Research Article (Araştırma Makalesi)

Protective Effect of Metformin Alone or in Combination with Valproic acid on Pentylenetetrazole-Induced Seizures in Mice

Metforminin Tek Başına veya Valproik asit ile Beraber Farelerde Pentilentetrazol ile İndüklenen Nöbetler Üzerine Koruyucu Etkisi

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Özet

Amaç: Bu çalışmanın amacı, metforminin pentilentetrazol (PTZ) ile indüklenen nöbet davranışı üzerindeki etkilerini ve nöronal hasar üzerindeki nöroprotektif etkisini araştırmaktır.

Gereç ve Yöntemler: 35-38 gram ağırlığındaki otuz beş (35) erkek BALB-c Albino fare rastgele beş gruba ayrıldı: Kontrol grubu (1), Salin+PTZ grubu (2), Valproik Asit (VPA 200 mg/kg i.p.)+PTZ grubu (3), Metformin (200 mg/kg i.p.)+PTZ grubu (4) ve VPA+Metformin+PTZ grubu (5). PTZ (60 mg/kg, intraperitoneal-i.p.), nöbetleri indüklemek için ilaç enjeksiyonundan 30 dakika sonra enjekte edildi ve nöbet aşamaları ve davranışsal skorlama değerlendirildi. İşlem tamamlandıktan sonra beyin dokuları çıkarıldı ve biyokimyasal ve histopatolojik prosedürlerle analiz edildi. Hipokampal Cornu Ammonis (CA)1, CA2, CA3 ve DG (dentat girus) bölgeleri histopatolojik olarak değerlendirildi ve oksidatif stres belirteçleri (toplam antioksidan durum (TAS), toplam oksidan durum (TOS)) ölçüldü.

Bulgular: Salin+PTZ grubuyla karşılaştırıldığında, Metformin tek başına ilk miyoklonik jerk (FMJ) başlangıç süresini etkilemedi, ancak VPA ve metformin kombinasyonun FMJ başlangıç süresini anlamlı derecede artırdığı izlendi ($p<0.05$). Ek olarak, VPA ile beraber ve/veya VPA olmadan metformin tedavisi beyin oksidatif stresini önemli ölçüde azalttı ($p<0.05$). Ayrıca, histopatolojik değerlendirme ile metformin uygulamasının ve VPA+metformin kombinasyonunun hipokampal CA1, CA2, CA3 ve DG alanlarındaki dark nöron oluşumunu azalttığı saptandı ($p<0.05$).

Sonuç: Metforminin, epileptik nöbetleri ve beyin oksidatif stresini azalttığı ve PTZ ile indüklenen nöbet sonrası nöral hasarı önlediği tespit edilmiştir.

Anahtar kelimeler: Epilepsi, Metformin, Nöronal hasar, Pentylenetetrazole

Abstract

Objective: The aim of this study was to investigate the effects of metformin on pentylenetetrazole (PTZ)-induced seizures and the neuroprotective effect of metformin on neuronal damage after pentylenetetrazole administration.

Material and Methods: Thirty-five (35) Male BALB-c Albino mice weighing 35-38 g were divided randomly into five groups: Control group (1), Saline+PTZ group (2), Valproic Acid (VPA, 200 mg/kg intraperitoneal-i.p.)+PTZ group (3), Metformin (200 mg/kg i.p.)+PTZ group (4), and VPA+Metformin+PTZ group (5). The PTZ (60 mg/kg, i.p.) was injected 30 min after drugs injection to induce seizures and seizure stages and behavioral scoring were evaluated. After completing procedure, brain tissues were removed and analyzed with biochemical and histopathological procedures. The hippocampal Cornu Ammonis (CA) 1, CA2, CA3 and DG (dentate gyrus) regions were histopathologically evaluated and oxidative stress markers (total antioxidant status (TAS), total oxidant status (TOS)) were measured.

Results: Compare to Saline+PTZ group, metformin administration alone did not affect the onset time of the first myoclonic jerk (FMJ), but combination of VPA and metformin significantly increased FMJ onset time ($p<0.05$). Additionally, the treatment of metformin with or without VPA reduced the brain oxidative stress ($p<0.05$). Furthermore, histopathological assessment demonstrated that metformin administration and the combination of VPA and metformin decreased dark neuron formation in the hippocampal CA1, CA2, CA3, and DG areas ($p<0.05$).

Conclusion: Metformin was found to be significantly effective in reducing epileptic seizures, brain oxidative stress, and preventing neural damage after PTZ-induced seizure

Keywords: Epilepsy, Metformin, Neuronal damage, Pentylenetetrazole

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INTRODUCTION

Epilepsy is a chronic neurological disease caused by the excessive irregular discharge of cerebral neurons (1). It is the second widespread neurological disease with an annual incidence of 0.05/1000 per year. The incidence and prevalence of epilepsy varies across countries. It accounts for 1% of the disease burden all over the world (2). The pathogenesis of epilepsy, particularly temporal lobe epilepsy, is complex and this process involves an initial neuronal degeneration and then sclerosis. Moreover, besides the loss of neurons, the changes in both receptors and function of channel can be observed during the course of the epileptic discharge (3). Evidence indicates that besides these neurodegenerative changes, epileptic seizures also induce oxidative stress and cause the damage of hippocampus (4-6). Previous studies have demonstrated that high ROS levels in the hippocampus leads to loss of memory function and hippocampal impairment (7,8).

Metformin (N', N'-dimethylbiguanide) is an orally administered hypoglycemic drug used widely for the treatment of type 2 diabetes mellitus (9). Metformin has been found to be beneficial in not only type 2 diabetes mellitus and also heart disease and stroke. Moreover, it has been shown in a clinical study that metformin significantly reduces a risk of stroke independent from low blood glucose levels (10). The pharmacokinetics of metformin in humans are well described. Metformin (acute and chronic administration) has been shown to cross the blood-brain barrier (11,12). The effects of metformin on the central nervous system are contradictory (13). Some studies have reported the negative effects of metformin, such as an increased risk of Alzheimer's disease and cognitive dysfunction (14,15). However, various studies on central nervous system function and pathology have shown that metformin improves hippocampal neurogenesis and enhanced spatial learning, and cognitive changes. In addition, metformin has been reported to be effective in the treatment of Huntington's disease (16,17). Moreover, metformin has been claimed to have beneficial effects in different neuroinflammatory and neurodegenerative disease models and has protective effects against apoptosis of neuronal cell (18,19). It is suggested that the beneficial effects of metformin may be related to its antioxidant activity (11).

This present study aimed to investigate the effects of metformin on pentylenetetrazole- induced seizures and to indicate the neuroprotective effect of metformin on neuronal damage after pentylenetetrazole administration.

MATERIALS AND METHODS

Animals

Male BALB-c Albino mice (n=35) weighing 35-38 g were procured from Sivas Cumhuriyet University, Sivas, Turkey. Animals were kept under a 12-hlight-dark cycle with the temperature of 20–22 °C with a standard pellet diet and fed and tap water ad libitum during the treatment period. All experiments were carried out blind between 09:00 and 17:00 h (n=7 in each experimental group). The experimental protocols were approved by the Cumhuriyet University Animal Ethics Committee (Approval no: 65202830-050.04.04-157). This study was conducted in accordance with the principles of Guide for the Care and Use of Laboratory Animals.

Drug Administration

Pentylenetetrazole (PTZ), metformine and valproic acid were dissolved in physiological saline. The drugs were purchased from Sigma-Aldrich Co., St Louis, MO, USA. Solutions were freshly prepared on the days of the experiments.

Experimental Protocols

Thirty-five mice were divided randomly into five groups for behavioral assessments (n=7 for each groups). Control group (1), Saline+PTZ group (2), Valproic Acid (VPA, 200 mg/kg i.p.)+PTZ group (3), Metformin (200 mg/kg i.p.)+PTZ group (4), and VPA+Metformin+PTZ group (5). Group 1 was defined as basic control group. The mice in group 2 received saline (10 ml/kg, i.p.). The mice in group 3 received metformin (200 mg/kg i.p.). The mice in group 4 (positive control) received VPA (200 mg/kg i.p.). The mice in group 5 received combination of Metformin and VPA at indicated doses. The pentylentetrazol (PTZ) (60 mg/kg, i.p.) was injected 30 min after drugs injection to induce seizures. Pentylenetetrazole (PTZ) was given to mice to induce epileptic seizures (60 mg/kg, i.p.) 30 min after the administration of the last dose of the medicine. Racine's Convulsion Scale (RCS) were used to evaluate the seizures stages. Seizure stages are defined by RCS as follows: no convulsion (0); twitching of vibrissae and pinnae (1); motor arrest with more pronounced twitching (2); motor arrest with generalized myoclonic jerks (3); tonic clonic seizure while the animal remained on its feed (4); tonic-clonic seizure with loss of the righting reflex (5); and lethal seizure (6). Mice were observed for 30 minutes after PTZ injection both for behavioral scoring according to RCS and for determining the time of FMJ, which indicated the seizu-

re onset (20). The observation period for PTZ-induced seizures were limited with 30 minutes duration (21). The animals were sacrificed by decapitation after two hours and the brain tissues were removed for biochemical and histopathological evaluations.

Biochemical Analyses

Preparation of Brain Tissue Homogenates

The brain tissue samples were homogenized in cold phosphate buffered saline solution (PBS, pH: 7.2) using a manual homogenizer. The homogenates were centrifuged at 10000×g for 10 min at 4 ºC and the supernatant was collected for protein concentration determination by a Bradford protein assay kit (Merck, Germany) and for TAS and TOS determination by using TAS, TOS kit (Total Oxidant Status Assay Kit, sample code: RL0024, Rel Assay Diagnostics® Mega Tıp Ltd., Gaziantep, Turkey).

Measurement of Total Antioxidant Status (TAS)

Tissue TAS concentrations were measured with an automated assay method developed by Erel. This method is based on monitoring the reaction rate of free radicals which produced during the Fenton reaction. Antioxidants in the tissue samples should suppress coloring proportionally to their concentration (22). The results were expressed in micromole Trolox equivalents per milligram tissue protein (μmol Trolox Eq/mg protein).

Measurement of total oxidant status (TOS)

Tissue TOS concentrations were measured with previous described method developed by Erel (23). Since the ferrous ion is oxidized to the ferric ion when sufficient oxidant is present in the environment, the method allows for determining TOS levels by measuring tissue levels of ferric ions (23). The results of the assay were expressed in micromole hydrogen peroxide equivalents per milligram tissue protein (μmol H_2O_2 Eq/mg protein).

Histopathologic Examination

Two hours after finishing the experimental protocols, the animals were given a high dose of urethane and transcardially perfused with 100 ml of saline followed by 100 ml fixative solution (formaldehyde %4 in 0.2 M buffer phosphate at pH: 7.4). After perfusion, all mice were sacrificed and the brains removed. After 72 h fixation with 4% formaline, brain tissues were then processed for histological analysis. After routine histological procedures, the 5-μm thick coronal serial sections were obtained using a rotary microtome (Leica model RM 2145, Germany). Ten sections including hippocampus tissue from each group were chosen by systematic randomized method and mounted on glass slides to determine the hippocampus dark neurons with Toluidine blue staining. The slides were examined with light microscope (BX51, Japan) at magnification of X40 objective lens (UPlan FI, Japan) and digital photographs were taken from hippocampal CA1, CA2, CA3 and dentate gyrus (DG) areas of both hemispheres. For quantitative analysis of dark neurons, the physical dissector method was used.

Statistical Analysis

The data were expressed as mean±SEM. For all data, the one-way ANOVA test were run and Tukey post hoc test were used for pairwise comparisons. A p value less than 0.05 was used for statistical significance.

RESULTS

Evaluation of Groups in Terms of Epileptic Behavioral Assessment

Epileptic seizure stages were determined according to Racine scores between the groups. There were statistically significant differences between the saline+PTZ group and VPA+PTZ group ($p<0.05$). However, there were no statistically significant differences between the saline+PTZ group and the Metformin+PTZ group ($p>0.05$; **Table 1**).

In terms of FMJ onset times, there were statistically significant differences between the saline+PTZ group and VPA+PTZ group ($p<0.05$; **Table 1**). Moreover, combination of VPA and Metformin significantly increased FMJ onset time compared to Metformin+PTZ group ($p<0.05$; **Table 1**).

Table 1. Effect of metformin, VPA and their combination on seizures threshold (latency) in PTZ-induced seizures in mice

Group	Racine Scale	Latency to the 1st myoclonic seizures (min)
Control	None	None
Saline (1 ml/kg serum physiologic)+PTZ (60 mg/kg)	5.33±0.21	1.44±0.36
VPA (200 mg/kg)+PTZ	2.83±0.16*#	3.01±0.38*#
Metformin (200 mg/kg)+PTZ	5.00±0.44	1.26±0.08
VPA+Metformin+PTZ	2.66±0.21*	5.49±0.96*+

Values are presented as mean±SEM. *p<0.05 compared to saline+PTZ group, #p<0.05 and +p<0.05 compared to metformin group. VPA: Valproic acid, PTZ: Pentylenetetrazole

Evaluation of Groups in Terms of Biochemical Assessment

The TAS and TOS levels in the brain were measured using commercial kits. There were no statically significant in the TAS levels in the brain tissues between each group (p>0.05; **Table 2**). There were increase in the TOS levels in the brain tissues after PTZ-induced seizures compared with the control group (p<0.05; **Table 2**). The 200 mg/kg VPA, 200 mg/kg metformin, and combination of VPA and metformin significantly reduced TOS levels in the brain tissues compared to saline+PTZ group (p<0.05; **Table 2**).

Table 2. Effect of metformin, VPA and their combination on TAS and TOS levels after PTZ-induced seizures in mice

Group	TAS (µmol/mg protein)	TOS (µmol/mg protein)
Control	0.57±0.13	1.05±0.10
Saline (1 ml/kg serum physiologic)+PTZ (60 mg/kg)	0.57±0.01	1.45±0.07*
VPA (200 mg/kg)+PTZ	0.55±0.10	1.18±0.03#
Metformin (200 mg/kg)+PTZ	0.54±0.00	1.20±0.04#
VPA+Metformin+PTZ	0.55±0.01	1.19±0.02#

Values are presented as mean ± SEM. *p<0.05 compared to control group, #p<0.05 compared to saline+PTZ group. VPA: Valproic acid, PTZ: Pentylenetetrazole, TAS: Total antioxidant status TOS: Total oxidant status

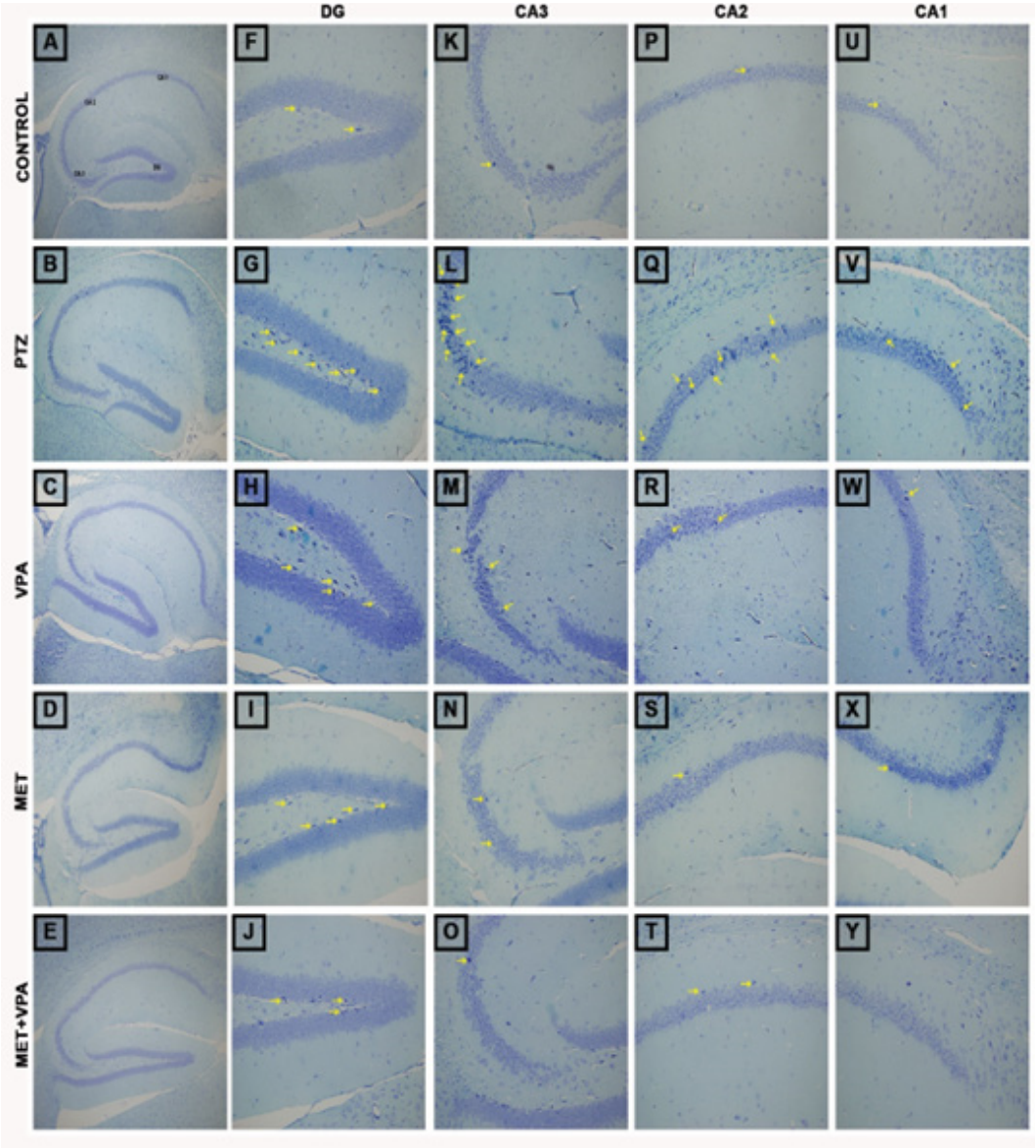

Figure 1. Histopathological evaluation of dark neurons

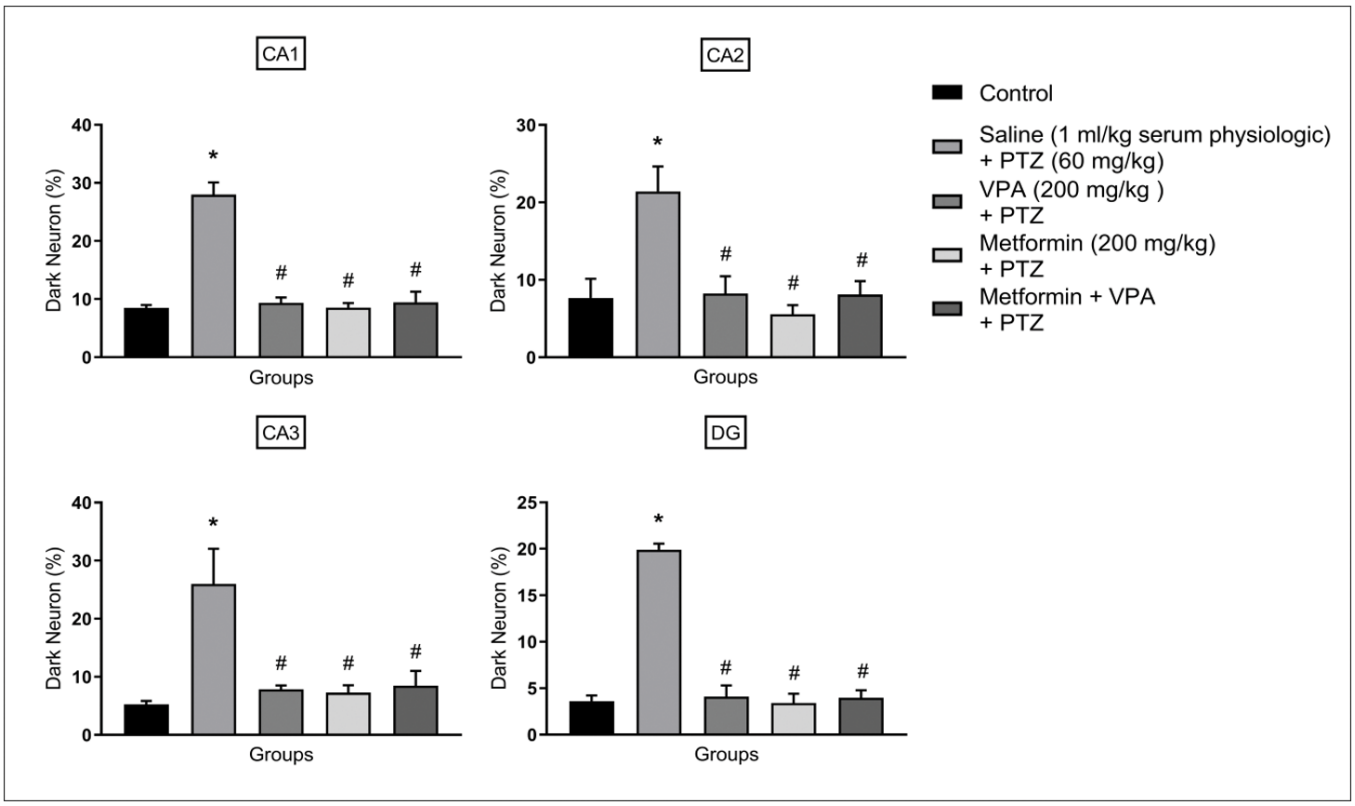

Figure 2. The Effect of Metformin on dark neuron in CA1, CA2, CA3, and DG hippocampal regions after PTZ induced seizures. The values are presented as mean ± SEM. *P<0.05 vs. PTZ group. VPA: Valproic acid, PTZ: Pentylenetetrazole

Histopathological Evaluation

In this study, the identification of the neuroprotective effect of metformin was provided by histopathological evaluation of dark neurons (**Figure 1**). The dark neurons are defined by various morphological features such as, neuronal shrinkage, nuclear pyknosis, chromatin aggregation, intense (dark) staining of perikaryal, dendritic and axonal cytoplasm (**Figure 1**, arrows).

The sections of the rat coronal hippocampus with toluidine blue staining. Basophilic (dark) neurons (arrow) distributed between normal pyramidal neurons. General hippocampal images of control, PTZ (saline), VPA, MET, and MET+VPA groups (A-E, respectively); DG (dentate gyrus) region of control, PTZ (saline), VPA, MET, and MET+VPA groups (F-J, respectively); CA3 region of control, PTZ (saline), VPA, MET, and MET+VPA groups (K-O, respectively); CA2 region of control, PTZ (saline), VPA, MET, and MET+VPA groups (P-T, respectively); CA1 region of control, PTZ (saline), VPA, MET, and MET+VPA groups (U-Y, respectively).

The 200 mg/kg VPA, 200mg/kg Metformin, and the combination of VPA and Metformin significantly decreased percentage of dark neuron formation in the hippocampal CA1, CA2, CA3, and DG areas as compared to saline+PTZ group (p<0.05, **Figure 2**). In addition, there were no statistically differences in terms of the percentage of dark neuron formation in the hippocampal areas between the VPA+PTZ, Metformin+PTZ, and VPA+-Metformin+PTZ groups (>0.05; **Figure 2**).

DISCUSSION

In this present study, we have studied the neuroprotective effect of metformin against PTZ-induced epileptic seizures and epileptogenesis. Previous studies have represented that PTZ induced neuronal death and neuronal damage in the hippocampus (4,24,25). PTZ exposure is known to trigger epileptic seizures as well as increase the permeability of the blood-brain barrier (26). Metformin, an oral anti-hyperglycemic agent, is primarily used in the treatment of type 2 diabetes. Metformin can able to cross the blood-brain barrier and has neuroprotective, anti-inflammatory, and antioxidant properties (27,28). Takata et al. (29) have demonstrated that metformin has therapeutic effects that prevent the breakdown of the blood-brain barrier. Furthermore, metformin has beneficial effects on multiple sclerosis,

stroke, and neurodegenerative disease such as Alzheimer's and Huntington's diseases (16,17,30). In addition, it is claimed that metformin activates neural progenitor cells in hypoxia-ischemia injury and enhances neurogenesis through BMP, Shh, and aPKC signaling pathways (31).

In this current study, we confirmed that PTZ administration induced behavioral seizures, oxidative stress, and hippocampal neuronal damage. PTZ kindling is associated with behavioral seizures in the form of a long seizure duration and a short latency for the first jerk. According to our findings, Metformin alone did not affect the FMJ onset time, but combination of VPA and metformin significantly increased FMJ onset time. Additionally, metformin exhibited anticonvulsant activity and caused reduced oxidative stress after PTZ-induced seizures in brain tissue. It is known that PTZ-induced changes are associated with oxidative stress and excessive ROS production or decreased antioxidant activity leads to oxidative stress. However, increased oxidative stress (ROS production) plays an important role in neuronal death (4,32). Some previous studies have claimed that oxidative damage increases in post-seizure brain tissues in rodents after a single dose of PTZ administration (4, 32,33). Similarly, to these studies, we observed high TOS levels in the post-seizure brain tissues (**Table 2**). In this study, Metformin was used due to neuroprotective, anti-inflammatory, and antioxidant properties. Indeed, the treatment of Metformin with or without VPA significantly reduced the brain oxidative stress (**Table 2**). It is well known that metformin provides maintenance of mitochondrial integrity and treating damage caused by oxidative stress through activation of phosphatidylinositol-3-kinase (PI3K) and AKT phosphorylation. Thus, Metformin clears ROS from brain tissue by inducing the production of the antioxidant system and increasing the antioxidant system activity through this pathway (34,35). Additionally, previous studies have shown that PTZ-induced oxidative stress causes to the neuronal death in the nervous system (24,25). Therefore, increasing ROS production during epileptogenesis can cause permanent damage to the brain (36,37). In addition, the results of this current work showed that PTZ-induced seizures resulted in dark neuron production in the hippocampal regions, as observed in previous studies (4,38). These basophilic neurons are found among healthy neurons in the central nervous system and have been detected in hypoglycemia, ischemia, stress, as well as in epilepsy (39). In our results, we have shown that the Metformin administration and the combination of VPA and Metformin decreased dark neuron formation in the hippocampal CA1, CA2, CA3, and DG areas (**Figure 1** and **2**). This result has demonstrated that Metformin has a significant neuroprotective effect against neuronal damage.

The results of this current study showed that Metformin treatment decreased epileptic seizures as well as the brain oxidative stress and preventing neural damage after PTZ- induced seizure. These results support the beneficial effect of Metformin on the nervous system.

Conflict of Interest and Financial Status: The authors of the current paper declare no conflict of interest.

Ethical Approval: The study was approved by Cumhuriyet University Animal Ethics Committee (Approval no: 65202830-050.04.04-157).

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Animal Rights statement: The authors declare that protect the animal rights in their studies in accordance with the principles of Guide for the Care and Use of Laboratory Animals

REFERENCES

1. Maiha BB, Magaji MG, Yaro AH, Hamza AH, Ahmed SJ, Magaj AR. Anticonvulsant studies on Cochlospermum tinctorium and Paullinia pinnata extracts in laboratory animals. Nig J Pharm Sci 2009;8:102-108.
2. Carpio A, Hauser WA. Epilepsy in the developing world. Curr Neurol Neurosci Rep 2009;9:319-326.
3. Curia G, Lucchi C, Vinet J, Gualtieri F, Marinelli C, Torsello A et al. Pathophysiogenesis of mesial temporal lobe epilepsy: is prevention of damage antiepileptogenic? Curr.Med.Chem 2014;21:663-688.
4. Akkaya R, Gümüş E, Akkaya B, Karabulut S, Gülmez K, Karademir M et al. Wi-Fi decreases melatonin protective effect and increases hippocampal neuronal damage in pentylenetetrazole induced model seizures in rats. Pathophysiology 2019;26:375-379.
5. Tastemur Y, Gumus E, Ergul M, Ulu M, Akkaya R, Ozturk A et al. Positive effects of angiotensin-converting enzyme (ACE) inhibitor, captopril, on pentylenetetrazole-induced epileptic seizures in mice. Trop J Pharm Res 2020;19:637-643.
6. Thom M, Zhou J, Martinian L, Sisodiya S. Quantitative post-mortem study of the hippocampus in chronic epilepsy: Seizures do not inevitably cause neuronal loss. Brain 2005;128:1344–1357.
7. Sato H, Takahashi T, Sumitani K, Takatsu H, Urano S. Gluco-

corticoid generates ROS to induce oxidative injury in the hippocampus, leading to impairment of cognitive function of rats. J Clin Biochem Nutr 2010;47:224-232.
8. Lim DW, Park J, Jung J, Kim SH, Um MY, Yoon M et al. Dicaffeoylquinic acids alleviate memory loss via reduction of oxidative stress in stress-hormone-induced depressive mice. Pharmacol Res 2020;161:105252.
9. Davidson MB, Peters AL. An overview of metformin in the treatment of type 2 diabetes mellitus. Am J Med 1997;102:99-110.
10. Cheng YY, Leu HB, Chen TJ, Chen CL, Kuo CH, Lee SD et al. Metformin-inclusive therapy reduces the risk of stroke in patients with diabetes: A 4-year follow-up study. J Stroke Cerebrovasc Dis 2014;23:99-105.
11. Thangthaeng N, Rutledge M, Wong JM, Vann PH, Forster MJ, Sumien N. Metformin impairs spatial memory and visual acuity in old male mice. Aging Dis 2017;8:17-30.
12. Labuzek K, Liber S, Gabryel B, Okopień B. Metformin has adenosine-monophosphate activated protein kinase (AMPK)-independent effects on LPS-stimulated rat primary microglial cultures. Pharmacol Rep 2010;62:827-848.
13. Tasci I. Metformin: good or bad for the brain? Ann Transl Med 2014;6:53.
14. Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H et al. Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care 2013;36:2981-2987.
15. Imfeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. J Am Geriatr Soc 2012;60:916-921.
16. Li J, Deng J, Sheng W, Zuo Z. Metformin attenuates Alzheimer's disease-like neuropathology in obese, leptin-resistant mice. Pharmacol Biochem Behav 2012;101:564-574.
17. Wang J, Gallagher D, DeVito LM, Cancino GI, Tsui D, He L et al. Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. Cell Stem Cell 2012;11:23-35.
18. Labuzek K, Liber S, Gabryel B, Okopień B. Metformin has adenosine-monophosphate activated protein kinase (AMPK)-independent effects on LPS-stimulated rat primary microglial cultures. Pharmacol Rep 2010;62:827-848.
19. El-Mir MY, Detaille D, R-Villanueva G, Delgado-Esteban M, Guigas B, Attia S et al. Neuroprotective role of antidiabetic drug metformin against apoptotic cell death in primary cortical neurons. J Mol Neurosci 2008;34:77-87.
20. Racine RJ. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. Electroencephalogr Clin Neurophysiol 1972;32:269-279.
21. Uyanikgil Y, Özkeşkek K, Çavuşoğlu T, Solmaz V, Tümer MK, Erbas O. Positive effects of ceftriaxone on pentylenetetrazol-induced convulsion model in rats. Int J Neurosci 2016;126:70-75.
22. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112-119.
23. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103-1111.
24. Haggag BS, Hasanin AH, Raafat MH, Abdel Kawy HS. Lamotrigine decreased hippocampal damage and improved vascular risk markers in a rat model of pentylenetetrazole induced kindling seizure. Korean J Physiol Pharmacol 2014;18:269-278.
25. Shi Y, Miao W, Teng J, Zhang L. Ginsenoside Rb1 protects the brain from damage induced by epileptic seizure via Nrf2/ARE signaling. Cell Physiol Biochem 2018;45:212-225.
26. Danjo S, Ishihara Y, Watanabe M, Nakamura Y, Itoh K. Pentylentetrazole-induced loss of blood-brain barrier integrity involves excess nitric oxide generation by neuronal nitric oxide synthase. Brain Res 2013;1530:44-53.
27. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M et al. Metformin improves healthspan and lifespan in mice. Nat Commun 2013;4:2192.
28. Labuzek K, Suchy D, Gabryel B, Bielecka A, Liber S, Okopień B. Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. Pharmacol Rep 2010;62:956-965.
29. Takata F, Dohgu S, Matsumoto J, Machida T, Kaneshima S, Matsuo M et. al. Metformin induces up-regulation of blood-brain barrier functions by activating AMP-activated protein kinase in rat brain microvascular endothelial cells. Biochem Biophys Res Commun 2013;433:586-590.
30. Zhao RR, Xu XC, Xu F, Zhang WL, Zhang WL, Liu LM et al. Metformin protects against seizures, learning and memory impairments and oxidative damage induced by pentylenetetrazole-induced kindling in mice. Biochem Biophys Res Commun 2014;448:414-417.
31. Zeyghami MA, Hesam E, Khadivar P, Hesam HK, Ahmadnia A, Amini A. Effects of atorvastatin and metformin on development of pentylenetetrazole-induced seizure in mice. Heliyon 2020;6:3761.
32. Ilhan A, Iraz M, Gurel A, Armutcu F, Akyol O. Caffeic acid phenethyl ester exerts a neuroprotective effect on CNS against pentylenetetrazol-induced seizures in mice. Neurochem Res 2004;29:2287-2292.
33. Golechha M, Sarangal V, Bhatia J, Chaudhry U, Saluja D, Arya DS. Naringin ameliorates pentylenetetrazol-induced seizures and associated oxidative stress, inflammation, and cognitive impairment in rats: possible mechanisms of neuroprotection. Epilepsy Behav 2014;41:98-102.
34. Dehkordi AH, Abbaszadeh A, Mir S, Hasanvand A. Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. Journal of Renal Injury Prevention 2019;8:54–61.
35. Tang G, Yang H, Chen J, Shi M, Ge L, Ge X et al. Metformin ameliorates sepsis-induced brain injury by inhibiting apoptosis, oxidative stress and neuroinflammation via the PI3K/Akt signaling pathway. Oncotarget 2017;8:97977-97989.
36. Aguiar CC, Almeida AB, Araújo PV, de Abreu RN, Chaves EM, do Vale OC et al. Oxidative stress, and epilepsy: literature review. Oxid Med Cell Longev 2012;2012:795259.
37. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009;7:65-74.
38. Gumus E, Taskiran AS, Toptas HA, Güney Ö, Kutlu R, Gunes H et al. Effect of the cyclooxygenase-2 inhibitor tenoxicam on pentylenetetrazole-induced epileptic seizures in rats. Cumhuriyet Med 2017;39:652-658.
39. Kherani ZS, Auer RN. Pharmacologic analysis of the mechanism of dark neuron production in cerebral cortex. Acta Neuropathol. 2008;116:447-452.