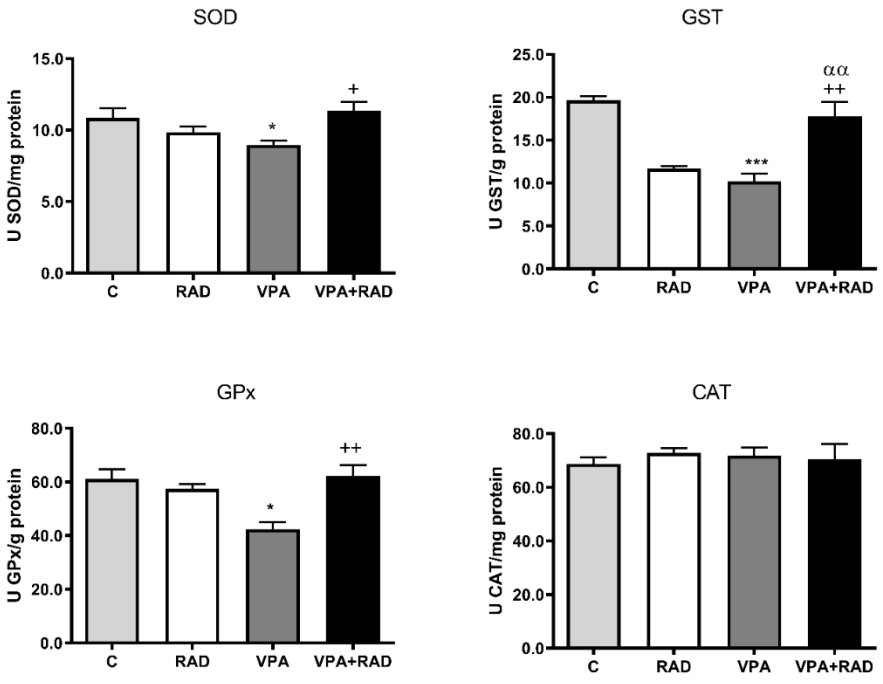


R: Radicut-given group; V: Valproic acid-given group; V+R: Valproic acid and Radicut-given group. **$p<0.01$ significantly different from Controls; $^{aa}p<0.01$ significantly different from group R; +$p<0.05$, +++$p<0.001$ significantly different from group V.

Activities of SOD, GST, GPx, and CAT

Activities of SOD, GST, GPx, and CAT are shown in Figure 3. Significant decreases in spleen SOD, GST, and GPx activities were detected in the V group compared to the controls ($p<0.05$, $p<0.001$, $p<0.05$, respectively), and RAD administration caused a significant increase in SOD, GST, and GPx activities in the V+R group compared to the V group ($p<0.05$, $p<0.01$, $p<0.01$, respectively). CAT activity increased slightly in the V group compared to the controls, however, the result was statistically insignificant.

Figure 3. Activities of SOD, GST, GPx, and CAT in spleen tissue.



R: Radicut-given group; V: Valproic acid-given group; V+R: Valproic acid and Radicut-given group. *p<0.05, ***p<0.001 significantly different from controls; ^{aa}p<0.01 significantly different from group R; +p<0.05, ++p<0.01 significantly different from group V.

Discussion

The spleen is closely related to the liver due to the blood flow connection between them. As a result, disorders affecting the liver impair the spleen. It was known that VPA has many side effects on liver function and it may cause serious and life-threatening damage to the liver which results in liver toxicity, cirrhosis, and hepatic encephalopathy[18]. VPA also affects other organs by increasing free radicals, triggering oxygen-dependent tissue injury, and causing oxidative damage in the body[19]. Since there are limited studies on the effects of VPA on the spleen, the findings of this study will form the basis for new studies.

Oxidative stress occurs when there are too many free radicals and/or low antioxidant defense. LPO is considered a good biomarker of oxidative stress due to its production by free radicals[20]. In previous studies, it was shown that VPA treatment increases the production of free radicals and elevates LPO levels[21-23]. In the present study, VPA elevated LPO levels significantly. This increase in LPO levels may be a result of increased

ROS production due to VPA administration and may also represent VPA's negative effects on antioxidant homeostasis in the spleen, which might result in reduced immunological function. The administration of RAD to the V group reversed the increased LPO levels which might be attributed to RAD's ability to function as a radical scavenger and as a tissue protector against oxidative stress in spleen tissue. RAD scavenges the radicals by donating electrons and inhibits lipid peroxidation by scavenging peroxyl radicals in addition to hydroxyl radicals.

Sialic acids (Sas) are monosaccharides with a nine-carbon backbone localized on the surface of cell membranes. SAs are cytoprotective and altered SA level is used as a marker of various inflammatory diseases[24]. SA is suggested to be an inflammation marker and it was pointed that increased levels of SA in intestinal and cardiac tissues as a result of VPA treatment[25,26]. In our current study, VPA administration caused an elevation in the spleen SA level which reflects the self-protection of the organism. The SA-lowering effect of RAD might be attributed to its protective effect on membrane stability in the spleen.

GSH is an endogenous cellular protector in the antioxidant system. It is crucial in the detoxification of hydrogen peroxide and other peroxides[27]. VPA treatment slightly increased GSH level, which may be an initial adaptive response of the immune system to elevated oxidative stress in VPA-induced toxicity.

ACP is an enzyme that provides a phosphate group to tissues. It is also a useful parameter as its amount increases in diseases. Consistent with previous studies[28,29], VPA caused an increase in spleen ACP activity which may be a reason for the disruption in membrane integrity and deleterious effects of radicals. With the RAD administration, VPA-induced spleen ACP increase was reduced. Therefore, treatment with RAD may have been effective in reversing the VPA-induced spleen damage due to the curative effect of RAD.

ALP plays a role in inflammation, thus it can be used as a parameter in diseases. Besides, it is an important enzyme in the calcification process of the cell and it was suggested that ALP may be a target in the prevention of diseases[30]. In this study, a slight decrease in ALP activity was found in the VPA-given group. It may be the result of VPA's effect on spleen tissue ALP activity in the direction of inhibition. However, this result was statistically insignificant.

TF is an important cell membrane component and also a coagulation factor. TF activity is measured by the prothrombin time test and shortened clot formation time indicates increased activity[31]. VPA administration decreased clot formation time indicating

increased TF activity in the V group, which may be a consequence of altered membrane composition in spleen tissue. RAD reversed this situation by maintaining membrane stability and thereby decreasing spleen TF activity significantly in the V+R group.

The antioxidant enzymes SOD, GST, CAT, and GPx are crucial in defending against the deleterious effects of radicals to protect the cell. Immune cells are very susceptible to changes in antioxidant status because they play critical roles in the immune system by releasing large amounts of ROS. Furthermore, immunocytes have plasma membranes that are high in polyunsaturated fatty acids, making them susceptible to LPO[32]. SOD and CAT enzymes, in this perspective, are essential for first-line defense responses. VPA modulates the enzymatic antioxidant activity negatively or positively[33]. Thus, the results of antioxidant levels associated with VPA treatment have varied in various studies. This may also be due to the difference in the dose and duration of treatment used in the treatment of VPA. Although Kurekci et al.[34] and Cengiz et al.[19] found increased activities of these enzymes, decreased activities of SOD, GST, and GPx in several tissues caused by VPA administration were reported in previous studies[35-38]. Based on these findings, in our study, although GSH and CAT were not affected by VPA or RAD treatments, decreased SOD, GST, and GPx activities were found in the V group compared to the controls. Besides, RAD treatment increased these antioxidant enzyme activities in the V group. This diminishment in SOD, GST, and GPx activities may be related to higher levels of ROS, as well as the depletion of antioxidant enzymes that are in defense against ROS. RAD was potentially effective in eliminating the detrimental effects of VPA administration on tissues. Administration of RAD to the V group reversed the results probably by its ameliorative effect on spleen tissue. RAD-given V group reversed these defects which may have done with the feature of RAD being a radical scavenger and it acts as a tissue protector against oxidative stress in the spleen. In previous studies, RAD also showed its tissue protective property on different tissues[3,4,28,29,39].

Conclusion

We suggest that RAD may be potentially useful for preventing the spleen from oxidative stress during VPA treatment which may restore the functions of the spleen in the immune response.

Acknowledgements

We thank Dr. Leyla Koc Ozturk for her valuable support in the present study.

REFERENCES

1. Tunali S. The effects of vitamin B6 on lens antioxidant system in valproic acid-administered rats. *Hum Exp Toxicol*. 2014;33:623-628.
2. Soria-Castro R, Chávez-Blanco AD, García-Pérez BE, et al. Valproic acid inhibits interferon-γ production by NK cells and increases susceptibility to Listeria monocytogenes infection. *Sci Rep*. 2020;10:1-14.
3. Higashi Y, Jitsuiki D, Chayama K, Yoshizumi M. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a novel free radical scavenger, for treatment of cardiovascular diseases. *Recent Pat Cardiovasc Drug Discov*. 2006;1:85-93.
4. Cakmak NH, Yanardag R. Edaravone, a free radical scavenger, protects liver against valproic acid induced toxicity. *J Serb Chem Soc*. 2015;80:627-637.
5. Nolte MA, Hamann A, Kraal G, Mebius RE. The strict regulation of lymphocyte migration to splenic white pulp does not involve common homing receptors. *Immunology*. 2010;106:299-307.
6. Mebius RE, Kraal G. Structure and function of the spleen. *Nat Rev Immunol*. 2005;5:606-616.
7. Espandiari P, Zhang J, Schnackenberg LK, et al. Age-related differences in susceptibility to toxic effects of valproic acid in rats. *J Appl Toxicol*. 2008;28:628-637.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193:265-275.
9. Yagi K. Assay for blood plasma or serum. *Methods Enzymol*. 1984;105:328-337.
10. Warren L. The thiobarbituric acid assay of sialic acids. *J Biol Chem*. 1959;234:1971-1975.
11. Beutler E. Gluthatione in red blood cell metabolism: In: *Red Cell Metabolism*. A Manual Biochemical Methods. New York: Grune and Stratton; 1975; pp. 112-4.
12. Walter K, Schült C. Acid and alkaline phosphatase in serum (two point method). In: *Methods of Enzymatic Analysis* Ed: Bergmeyer HU, 2nd ed. FL, 1974;856-86.
13. Ingram GIC, Hills M. Reference method for the one stage prothrombin time test on human blood. *Thromb Haemostas*. 1976;36:237-238.
14. Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte SOD activity and other parameters of copper status in rats ingesting lead acetate. *Toxicol Appl Pharmacol*. 1986;82:512-520.

15. Habig WH, Jacoby WB. Assays for differentation of glutathione-s-transferases. *Methods in Enzymol*. 1981;77:398-405.

16. Paglia DE, Valentine WN. Studies on the quantitative and quantitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967;70:158-168.

17. Aebi H. Catalase in vitro. *Methods in Enzymol*. 1984;105:121-126.

18. Gayam V, Mandal AK, Khalid M, Shrestha B, Garlapati P, Khalid M. Valproic acid induced acute liver injury resulting in hepatic encephalopathy-a case report and literature review. *J Community Hosp Intern Med Perspect*. 2018;8:311-314.

19. Cengiz M, Yüksel A, Seven M. The effects of carbamazepine and valproic acid on the erythrocyte glutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children. *Pharmacol Res*. 2000;41:423-425.

20. Messaoudi I, El Heni J, Hammouda F, Saïd K, Kerkeni A. Protective effects of selenium, zinc, or their combination on cadmium-induced oxidative stress in rat kidney. *Biol Trace Elem Res*. 2009;130:152-161.

21. Tong V, Teng XW, Chang TK, Abbott FS. Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. *Toxicol Sci*. 2005;86:427-435.

22. Chaudhary S, Ganjoo P, Raiusddin S, Parvez S. Nephroprotective activities of quercetin with potential relevance to oxidative stress induced by valproic acid. *Protoplasma*. 2015;252:209-217.

23. Sokmen BB, Tunali S, Yanardag R. Effects of vitamin U (S-methyl methionine sulphonium chloride) on valproic acid induced liver injury in rats. *Food Chem Toxicol*. 2012;50:3562-3566.

24. Schauer R, Kamerling JP. Exploration of the sialic acid world. *Adv Carbohydr Chem Biochem*. 2018;75:1-213.

25. Oktay S, Alev B, Tunali S, et al. Edaravone ameliorates the adverse effects of valproic acid toxicity in small intestine. *Hum Exp Toxicol*. 2015;34:654-61.

26. Ustundag UV, Tunali S, Alev B, et al. Effects of Chard (Beta Vulgaris L. Var. Cicla) on cardiac damage in valproic acid–induced toxicity. *J Food Biochem*. 2016;40:132-139.

27. Pastore A, Piemonte F, Locatelli M, et al. Determination of blood total, reduced, and oxidized glutathione in pediatric subjects. *Clin Chem*. 2001;47:1467-1469.

28. Oktay S, Alev B, Ozturk LK, et al. Edaravone ameliorates valproate-induced gingival toxicity by reducing oxidative-stress, inflammation and tissue damage. *Marmara Pharm J*. 2016;20:243-251.

29. Çelik Ç, Bayrak BB, Hacıhasanoğlu Çakmak N, Yanardağ R. Protective effect of edaravone on rat testis after valproic acid treatment. *J Res Pharm*. 2022;26:52-62.

30. Haarhaus M, Brandenburg V, Kalantar-Zadeh K, Stenvinkel P, Magnusson P. Alkaline phosphatase: a novel treatment target for cardiovascular disease in CKD. *Nat Rev Nephrol*. 2017;13(7):429-442.

31. Camerer E, Huang W, Coughlin SR. Tissue factor-and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *PNAS*. 2000;97:5255-5260.

32. Farag MR, Moselhy AAA, El-Mleeh A, et al. Quercetin alleviates the immunotoxic impact mediated by oxidative stress and inflammation induced by doxorubicin exposure in rats. *Antioxidants (Basel)*. 2021;10(12):1906. doi: 10.3390/antiox10121906.

33. Cárdenas-Rodríguez N, Coballase-Urrutia E, Rivera-Espinosa L, et al. Modulation of antioxidant enzymatic activities by certain antiepileptic drugs (valproic acid, oxcarbazepine, and topiramate): evidence in humans and experimental models. *Oxid Med Cell Longev*. 2013;2013. doi: 10.1155/2013/598493.

34. Kurekci AE, Alpay F, Tanindi S, et al. Plasma trace element, plasma glutathione peroxidase, and superoxide dismutase levels in epileptic children receiving antiepileptic drug therapy. *Epilepsia*. 1995;36:600-604.

35. Chaudhary S, Parvez S. An in vitro approach to assess the neurotoxicity of valproic acid-induced oxidative stress in cerebellum and cerebral cortex of young rats. *Neurosci*. 2012;225:258-268.

36. Yis U, Seckin E, Kurul SH, Kuralay F, Dirik E. Effects of epilepsy and valproic acid on oxidant status in children with idiopathic epilepsy. *Epilepsy Res*. 2009;84:232-237.

37. Aranarochana A, Sirichoat A, Pannangrong W, Wigmore P, Welbat JU. Melatonin ameliorates valproic acid-induced neurogenesis impairment: The role of oxidative stress in adult rats. *Oxid Med Cell Longev*. 2021:2021. doi: 10.1155/2021/9997582.

38. Turkyilmaz IB, Altas N, Arisan I, Yanardag R. Effect of vitamin B6 on brain damage in valproic acid induced toxicity. *J Biochem Mol Toxicol.* 2021;35:e22855. doi: 10.1002/jbt.22855.

39. Tajima S, Bando M, Ishii Y, et al. Effects of edaravone, a free-radical scavenger, on bleomycin-induced lung injury in mice. *Eur Respir J*. 2008;32:1337-1343.