Neurotoxicity Research
https://doi.org/10.1007/s12640-022-00622-3

RESEARCH ARTICLE

Valproic Acid Attenuated PTZ-induced Oxidative Stress, Inflammation, and Apoptosis in the SH-SY5Y Cells via Modulating the TRPM2 Channel

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Received: 21 November 2022 / Revised: 8 December 2022 / Accepted: 9 December 2022
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Abstract

Valproic acid (VPA) is one of the most widely used antiepileptic drugs. The protective role of VPA and the role of the TRPM2 channel in this mechanism in developing neuronal damage due to increased pentylenetetrazol (PTZ)-induced neurotoxicity in SH-SY5Y cells were not clarified. Here, we investigated the role of VPA via modulation of TRPM2 channel on cell death and oxidative neurotoxicity in SH-SY5Y cells. The SH-SY5Y cell toxicity model was constructed by treating SH-SY5Y cells with PTZ. The VPA and TRPM2 channel antagonist N-(p-amylcinnamoyl) anthranilic acid (ACA) were added to prevent neurotoxicity in PTZ-induced SH-SY5Y cells. The role of the VPA and TRPM2 channel was evaluated using an ELISA kit and patch-clamp. Primarily, antioxidant (GSH and GSH-Px) and oxidative stress (MDA and ROS) levels and inflammatory factors (IL-1β, IL-6, and TNF-α) in cells were determined by ELISA kits. Then, TRPM2 channel activation in cells was detected using both the ELISA kit and patch-clamp methods. In addition, apoptosis and cell viability levels in cells were determined by performing PARP1, caspase-3, caspase-9, and CCK-8 assays by ELISA kits. Our results showed that the TRPM2 channel is vital in damage formation in PTZ-induced cells. Furthermore, we observed that VPA attenuated PTZ-induced neurotoxicity by suppressing cells' oxidative stress and inflammation, and reducing TRPM2 channel activation. In our study, in which the protective effect of VPA and the role of the TRPM2 channel in PTZ-induced SH-SY5Y cells were investigated for the first time, we can conclude that VPA treatment and TRPM2 channel blockade can suppress PTZ-induced neurotoxicity.

Keywords Valproic acid · Pentylenetetrazole · TRPM2 · Oxidative stress · Apoptosis

Introduction

Epilepsy (EP) is a neurological disease. An epileptic seizure is a set of sudden symptoms and clinical signs resulting from an abnormal, excessive, hypersynchronous burst of electrical activity that impairs brain function. Valproic acid (VPA) is the most widely used drug in treating EP (Abdelkader et al. 2020). The most crucial feature of VPA in the treatment of epilepsy is that it has a broad spectrum of anticonvulsant action. This provides the convenience of using VPA in adults and children with EP (Romoli et al. 2019). However, although VPA is widespread, it is not known precisely how it works on neurons in the epileptic process.

Pentylenetetrazole (PTZ) is highly preferred in creating EP models because of its easy penetration through biological membranes, rapid distribution to organs after intraperitoneal (i.p.) injection, and brain distribution ability to stimulate epileptogenic activity by blocking γ-aminobutyric acid (GABA) (Samokhina and Samokhin 2018; Taskıran et al. 2020). It is known that oxidative stress (OS) has a vital role in the etiology of EP and behavioral disorders. In addition, animal studies have shown that OS is an important factor in epileptic seizures (Pearson-Smith and Patel 2017; Iqubal et al. 2018). Zhang et al., in a rat EP model, observed that MDA levels increased while SOD and GSH-Px levels decreased in the hippocampus of the rat induced by PTZ (Zhang et al. 2021). Taskiran et al. found that 8-hydroxy-2′-deoxyguanosine (8-OhdG) and caspase-3 (Casp/3) levels increased in the cortex and hippocampus after epileptic seizures in PTZ-induced rats. They also determined that TOS and apoptosis levels increased, and TAS levels decreased significantly in

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Published online: 20 December 2022

PTZ-induced SH-SY5Y cells (Taskiran et al. 2021). It has been reported that there are significant changes in the levels of proinflammatory cytokines in EP-related experimental animal studies. Khatoon et al. reported that IL-6 and TNF-α levels were significantly increased in the hippocampus and cortex of PTZ-induced mice compared to the control group (Khatoon et al. 2021). Like the studies above, many studies emphasize that PTZ application causes severe damage in vivo or in vitro models. However, it is unclear how PTZ, used to create an EP model, triggers damage to cells through which mechanisms.

Transient receptor potential (TRP) channels are cation channels that play an essential role in regulating Ca^{2+} concentration in various cell types and tissues, both neuronal and non-neuronal. The TRP Melastatin-2 (TRPM2) channel is a member of the TRP channel family (Naziroglu et al. 2020). Studies have proven that the TRPM2 channel is activated, especially by oxidative stress. Since the TRPM2 channel is Ca^{2+} permeable, excessive activation of the channel may trigger many pathophysiological events, up to apoptosis (Malko and Jiang 2020; Yildizhan and Naziroglu 2022).

In this study, we aimed to investigate the effect of PTZ, which is the most used in the experimental neurotoxicity model, on SH-SY5Y cells. In our literature review, we did not find any study on the role of the TRPM2 channel and the protective effect of VPA in PTZ-induced SH-SY5Y cells. Therefore, we aimed to examine in detail the role of the TRPM2 channel and the protective effect of VPA in PTZ-induced SH-SY5Y cells.

Materials and Methods

Chemicals and Cell Culture

VPA (Cat#13,033) and N-(p-amylcinnamoyl) anthranilic acid (ACA) (Cat#14,531) were obtained from Cayman Chemical Co. (Ann Arbor, USA). PTZ (Cat#P6500) was purchased by Sigma-Aldrich Co. (St Louis, USA). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Abbkine Scientific Co. (Wuhan, China). Reactive oxygen species (ROS) ELISA kit (Cat#E3524Hu) was obtained from BT-Lab Co. (Shanghai, China). A standard medium was prepared for SH-SY5Y cells. FBS (10%), L-glutamine (1 g/L), and penicillin/streptomycin (1%) were added into equivalent volumes of DMEM and Ham's F12 as medium content. The cells were seeded in a 5% CO_2 atmosphere at 37 °C (Ozkaya and Naziroglu 2020).

Study Groups and Drug Treatment

The cells were seeded in a T25 cm^2 flask. The medium of the cells was changed at appropriate intervals, and groups were formed as follows when the cells reached the desired confluence (70-80%).

Control group: The cells were kept in cell culture conditions for 24 h.

VPA group: The cells were incubated with VPA (1 mM for 24 h) (Terzïoğlu Bebïtoğlu et al. 2020).

ACA group: The cells were incubated with ACA (25 µM for 30 min) (Güzel et al. 2021).

PTZ group: The cells were incubated with PTZ (30 µM for 24 h) (Taskiran et al. 2021).

PTZ + ACA group: Cells were incubated with PTZ (30 µM for 24 h) and then incubated with ACA (25 µM) for the last 30 min of the 24 h of the study.

PTZ + VPA group: The cells were incubated by PTZ (30 µM) and VPA (1 mM) for 24 h.

PTZ + ACA + VPA group: The cells were incubated with PTZ (30 µM) and VPA (1 mM) for 24 h. and also incubated with ACA (25 µM) for the last 30 min of the 24 h of the study.

Cell Viability Assays

The CCK-8 (Abbkine, Cat#KTA1020) assay measured cell viability between the groups. The cell viability was measured on the BioTek EL_x808™ instrument at 450 nm with commercial ELISA kits, following the instructions in the kit procedure. The experiment was repeated three times to determine the cell viability rate in the groups, and the data were given as a percentage compared to the control group (% of control).

Measurement of Biochemical Parameters in SH-SY5Y Cells by ELISA Kits

The cells for each group were placed in separate sterile tubes. The tubes were centrifuged at 1000 rpm for 20 min by the kit's procedure. Following the removal of the supernatants, the cell pellets were suspended in PBS (pH 7.4) to produce a cell suspension at a concentration of approximately 1×10^6/ml. Cells were lysed through repeated freeze–thaw cycles to allow internal components to escape. It was centrifuged for 10 min at 4000 rpm at 4 °C. The supernatants were collected for biochemical analysis. The Bradford protein assay kit (Merck Millipore, Darmstadt, Germany) was used to determine the total protein levels in the samples. The levels of GSH (Abbkine, Cat#KTE62857), MDA (Abbkine, Cat#KTE61683), GSH-Px (Abbkine, Cat#KTE62766), IL-1β (Abbkine, Cat#KET6013), IL-6 (Abbkine, Cat#KET6017), TNF-α (Abbkine, Cat#KET6032), ROS (BT-Lab, Cat#E3524Hu), PARP1 (Abbkine, Cat#KTE61302), TRPM2 (Abbkine, Cat#KTE62279), Casp/3 (Abbkine, Cat#KTE62614), and Casp/9 (Abbkine, Cat#KTE62615) were measured on the

BioTek EL_x808™ instrument at 450 nm with commercial ELISA kits following the instructions in the kit procedure.

Electrophysiology

To determine TRPM2 channel activation in SH-SY5Y cells, recordings were taken and evaluated in the whole cell configuration. Standard extracellular fluid was prepared by adding the following chemicals (mM): 140 NaCl, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 5 KCl, 10 HEPES, pH 7.4 (pH adjusted with NaOH). Sodium-free N-methyl-D-glucamine (NMDG) extracellular solution was prepared using 150 mM NMDG instead of 140 NaCl. Standard intracellular fluid was prepared by adding the following chemicals (mM): 145 CsCl, 8 NaCl, 2 $MgCl_2$, 10 HEPES, pH 7.2 (Naziroglu 2007). TRPM2 is activated when intracellular Ca^{2+} is high (Kahya et al. 2017). Therefore, the intracellular Ca^{2+} concentration is high (1 µM (0.886 mM $CaCl_2$, 1 mM Cs-EGTA)) instead of the average Ca^{2+} concentration (50–100 nM). Intracellular Ca^{2+} density was calculated using the Max program (http://www.stanford.edu/~cpatton/maxc.html). SH-SY5Y cells found in the patch-clamp circle were added to the extracellular equivalent buffer. It was checked whether the resistance of the pipette was 3–7 MΩ on the computer software. Stock ADP-ribose (ADPR) and ACA solutions were dissolved in DMSO and diluted to the required concentrations in fluids of intracellular (ADPR = 1 mM) or extracellular ACA: 0.025 mM). With the help of a patch-pipette, a TRPM2 channel opener (1 mM ADPR) was introduced into the cells (Naziroglu and Luckhoff 2008; Celik and Naziroglu 2012). After activating TRPM2 channels, channel activity and current variability waited until a certain plateau was reached. When the plateau occurred, it was sent to the circle with the ACA perfusion system at a concentration of 0.025 mM, which was reported as a TRPM2 blocker. Finally, to ensure the viability of our cells, NMDG extracellular equivalent buffer solution was sent to the circle, and recording continued. The recording was terminated by removing the chemicals (ACA and NMDG) applied to the living cells by washing them with a peristaltic pump. Results were calculated as pA/pF and given as current density. This part of the study was conducted in BSN Health, Analyses, Innov., Consult., Org., Agricul., Trade Ltd., Isparta, Turkey.

Statistical Analysis

The analyses of statistics were performed in the SPSS program. The data were presented as means ± standard deviation (SD) according to the one-way ANOVA and Student's *t*-test. All data are representative of at least three independent experiments performed in triplicate. Results with a *p*-value of 0.05 or less were considered significant.

Results

The PTZ-induced Changes in GSH, MDA, and GSH-Px Levels were Attenuated by The VPA and ACA Treatments

Studies have reported that PTZ treatment reduces antioxidant levels in cells by increasing oxidative stress in various neuronal cells, including SH-SY5Y (Esmaeili-Mahani et al. 2017; Taskiran and Ergul 2021; Taskiran et al. 2021). Therefore, in this study, we determined how GSH (A), MDA (B), and GSH-Px (C) levels changed in the cells after group incubations. GSH and GSH-Px values of Control, VPA, and ACA groups were similar, and there was no significant difference between them ($p > 0.05$). However, the GSH and GSH-Px values of the PTZ group were the lowest compared to the other groups ($p < 0.05$). In addition, the GSH and GSH-Px values of the PTZ + ACA + VPA group were higher than only the PTZ + ACA and PTZ + VPA groups ($p < 0.05$). When the MDA values between the groups were compared, it was found that the MDA levels of the PTZ group were significantly higher than the other groups ($p < 0.05$). The MDA value of the PTZ + ACA + VPA group was lower than the PTZ + ACA and PTZ + VPA groups ($p < 0.05$, Fig. 1).

The PTZ-induced Increase of Inflammatory Cytokine Levels was Diminished in SH-SY5Y Cells by VPA and ACA Treatments

Inflammatory cytokines such as IL-1 β, IL-6, and TNF-α are important markers of inflammation, and their levels have been reported to be significantly increased in neurodegeneration (Dinarello 2000; Yildizhan and Naziroglu 2020). The levels of IL-1 β, IL-6, and TNF-α in the cells were shown for all the study groups in Fig. 2. The IL-1 β, IL-6, and TNF-α levels in the PTZ group were higher than in the other groups ($p < 0.05$). The PTZ + ACA + VPA group observed a significant decrease in the IL-1 β, IL-6, and TNF-α levels according to the PTZ + ACA and PTZ + VPA groups ($p < 0.05$, Fig. 2).

The VPA and ACA Treatments Diminished PTZ-induced Upregulation of ROS, PARP1, and TRPM2 Channel Levels in SH-SY5Y Cells

The activation of the TRPM2 channel is increased depending on the increase of intracellular OS (via ROS activation) and ADPR level (via Poly (ADP-Ribose) Polymerase 1 (PARP1) activation). It is also well known that the level of its channel expression changes in neurodegeneration (Nazıroğlu 2022). The ROS, PARP1, and TRPM2 channel levels in cells for all study groups are shown in Fig. 3.

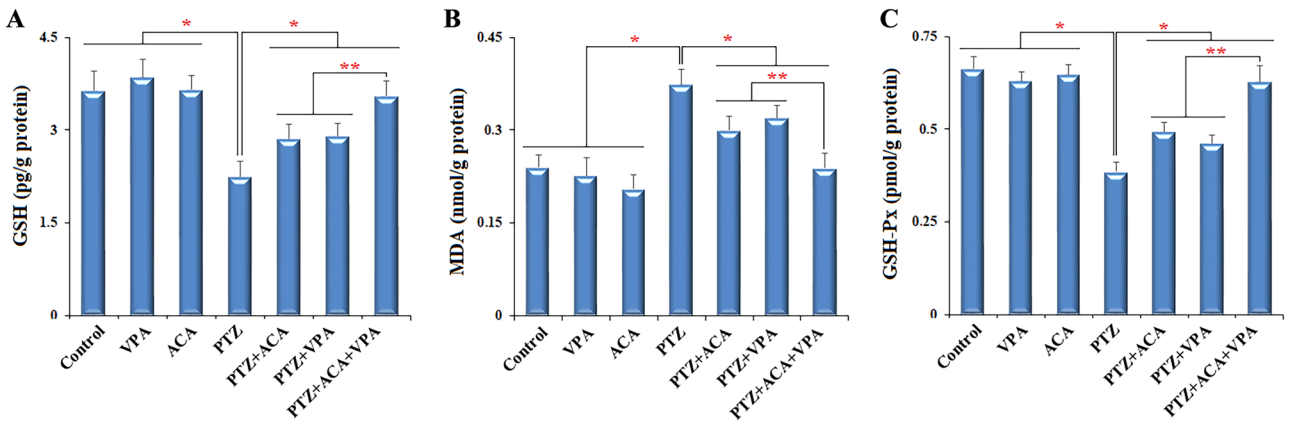


Fig. 1 The PTZ-induced changes in GSH, MDA, and GSH-Px levels were attenuated by the VPA and ACA treatments (mean ± SD). The PTZ-induced mean MDA level **A** was decreased via the upregulation of GSH level **B** and GSH-Px activity **C** in the SH-SY5Y cells by the VPA and ACA treatments. The analyses were performed by using the commercial ELISA kit (*$p \leq 0.05$ vs. PTZ group. **$p \leq 0.05$ vs PTZ + ACA + VPA group)

The ROS, PARP1, and TRPM2 channel levels in the PTZ group were higher than in the other groups ($p < 0.05$). The PTZ + ACA + VPA group observed a significant decrease in the ROS, PARP1, and TRPM2 channel levels according to the PTZ + ACA and PTZ + VPA groups ($p < 0.05$, Fig. 3).

Involvement of PTZ on the Increase of TRPM2 Current Density (Pa/Pf) in SH-SY5Y: Modulator Role of VPA

The patch-clamp technique is one of the essential techniques to determine the activation of cation channels, including the TRPM2 channel (Daldal and Nazıroğlu 2022). In patch-clamp analyses, we induced whole cell configuration in cells. We recorded TRPM2 channel stimulation using ADPR (in patch-pipette and 1 mM) to determine TRPM2 channel activation in the groups. SH-SY5Y cells had limited flow in the absence of cytosolic ADPR (Fig. 4A). Although downregulated to control levels by inhibition of TRPM2 (ACA and NMDG treatments), TRPM2 was stimulated to 0.64 nA in the Control + ADPR group by cytosolic ADPR stimulation (Fig. 4B). In the PTZ group, cytosolic ADPR stimulation induced more gates of TRPM2. We observed increased current density (up to 1.2 nA) in cells (Fig. 4C). Again, the current density in the PTZ group was reduced to the Control value ($p < 0.05$) by processing the ACA and NMDG treatments (Fig. 4C). In the VPA + ADPR (Fig. 4D) and PTZ + VPA + ADPR (Fig. 4E) groups, we observed limited TRPM2 activation by cytosolic ADPR stimulation. Available electrophysiology observation data confirmed VPA's modulator on PTZ-induced TRPM2

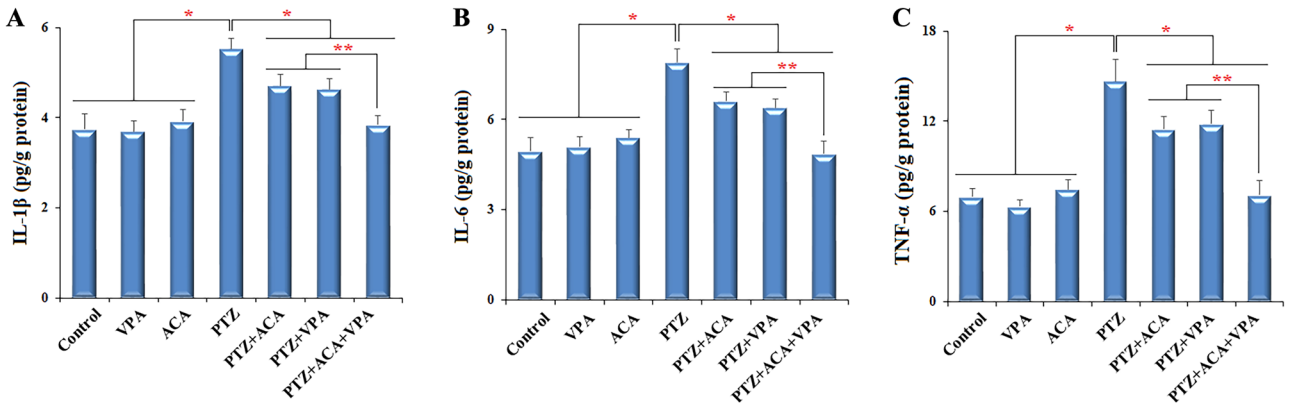


Fig. 2 The PTZ-induced increase of inflammatory cytokine levels was diminished in the SH-SY5Y cells by the VPA and ACA treatments (mean ± SD). The PTZ-induced increase of IL-1β **A**, IL-6 **B**, and TNF-α **C** was decreased in the SH-SY5Y cells by the VPA and ACA treatments. The analyses were performed by using the commercial ELISA kit (*$p \leq 0.05$ vs. PTZ group. **$p \leq 0.05$ vs PTZ + ACA + VPA group)

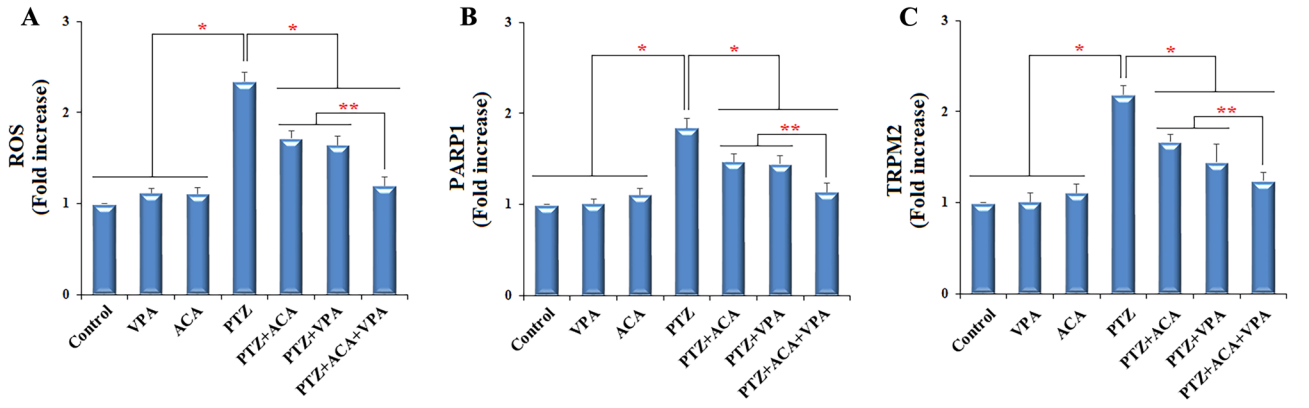


Fig. 3 The VPA and ACA treatments diminished PTZ-induced upregulation of ROS **A**, PARP1 **B**, and TRPM2 **C** channel levels in the SH-SY5Y cells (mean ± SD). The analyses were performed by using the commercial ELISA kit (*$p \leq 0.05$ vs. PTZ group. **$p \leq 0.05$ vs PTZ + ACA + VPA group)

stimulation in SH-SY5Y cells. In addition, we also observed that stimulation with ADPR increased TRPM2 channel activation in Control + ADPR and PTZ groups. In contrast, these groups' ACA incubation (ADPR + ACA) significantly decreased the current by blocking the channel (Fig. 4F).

The PTZ-caused Increase of Caspase Activations and Apoptosis Induction Through Decreased Cell Viability in SH-SY5Y Cells Modulated by VPA Treatment

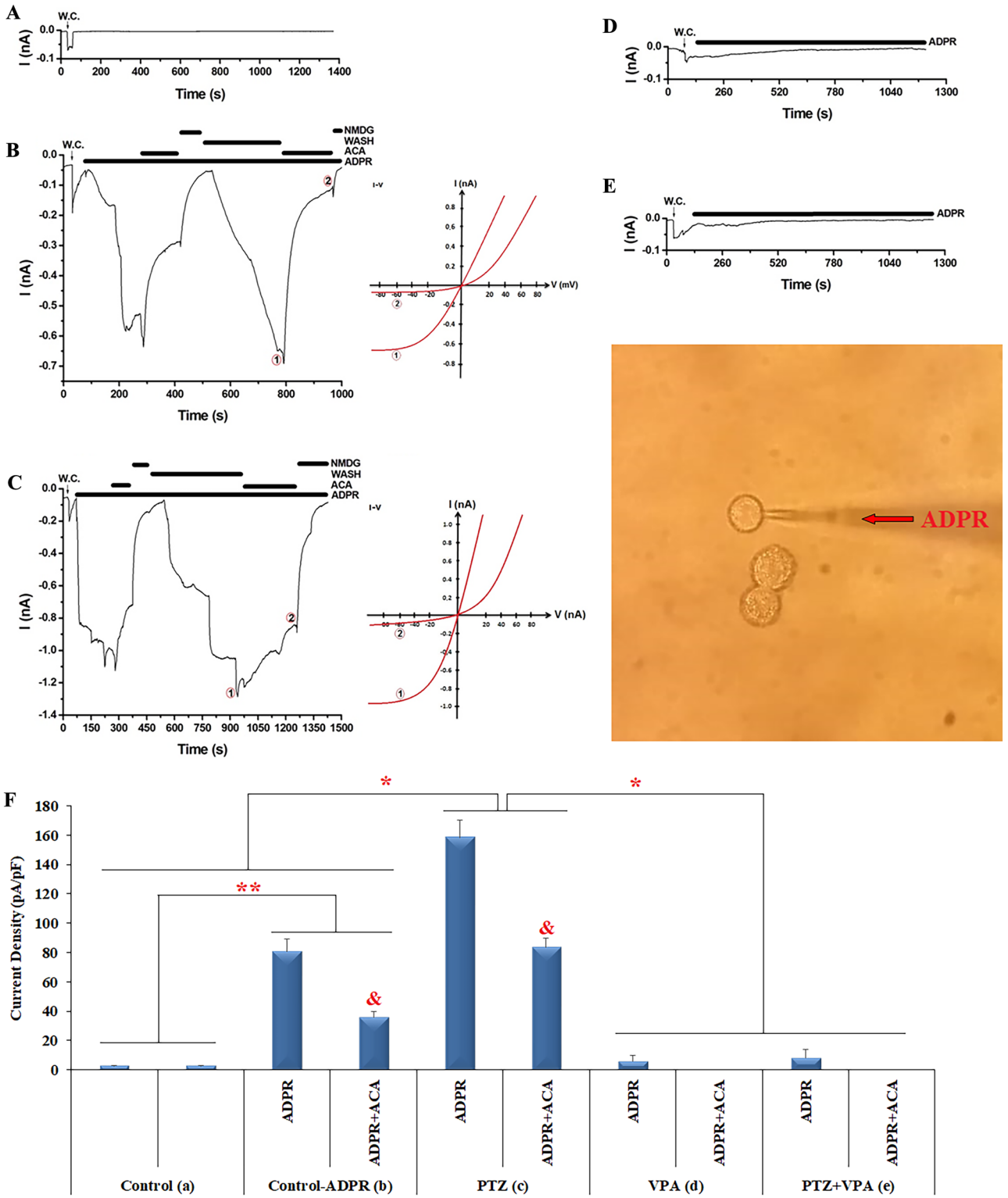
Studies have stated that an excessive increase in TRPM2 channel activation may cause a large amount of Ca^{2+} ions to enter the cell. Intracellular Ca^{2+} ion accumulation can trigger caspase activation by activating different mechanisms, which may cause the death of cells (Daldal and Nazıroğlu 2022; Yıldızhan and Nazıroğlu 2022). In our study, after determining the increase in TRPM2 channel activation in SH-SY5Y cells with patch-clamp analyses after PTZ incubation, we examined Casp/3, Casp/9, and cell viability levels among groups. We observed that Casp/3 and Casp/9 levels were significantly higher in the PTZ group compared to the other groups ($p < 0.05$). However, Casp/3 and Casp/9 levels were substantially lower in the PTZ + ACA and PTZ + VPA groups compared to the PTZ group ($p < 0.05$). The lowest Casp/3 and Casp/9 levels in the PTZ treatment groups (PTZ, PTZ + ACA, and PTZ + VPA) were in the PTZ + ACA + VPA group ($p < 0.05$, Fig. 5A and B). When cell viability was examined among the groups (% of the control group), the cell viability rate was the lowest in the PTZ group compared to the other groups. However, the cell viability rate was significantly higher in the PTZ + ACA and PTZ + VPA groups compared to the PTZ group. In the PTZ treatment groups, the highest cell viability rate was in the PTZ + ACA + VPA group ($p < 0.05$, Fig. 5C).

Discussion

Epilepsy is characterized by spontaneously recurrent seizures, unprovoked (without any stimulatory factor) (Moshé et al. 2015). One of the rodent experimental seizure models widely used for antiepileptic drug research is the pentylenetetrazole (PTZ)–induced ignition model (Samokhina and Samokhin 2018). PTZ is a selective antagonist of the γ-aminobutyric acid type A ($GABA_A$) chloride ionophore complex. Studies have emphasized that it stimulates epileptogenic activity by blocking GABA-mediated transmission (Luszczki et al. 2005). VPA is one of the most widely used drugs in treating EP (Abdelkader et al. 2020). One of the essential points in choosing VPA in EP is its broad spectrum of anticonvulsants (Romoli et al. 2019). Together with the studies to be carried out to understand this feature of VPA and its mechanism of action in neuronal cells, it will contribute to increasing the drug's effect by mediating the design of the combined drug to increase the drug's effect in the future.

This study examined how oxidative stress, inflammation, and apoptosis markers changed in SH-SY5Y cells after PTZ administration. We determined that after PTZ application, especially antioxidant levels in cells decreased while oxidant and inflammatory parameters increased significantly. In addition, we determined that caspase activity and cell death increased in cells in the PTZ group, which was associated with increased oxidant and inflammatory parameters. We formed treatment groups with known antioxidant properties of VPA and TRPM2 channel antagonist ACA for our possible hypothesis. Our results showed that VPA treatment and TRPM2 channel inactivation could reduce the increased neurotoxicity in PTZ-induced SH-SY5Y cells.

Studies have shown that oxidative stress and inflammation play a vital role in the etiology of EP (Khatoon et al. 2021; Taskiran et al. 2021; Zhang et al. 2021). A common feature of



EP and neurodegenerative disorders is oxidative stress, the most prominent marker responsible for the damage or death of neuronal cells that contribute to the pathogenesis of these diseases (Geronzi et al. 2018). Hassanzadeh et al. determined that in the kindling model of EP, oxidative stress and cognitive impairment were increased in PTZ-induced rats (Hassanzadeh et al. 2017). Alachkar et al. observed an increase in MDA level in an experimental EP model, PTZ-induced rats, while they observed a significant decrease in GSH, CAT, and SOD levels (Alachkar et al. 2020). Tamer et al., in their study investigating oxidative brain

◂**Fig. 4** The ADPR-induced stimulation of TRPM2 was increased in the SH-SY5Y cells by the treatment of PTZ, although the treatment of VPA and ACA diminished TRPM2 activity (mean ± SD). The patch-clamp records were taken as whole cell (W.C.) configuration after inducing the voltage-clamp (at − 60 mV). **A** Control group without ADPR stimulation. **B** Control + ADPR group with ADPR stimulation. **C** PTZ group. **D** VPA group. **E** PTZ + VPA group. **B** (I/V) and **C** (I/V) are corresponding I/V relations of currents recorded in **B** and **C** at the indicated time points of ADPR (1) and ACA (2), respectively. The cytosolic ADPR (1 mM)–induced TRPM2 currents were inhibited in the **B** and **C** by the treatment of 25 μM ACA and Na^+-free solution (NMDG). **F** Mean current densities of four groups after ADPR and ACA treatments (*$p \leq 0.05$ vs. PTZ group. **$p \leq 0.05$ vs Control group with ADRP. $^{\&}p \leq 0.05$ vs with ADPR in-groups)

damage and memory impairment in PTZ-induced rats, emphasized that PTZ-induced seizure activity causes neuronal and astrocytic damage accompanied by oxidative stress, including increased lipid peroxidation, neutrophil infiltration, and oxygen derivative production. The results confirmed that PTZ application caused free radicals, apoptosis, and memory impairment in the brain tissue of rats (Tamer et al. 2022). Taskiran et al. investigated the effect of thiamine against seizures, apoptosis, and nitric oxide and oxidative stress caused by PTZ. They reported increased neurotoxicity in PTZ-induced SH-SY5Y cells. The same study noted that PTZ increased TOS and apoptosis while TAS and cell viability decreased in SH-SY5Y cells (Taskiran and Ergul 2021). In this study, we determined that MDA levels increased and GSH and GSH-Px levels decreased in PTZ-induced SH-SY5Y cells. We decided that the use of VPA and ACA separately in VPA and ACA incubations with PTZ decreased the MDA level and increased the GSH and GSH-Px levels compared to the PTZ group. We found that MDA, GSH, and GSH-Px levels in the PTZ + ACA + VPA group were closest to the Control group's MDA, GSH, and GSH-Px levels (Fig. 1). The inflammatory cytokines are essential markers in the processes of major neurological diseases. In EP, different studies have shown that brain inflammation contributes to seizure susceptibility and epileptogenesis in various types of seizures and EP. In the central nervous system, expressions of inflammatory cytokines such as TNF-α, IL-6, and IL-1 β are expressed in both neurons and glial cells. Khatoon et al. emphasized that increased inflammatory and apoptosis molecules released during experimental EP in PTZ-induced mice cause neurotoxicity. In addition, the same study determined that IL-1 β, IL-6, and TNF-α levels were significantly increased in the hippocampus and cortex of mice induced by PTZ (Khatoon et al. 2021). In the study of the EP model created in HT-22 cells, an increase was observed in the levels of MDA and MPO, while the levels of SOD and GSH decreased in the cells in the group in which the EP model was created. In addition, this study reported an increase in IL-1 β, IL-6, and TNF-α levels in the cells in the EP model group (Qi and Liu 2022). The present study observed a significant increase in IL-1 β, IL-6, and TNF-α in PTZ-induced cells. It was observed that the IL-1 β, IL-6, and TNF-α levels of the groups that received VPA and ACA treatment were significantly reduced compared to the PTZ group. In addition, we determined that cytokine levels decreased to the control group levels in the group treated with VPA and ACA combined treatment (Fig. 2).

The ion channels are important in the pathogenesis of EP. It has been emphasized that activation of voltage-sensitive Ca^{2+} channels or intracellular Ca^{2+} accumulation in these channels may trigger seizures by causing synchronized discharge of neurons (Zheng et al. 2020). The TRPM2 channel is a non-selective Ca^{2+} permeable cation channel. Many studies have reported that raised OS and intracellular ADPR levels increase TRPM2 channel activation and expression (Malko and Jiang 2020; Yildizhan and Naziroglu 2022). Hu et al. determined that TRPM2 knockout mice reduced inflammatory responses in the hippocampus compared to wild-type mice in their EP model. They also reported that it reduced EP-induced neurodegeneration and autophagy in TRPM2 knockout mice and improved EP-induced cognitive impairment (Hu et al. 2020). An et al. stated that PARP1-dependent activation of TRPM2 channel–mediated ROS-induced cell death and increased TRPM2 expression increased susceptibility to ROS-induced death in human neuroblastoma SH-SY5Y cells (An et al. 2019). Akyuva et al. reported that mitochondrial oxidative stress, inflammation, and apoptosis were increased via the TRPM2 channel in SH-SY5Y cells where the hypoxia model was created. In addition, by using TRPM2 channel antagonists in hypoxia-modelled SH-SY5Y cells, it was confirmed by patch-clamp and confocal imaging methods that calcium influx into the cell via the TRPM2 channel (Akyuva and Nazıroğlu 2020). Increased TRPM2 activation is a well-known adverse factor in neurotoxicity and neurodegenerative diseases. In SH-SY5Y cells with interferon-gamma-mediated OS, apoptosis, and neuroinflammation, the patch-clamp technique showed that increased TRPM2 channel activation increases OS, apoptosis, and neuroinflammation. In addition, this study showed us that apoptotic factors and increased OS also increase TRPM2 channel expression (Güzel et al. 2021). In our research, we found that, supporting the literature data, we determined that ROS and PARP1 levels increased in PTZ-induced SH-SY5Y cells. While the ROS level increases the PARP1 level in the cell, we observed that it increases the TRPM2 channel activation in PARP1-induced ADPR. In addition, as can be seen in the publications we gave as a reference, channel activation and expression showed parallelism. We determined that TRPM2 channel expression and activation were decreased by the patch-clamp (Fig. 4) and the ELISA kit (Fig. 3) in SH-SY5Y cells treated by VPA and ACA. A recent study investigated how neurotoxicity parameters changed after PTZ administration in rats and SH-SY5Y cells. The study determined that levels of 8-OHdG, caspase-3, and brain-derived neurotrophic factor were significantly increased in the cortex and hippocampus of PTZ-treated rats. In SH-SY5Y cells, a decrease in cell viability and total antioxidant levels and an increase in total oxidant and apoptosis levels were reported after

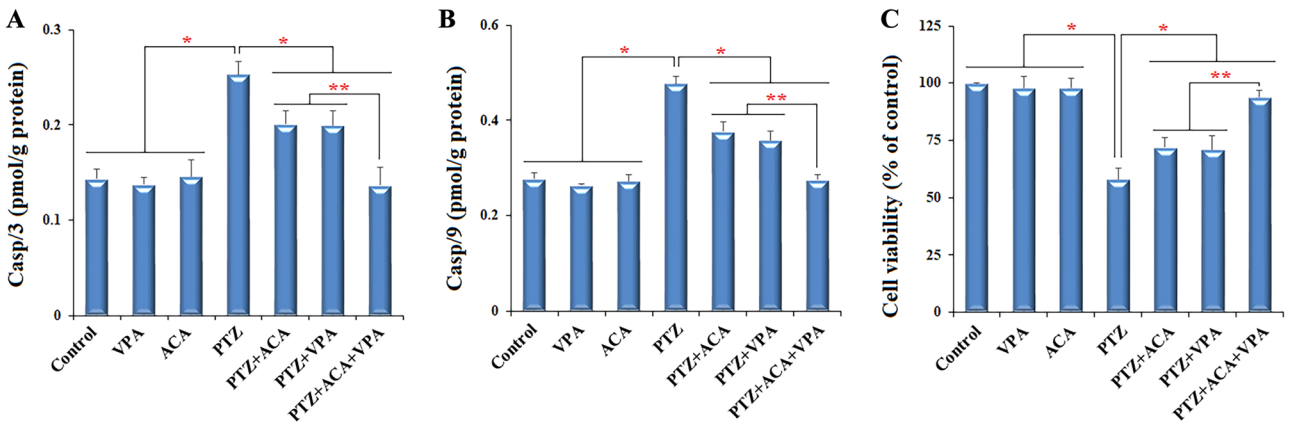


Fig. 5 VPA and ACA protect SH-SY5Y cells against PTZ-induced damage (mean ± SD). In SH-SY5Y cells with VPA and ACA treatments, PTZ-induced Casp/3 **A** and Casp/9 **B** levels were decreased, while viable cell numbers **C** increased. The Casp/3 and Casp/9 levels were performed using the commercial ELISA kit, and the CCK-8 assay kit assessed the cell viability (*$p \leq 0.05$ vs. PTZ group. **$p \leq 0.05$ vs PTZ + ACA + VPA group)

PTZ incubation [8]. A different study reported that controlling the activation or expression level of the TRPM2 channel can reduce the formation of proinflammatory cytokines and pro-apoptotic factors in SH-SY5Y cells with neurotoxicity (Ding et al. 2019). In this study, we determined that Casp/3 and Casp/9 levels were increased, and cell viability decreased in PTZ-induced SH-SY5Y cells. We observed that using VPA and ACA separately in PTZ and VPA and ACA incubations decreased Casp/3 and Casp/9 levels and increased cell viability compared to the PTZ group.

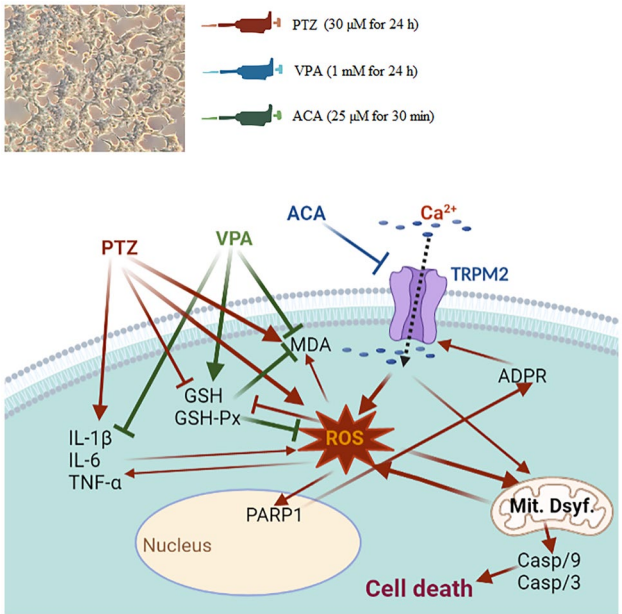


Fig. 6 Possible molecular pathways of VPA and TRPM2 antagonist on oxidative stress, inflammatory factors, and apoptosis in PTZ-induced neurotoxicity model in SH-SY5Y cells (Created by BioRender)

We determined that the combined use of VPA and ACA reduced PTZ-induced neurotoxicity and brought Casp/3, Casp/9, and cell viability to the control group levels.

Conclusion

In this study, we determined that the antioxidant level decreased and the increase in OS increased in PTZ-induced SH-SY5Y cells. In addition, we observed an increase in proinflammatory cytokines and the deterioration of the oxidant/antioxidant balance in PTZ-induced SH-SY5Y cells. Increased intracellular OS increased intracellular PARP1-induced ADPR increase, which is an intracellular TRPM2 agonist, resulting in TRPM2 channel activation. TRPM2 channel activation in cells caused too much Ca^{2+} entry into the cell, disrupting intracellular ion concentration, increasing intracellular caspase activity, and thus apoptosis. However, by reducing TRPM2 channel activation, VPA and ACA improved PTZ-induced oxidative damage and decreased proinflammatory cytokines and neurotoxicity values. These results showed that VPA and ACA administration could suppress PTZ-induced neurotoxicity (Fig. 6).

Acknowledgements This study was presented as an oral presentation to the 7th International Brain Research School organized on 27 June-03 July 2022, Isparta, Turkey, by Autor's. https://dergipark.org.tr/tr/download/issue-file/60314. The results of the current study were summarized from a Ph.D. thesis of AA.

Author Contribution M.B. and A.A. reviewed the current literature, formulated the hypothesis, and designed the project. Y.T. and K.Y. contributed to in vitro experimental studies. K.Y. contributed to patch-clamp, biochemistry analysis, and manuscript writing. All authors read and approve the manuscript, and all data were generated in-house and that no paper mill was used.

Funding This work was supported by the Research Fund of Istanbul University. Project No. 38188.

Data Availability Data will be made available on request.

Declarations

Competing Interests The authors declare no competing interests.

Conflict of Interest The authors declare no competing interests.

References

Abdelkader NF, Elyamany M, Gad AM, Assaf N, Fawzy HM, Elesawy WH (2020) Ellagic acid attenuates liver toxicity induced by valproic acid in rats. J Pharmacol Sci 143(1):23–29

Akyuva Y, Nazıroğlu M (2020) Resveratrol attenuates hypoxia-induced neuronal cell death, inflammation and mitochondrial oxidative stress by modulation of TRPM2 channel. Sci Rep 10(1):1–16

Alachkar A, Azimullah S, Lotfy M, Adeghate E, Ojha SK, Beiram R, Łażewska D, Kieć-Kononowicz K, Sadek B (2020) Antagonism of histamine H3 receptors alleviates pentylenetetrazole-induced kindling and associated memory deficits by mitigating oxidative stress, central neurotransmitters, and c-Fos protein expression in rats. Molecules 25(7):1575

An X, Fu Z, Mai C, Wang W, Wei L, Li D, Li C, Jiang L-H (2019) Increasing the TRPM2 channel expression in human neuroblastoma SH-SY5Y cells augments the susceptibility to ROS-induced cell death. Cells 8(1):28

Celik O, Naziroglu M (2012) Melatonin modulates apoptosis and TRPM2 channels in transfected cells activated by oxidative stress. Physiol Behav 107(3):458–465

Daldal H, Nazıroğlu M (2022) Carvacrol protects the ARPE19 retinal pigment epithelial cells against high glucose-induced oxidative stress, apoptosis, and inflammation by suppressing the TRPM2 channel signaling pathways. Graefes Arch Clin Exp Ophthalmol 260(8):2567–2583

Dinarello CA (2000) Proinflammatory cytokines. Chest 118(2):503–508

Ding X-M, Zhao L-J, Qiao H-Y, Wu S-L, Wang X-H (2019) Long non-coding RNA-p21 regulates MPP+-induced neuronal injury by targeting miR-625 and derepressing TRPM2 in SH-SY5Y cells. Chem Biol Interact 307:73–81

Esmaeili-Mahani S, Sheibani V, Najafipour H (2017) Apelin-13 protects rat primary cortical glia-neuron co-culture against pentylenetetrazole-induced toxicity. Biomed Pharmacother 87:661–668

Geronzi U, Lotti F, Grosso S (2018) Oxidative stress in epilepsy. Expert Rev Neurother 18(5):427–434

Güzel M, Nazıroğlu M, Akpınar O, Çınar R (2021) Interferon gamma-mediated oxidative stress induces apoptosis, neuroinflammation, zinc ion influx, and TRPM2 channel activation in neuronal cell line: modulator role of curcumin. Inflammation 44(5):1878–1894

Hassanzadeh P, Arbabi E, Atyabi F, Dinarvand R (2017) Ferulic acid exhibits antiepileptogenic effect and prevents oxidative stress and cognitive impairment in the kindling model of epilepsy. Life Sci 179:9–14

Hu H, Zhu T, Gong L, Zhao Y, Shao Y, Li S, Sun Z, Ling Y, Tao Y, Ying Y et al (2020) Transient receptor potential melastatin 2 contributes to neuroinflammation and negatively regulates cognitive outcomes in a pilocarpine-induced mouse model of epilepsy. Int Immunopharmacol 87:106824

Iqubal A, Sharma S, Sharma K, Bhavsar A, Hussain I, Iqubal MK, Kumar R (2018) Intranasally administered pitavastatin ameliorates pentylenetetrazol-induced neuroinflammation, oxidative stress and cognitive dysfunction. Life Sci 211:172–181

Kahya MC, Nazıroğlu M, Övey İS (2017) Modulation of diabetes-induced oxidative stress, apoptosis, and Ca2+ entry through TRPM2 and TRPV1 channels in dorsal root ganglion and hippocampus of diabetic rats by melatonin and selenium. Mol Neurobiol 54(3):2345–2360

Khatoon S, Agarwal NB, Samim M, Alam O (2021) Neuroprotective effect of fisetin through suppression of IL-1R/TLR axis and apoptosis in pentylenetetrazole-induced kindling in mice. Front Neurol 12:1152

Luszczki JJ, Wojcik-Cwikla J, Andres MM, Czuczwar SJ (2005) Pharmacological and behavioral characteristics of interactions between vigabatrin and conventional antiepileptic drugs in pentylenetetrazole-induced seizures in mice: an isobolographic analysis. Neuropsychopharmacology 30(5):958–973

Malko P, Jiang L-H (2020) TRPM2 channel-mediated cell death: an important mechanism linking oxidative stress-inducing pathological factors to associated pathological conditions. Redox Biol 37:101755

Moshé SL, Perucca E, Ryvlin P, Tomson T (2015) Epilepsy: new advances. The Lancet 385(9971):884–898

Naziroglu M (2007) New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res 32(11):1990–2001

Nazıroğlu M (2022) A novel antagonist of TRPM2 and TRPV4 channels: Carvacrol. Metab Brain Dis 37(3):711–728

Naziroglu M, Luckhoff A (2008) A calcium influx pathway regulated separately by oxidative stress and ADP-Ribose in TRPM2 channels: single channel events. Neurochem Res 33(7):1256–1262

Naziroglu M, Oz A, Yildizhan K (2020) Selenium and neurological diseases: focus on peripheral pain and TRP channels. Curr Neuropharmacol 18(6):501–517

Ozkaya D, Naziroglu M (2020) Curcumin diminishes cisplatin-induced apoptosis and mitochondrial oxidative stress through inhibition of TRPM2 channel signaling pathway in mouse optic nerve. J Recept Signal Transduct Res 40(2):97–108

Pearson-Smith JN, Patel M (2017) Metabolic dysfunction and oxidative stress in epilepsy. Int J Mol Sci 18(11)

Qi H, Liu L (2022) Rhoifolin attenuates damage to hippocampal neuronal culture model of acquired epilepsy in vitro by regulating NF-κB/iNOS/COX-2 axis. Quality Assurance and Safety of Crops & Foods 14(3):116–123

Romoli M, Mazzocchetti P, D'Alonzo R, Siliquini S, Rinaldi VE, Verrotti A, Calabresi P, Costa C (2019) Valproic acid and epilepsy: from molecular mechanisms to clinical evidences. Curr Neuropharmacol 17(10):926–946

Samokhina E, Samokhin A (2018) Neuropathological profile of the pentylenetetrazol (PTZ) kindling model. Int J Neurosci 128(11):1086–1096

Tamer SA, Koyuncuoğlu T, Köroğlu AK, Akakın D, Yüksel M, Yeğen BÇ (2022) Nesfatin-1 ameliorates oxidative brain damage and memory impairment in rats induced with a single acute epileptic seizure. Life Sci 294:120376

Taskiran AS, Ergul M (2021) The modulator action of thiamine against pentylenetetrazole-induced seizures, apoptosis, nitric oxide, and oxidative stress in rats and SH-SY5Y neuronal cell line. Chem Biol Interact 340:109447

Taskiran AS, Ergul M, Gunes H, Ozturk A, Sahin B, Ozdemir E (2021) The effects of proton pump inhibitors (pantoprazole) on pentylenetetrazole-induced epileptic seizures in rats and neurotoxicity in the SH-SY5Y human neuroblastoma cell line. Cell Mol Neurobiol 41(1):173–183

Taskıran AS, Ozdemir E, Gumus E, Ergul M (2020) The effects of salmon calcitonin on epileptic seizures, epileptogenesis, and postseizure hippocampal neuronal damage in pentylenetetrazole-induced epilepsy model in rats. Epilepsy Behav 113:107501

Terzïoğlu Bebïtoğlu B, Oğuz Ef, Acet G, (2020) Effect of valproic acid on oxidative stress parameters of glutamate-induced excitotoxicity in SH-SY5Y cells. Exp Ther Med 20(2):1321–1328

Yildizhan K, Naziroglu M (2020) Glutathione depletion and parkinsonian neurotoxin MPP(+)-induced TRPM2 channel activation play central roles in oxidative cytotoxicity and inflammation in microglia. Mol Neurobiol 57(8):3508–3525

Yildizhan K, Naziroglu M (2022) Protective role of selenium on MPP(+) and homocysteine-induced TRPM2 channel activation in SH-SY5Y cells. J Recept Signal Transduct Res 42(4):399–408

Zhang Y, Zhao J, Afzal O, Kazmi I, Al-Abbasi FA, Altamimi AS, Yang Z (2021) Neuroprotective role of chrysin-loaded poly (lactic-co-glycolic acid) nanoparticle against kindling-induced epilepsy through Nrf2/ARE/HO-1 pathway. J Biochem Mol Toxicol 35(2):e22634

Zheng Q, Zhu T, Hu H, Zhao Y, Ying Y, Luo X, Ling Y, Chen Z, Ji H, Jiang P (2020) TRPM2 ion channel is involved in the aggravation of cognitive impairment and down regulation of epilepsy threshold in pentylenetetrazole-induced kindling mice. Brain Res Bull 155:48–60

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