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THE EFFECTS OF ALPHA LIPOIC ACID ON LENS INJURY IN RATS ADMINISTERED WITH VALPROIC ACID

VALPROİK ASİT UYGULANAN SIÇANLARDA ALFA LİPOİK ASİDİN LENS HASARI ÜZERİNE ETKİLERİ

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ABSTRACT

Objective: Valproic acid (2-propyl valeric acid; VPA) is an effective short-chained fatty acid which is used for the treatment of migraine and schizophrenia. Though it provides effective treatment, its side effects are associated with free radicals and in this way it affects many organs and tissues. Alpha lipoic acid (ALA) is known to be a powerful antioxidant.

Material and Methods: The aim of this current study was to investigate the protection of ALA on VPA induced lens injury. Female rats were split into four groups as follows: 1st group, control animals (corn oil per day for 15 days); 2nd group, ALA administered group (50 mg per kg each day for 15 days); 3rd group, VPA administered group (500 mg per kg each day for 15 days) and 4th group, VPA and ALA administered group to which the same dose was given at the same time each day. On the 16th day, lens tissues were taken.

Results: Lens glutathione levels and glutathione-S-transferase activities were decreased while lipid peroxidation and protein carbonyl levels, superoxide dismutase, glutathione peroxidase and reductase, aldose reductase and sorbitol dehydrogenase activities were elevated after VPA administration. ALA reversed these levels and activities in the VPA group.

Conclusion: We can conclude that ALA used its antioxidant property and ameliorated VPA induced lens injury.

Keywords: Valproic acid, alpha lipoic acid, lens tissue, oxidative stress

ÖZET

Amaç: Valproik asit (VPA) kısa zincirli bir yağ asididir ve migren ile şizofreni tedavisinde kullanılır. Tedavi edici etkilerinin olmasına rağmen, bu ilacın yan etkileri serbest radikaller ile ilişkilidir ve bu yol ile birçok organ ve dokuyu etkilemektedir. Alfa lipoik asit (ALA), güçlü bir antioksidandır.

Gereç ve Yöntem: Bu çalışmada, ALA'in VPA ile oluşturulan lens hasarı üzerine koruyucu etkileri araştırıldı. Dişi sıçanlar dört gruba ayrıldı: 1. grup, kontrol hayvanları (15 gün boyunca her gün mısır özü yağı verildi); 2. grup, ALA verilen grup (15 gün boyunca her gün 50 mg/kg verildi); 3. grup, VPA uygulanan grup (15 gün boyunca her gün 500 mg/kg uygulandı), 4. grup; Her gün aynı saatte ve dozda VPA ile ALA uygulanan grup, 16'ıncı günde, lens dokuları alındı.

Bulgular: VPA uygulanmasından sonra, lens glutatyon düzeyleri ve glutatyon-S-transferaz aktiviteleri azalırken lipid peroksidasyonu ve protein karbonil seviyeleri, süperoksit dismutaz, glutatyon peroksidaz ve redüktaz, aldoz redüktaz ve sorbitol dehidrojenaz aktiviteleri artış gösterdi. ALA, VPA grubundaki bu seviyeleri ve artışları tersine çevirdi.

Sonuç: ALA'in antioksidan özelliğini kullandığı ve VPA ile oluşturulan lens hasarını iyileştirdiği sonucuna varılmıştır.

Anahtar Kelimeler: Valproik asit, alfa lipoik asit, lens dokusu, oksidatif stres

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INTRODUCTION

Valproic acid (VPA) is a short-chained and branched fatty acid having the chemical formula of "2-propyl valeric acid". It is derived from valeric acid which exists in *Valeriana officinalis* and was synthesized in the late 19th century by Burton (1). Nowadays, this molecule is used and widely preferred due to its anticonvulsant effect in the treatment of many seizures such as complex partial/tonic-clonic, depression, strong headaches like migraine, and schizophrenia (2-4). Despite its protective and preventive effects, VPA has been declared to have many serious side effects and this drug has taken the lead in the formation and construction of free radical species which damage many tissues and organs like heart, lung, liver, brain, testis, etc. (5-8).

Researchers have been trying to eliminate the harmful effect of VPA by using different protective agents. One of the solutions was put forward by Sokmen et al. and this involved using a sulphur containing compound like S-methyl methionine sulfonium chloride (MMSC), also known as Vitamin U and a powerful antioxidant. This was then proven to be effective by Gezginci-Oktayoglu et al. and Oztay et al. (9-11).

Based on this approach, we used alpha lipoic acid (ALA) in our study. ALA occurs in many types of nutrition such as meat and green vegetables (12). This sulphur containing compound has a unique structure in that it carries lipid while at the same time having water-soluble properties (13). ALA has a stimulant effect on insulin-sensitive cells, thereby facilitating glucose uptake. It has a stabilizing effect on cell redox system, proteins and some molecules such as glutathione (14). In addition, ALA has been described as being involved in the most important energy production pathway, the Krebs cycle, where ALA plays a vital role as a coenzyme assisting in the transfer of acyl groups (15). Its protective effects on different systems of VPA and α-cypermethrin have been reported by many researchers, respectively (7, 16, 17).

The lens has a special closed system and is well designed for transmitting light. It is made up of proteins at a much higher level when compared with other organs (18). In the crystalline structure of proteins, many cysteine residues are found (19). The biological composition of these crystalline structures are sometimes affected by free radical attacks, and as a result become damaged by processes such as oxidation (20). Moreover, VPA affects the lens by increasing free radical levels. It may lead to cell death since it affects glucose levels and forms hypoxia in human epithelial cells (21-23).

In the light of this, the aim of our study was to investigate the protection provided by ALA in VPA induced lens injury.

MATERIAL AND METHODS

Animals

The rats were supplied by the Experimental Animal Implementation and Research Centre, DEHAMER, Marmara University. Ethical approval was obtained from the Marmara University Animal Care and Use Committee (Date: 23.03.2015, No: 34.mar). Female Sprague-Dawley rats (6 months old) were used. The animals received standard food as pellets. They had access to tap water *ad libitum*.

Experimental procedures

The Sprague-Dawley rats were split into four groups. The 1st group consisted of control animals which received corn oil; the 2nd group consisted of animals which received ALA (50 mg per kg); the 3rd group included animals to which VPA (0.5 g per kg) was administered; and the 4th group incluced animals which received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5g per kg). Corn oil and ALA were applied by gavage technique while VPA was applied intraperitoneally for 15 days. On the last day of experiment, all animals fasted overnight after which blood was taken from their hearts using a sterile injector. Their lens tissues were collected. The lens tissues were homogenized in physiological saline (0.9% NaCl) for the preparation of 10% (w/v) homogenate. The homogenates were centrifuged at 10000 x g at +4°C for 10 minutes and supernatants were used for the analysis.

Biochemical analyses

In clear supernatants, reduced glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PC) levels were taken according to Beutler, Ledwozyw et al. and Levine et al. (24-26). Glutathione-S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities were performed using the methods of Habig and Jakoby, Mylroie et al., Wendel, and Beutler (27-30). The lens marker enzymes aldose reductase (AR) and sorbitol dehydrogenase (SDH) were determined as per the methods of Hayman and Kinoshita, and Barretto and Beutler (31, 32). All the levels and enzyme activities were recorded according to the protein levels of the lens with reference to Lowry et al. (33).

Statistical analyses

Statistical analysis of biochemical results was performed via GraphPad Prism 6.0 (GraphPad Software, San Diego, California, USA). The values were expressed as means ± standard deviation (SD). The results were evaluated using an unpaired t-test and analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The value of $p<0.05$ was considered statistically significant.

RESULTS

The lens GSH levels are shown in Figure 1. VPA administration decreased GSH levels in the control group in a

significant manner (p<0.01). ALA significantly reversed this level in the VPA group (p<0.01) (Figure 1).

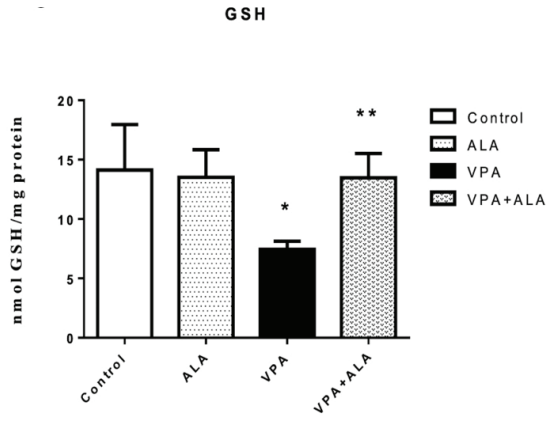


Figure 1: The lens GSH levels of control and experimental groups

GSH: reduced glutathione, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group; **p<0.01 vs VPA group.

The lens LPO levels are presented in Figure 2. According to the results, LPO levels of the VPA group were found to be increased significantly as compared to the control group (p<0.0001). Administration of ALA significantly decreased this level in the VPA group, (p<0.0001) (Figure 2).

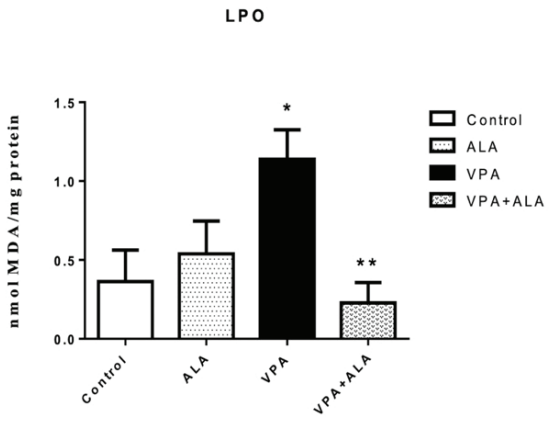


Figure 2: The lens LPO levels of the control and experimental groups

LPO: lipid peroxidation, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.0001 vs control group; **p<0.0001 vs VPA group.

The lens PC levels are given in Figure 3. VPA significantly increased the PC levels of the control groups (p<0.01). In the VPA+ALA group, this level was reversed in a significant manner as compared to the VPA group (p<0.0001) (Figure 3).

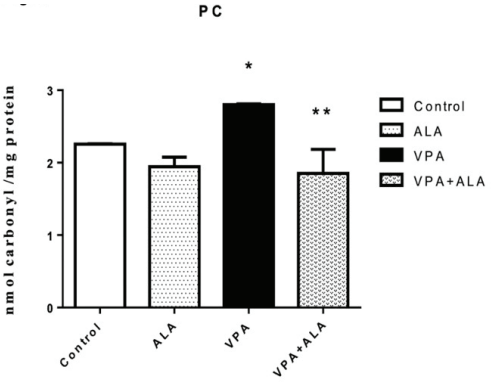


Figure 3: The PC levels of the control and experimental groups

PC: protein carbonyl, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group, **p<0.0001 vs VPA group.

The lens GST activities can be seen in Figure 4. ALA significantly decreased GST activities of the control group (p<0.001). Administration of VPA resulted in a diminishment in GST activities in the control group which was statistically significant (p<0.0001). In the VPA+ALA group, this activity was reversed as compared to the control group (p<0.0001) (Figure 4).

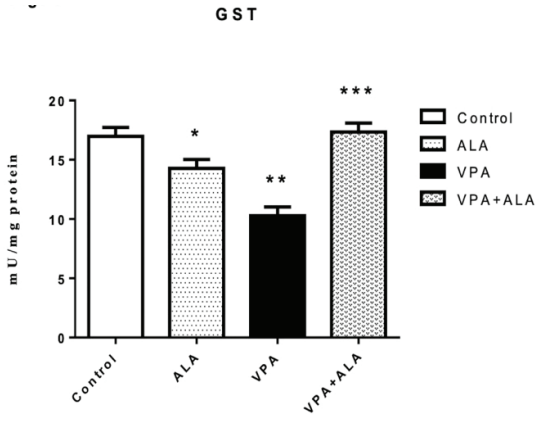


Figure 4: The GST activities of the control and experimental groups

GST: glutathione-S-transferase, ALA: alpha lipoic acid, VPA: valproic acid; Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.001 vs control group; **p<0.0001 vs control group; ***p<0.0001 vs VPA group.

The lens SOD activities are shown in Figure 5. An elevation in SOD activities was observed in the VPA group as compared to the control group ($p<0.01$). Administration of ALA reversed the activities of the VPA group which was statistically significant ($p<0.001$) (Figure 5).

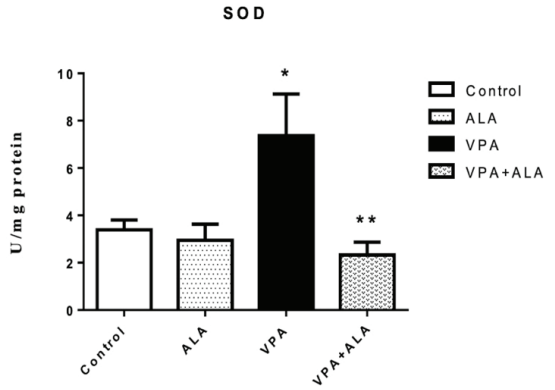


Figure 5: The SOD activities of the control and experimental groups
SOD: superoxide dismutase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group; **p<0.001 vs VPA group.

The lens GPx activities are shown in Figure 6. According to the results, a significant elevation was observed after ALA and VPA administration to the control group ($p<0.01$, $p<0.001$) respectively. These activities were significantly reversed in the VPA+ALA group as compared to the VPA group ($p<0.0001$) (Figure 6).

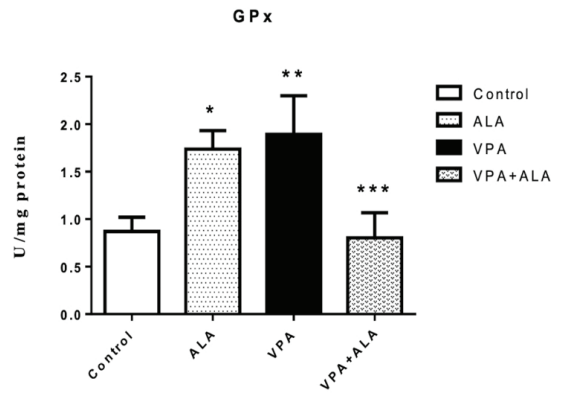


Figure 6: The GPx activities of the control and experimental groups
GPx: glutathione peroxidase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group; **p<0.001 vs control group; ***p<0.0001 vs VPA group.

The lens GR activities are seen in Figure 7. The GR activities of the VPA group were recorded as being elevated as compared to the control group ($p<0.05$). These activities were found to be significantly decreased after ALA administration to the VPA group ($p<0.05$) (Figure 7).

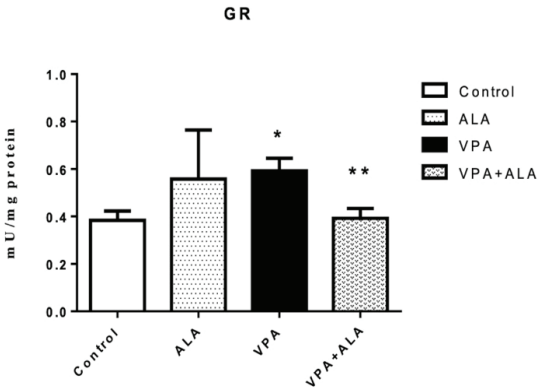


Figure 7: The GR activities of the control and experimental groups
GR: glutathione reductase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.05 vs control group; **p<0.05 vs VPA group.

The lens AR activities are presented in Figure 8. AR activities were increased after VPA administration to the control group which was statistically significant ($p<0.0001$). ALA significantly reversed these activities in the VPA group ($p<0.05$) (Figure 8).

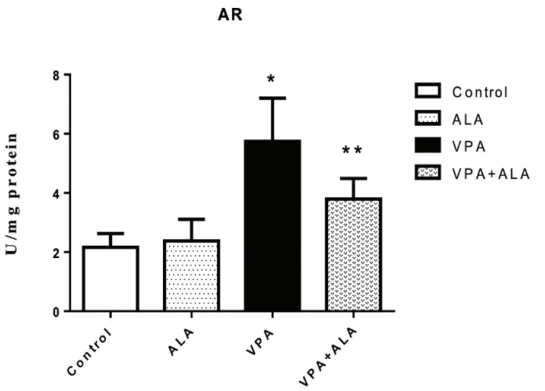


Figure 8: The AR activities of the control and experimental groups
AR: aldose reductase, ALA: alpha lipoic acid, VPA: valproic acid; Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.0001 vs control group; **p<0.05 vs VPA group.

The lens SDH activities are given in Figure 9. After VPA administration, SDH increased in the control group which was statistically significant ($p<0.01$). ALA insignificantly decreased these activities of the VPA group.

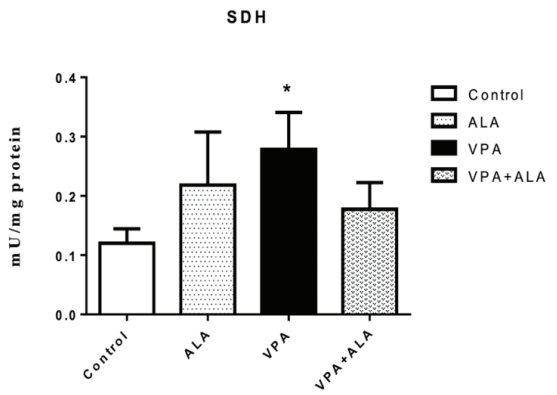


Figure 9: The SDH activities of the control and experimental groups
SDH: sorbitol dehydrogenase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *$p<0.01$ vs control group.

DISCUSSION

The main function of the lens is the transmission and focusing of light onto the retina. This high-speed transmission requires the highest protein levels (34). Due to the function of the eye being related to light, all layers of an eye, including the lens, are open to being affected by the presence of oxygen (35). This means that the lens layer is susceptible to damage induced by reactive oxygen species (ROS) . In addition, VPA has been widely described as having a triggering effect on free radicals in many tissues including lens tissue (5, 22, 36).

The lens has been documented as evolving an anaerobic biological system by consisting of glutathione at mM levels (37). The protective effect of GSH is essential for GPx activity, and in general for protection of protein structure against oxidant molecules (38). When GSH is oxidized by the presence of free radicals, it will transform into oxidized glutathione (GSSG). Their transformation should be kept under strict regulation because lens membrane is capable of permitting GSSG while it is impermeable to GSH (38). Besides, lens crystallin consists of many cysteine residues and Lou reported that their connection with GSH might be initially protective (39). In this study, GSH levels decreased in the lenses of VPA treated rats, probably due to the free radical triggering effect of VPA. Reduced form of ALA (dihydrolipoic acid) helps convert cystine to cysteine. The decreased GSH level of the VPA group may have been increased in the VPA+ALA group owing to effects of ALA on the conversion of cystine to cysteine (a substrate for GSH synthesis) (40, 41).

Moreover, a diminishing tendency of GSH levels via VPA affected the membrane stability and protein structure by increasing LPO and PC levels. This is because amino acids like cysteine and tyrosine and other proteins are responsible for the prevention of excess oxygen derived metabolites (38). In addition, VPA has been referred to as increasing LPO and PC levels in the lens by affecting membrane and protein composite (22). ALA reversed these levels in VPA administered rats. In addition to the radical scavenging activity of ALA, the ameliorative property of ALA has been described by Neal et al. as having an elevator effect on cystine levels which can be related to protein stabilization (23).

GSH-based redox systems in the lens carry a great importance due to high protein levels and a necessity of their thiol group stabilization (42). Likewise, GST, GPx, GR and SOD are important antioxidant enzymes in metabolism and, according to research , lens tissue hosts GPx and SOD at a very high level (43). VPA has been reported as having a lowering effect on mitochondrial membrane potential and oxygen levels and an elevating effect on cell death and depletion of ATP levels which in turn has resulted in an increase in ROS levels (44). In our study, we found elevated SOD, GPx and GR activities in VPA treated rat lenses, but GST activities were diminished. Increased SOD and GPx reveals the increased levels of superoxide anion and hydrogen peroxide levels and increased GR activity means the regeneration of GPx. Decreased GST activity is associated with a decrease in GSH concentration. This is usually due to increased levels of free radicals.The positive effect of ALA may have occurred due to the ability of a reduced form of ALA (dihydrolipoic acid, DHLA) to scavenge free radicals such as superoxide and hydroxyl. More so, DHLA participates in the recycling of GSH from GSSG (45). The present findings support these approaches.

The NADPH dependent enzymes, AR and SDH, are important markers of the polyol pathway. In this case, glucose gains importance by being reduced to sorbitol by AR, and in turn, sorbitol is catalyzed to fructose by SDH. Aldose reductase (AR) has a low affinity for glucose compared to hexokinase. However, AR activity does increase in lens tissue in cases where blood glucose levels rise (such as in diabetes) (46, 47). Chateauvieux et al. mentioned in their review publications that VPA had the effect of changing fasting glucose levels in young children/teenagers (48). In the light of this , we determined that VPA increased AR and SDH activities in the lens tissues of rats. ALA decreased these activities. ALA is known as having an ability

to regulate energy metabolism and glucose regulation through its cofactor property on pyruvate / α-ketoglutarate dehydrogenase in the Krebs cycle (49). ALA may be preferred due to this positive and regulative effect in diabetic conditions. In addition, ALA is reported to increase glucose uptake by insulin-sensitive/resistant muscles, which is related to it triggering glucose transporters activities of plasma membranes in the presence of glucose (50).

CONCLUSION

Based on these results, we can assume that ALA protects lens tissue by using it's antioxidant and ameliorative the effect which decreases oxidative stress on lens tissues which were affected by the VPA administration.

Ethics Committee Approval: This study was approved by Marmara University Animal Experiments Local Ethics Committe (Date: 23.03.2015, No: 34.mar).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- R.Y., İ.B.T., Y.Ö.; Data Acquisition- Y.Ö., İ.B.T.; Data Analysis/Interpretation- R.Y., İ.B.T., Y.Ö.; Drafting Manuscript- R.Y. İ.B.T.; Critical Revision of Manuscript- R.Y.; Final Approval and Accountability- R.Y., İ.B.T.; Material or Technical Support- R.Y., İ.B.T., Y.Ö.; Supervision- R.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

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