Original Article

Treatment of pilocarpine-induced epileptic seizures in adult male mice

Tratamento de crises epilépticas induzidas por pilocarpina em camundongos adultos machos

W. K. Abdelbasset[a,b]* ⓘ, S. A. Jasim[c] ⓘ, M. Rudiansyah[d] ⓘ, H. Huldani[e] ⓘ, R. Margiana[f,g] ⓘ, A. T. Jalil[h,i] ⓘ, H. J. Mohammad[j] ⓘ, H. SH. Ridha[k] ⓘ and G. Yasin[l] ⓘ

[a]Prince Sattam bin Abdulaziz University, College of Applied Medical Sciences, Department of Health and Rehabilitation Sciences, Al Kharj, Saudi Arabia

[b]Cairo University, Kasr Al-Aini Hospital, Department of Physical Therapy, Giza, Egypt

[c]Al-Maarif University College, Medical Laboratory Techniques Department, Al-anbar-Ramadi, Iraq

[d]Universitas Lambung Mangkurat, Faculty of Medicine, Department of Internal Medicine, Ulin Hospital, Banjarmasin, Indonesia

[e]Lambung Mangkurat University, Department of Physiology, Magister Management, Magister Immunology, Banjarmasin, South Borneo, Indonesia

[f]Universitas Indonesia, Faculty of Medicine, Department of Anatomy, Jakarta, Indonesia

[g]Universitas Indonesia, Faculty of Medicine, Master's Programme Biomedical Sciences, Jakarta, Indonesia

[h]Yanka Kupala State University of Grodno, Faculty of Biology and Ecology, Grodno, Belarus

[i]The Islamic University, College of Technical Engineering, Najaf, Iraq

[j]Al-Manara College for Medical Sciences, Maysan, Iraq

[k]Al-Nisour University College, Baghdad, Iraq

[l]Bahauddin Zakariya University, Department of Botany, Multan, Pakistan

Abstract

Epilepsy is one of the most common neurological disorders affecting most social, economic and biological aspects of human life. Most patients with epilepsy have uncontrolled seizures and drug side effects despite the medications. Patients with epilepsy often have problems with attention, memory, and information processing speed, which may be due to seizures, underlying causes, or anticonvulsants. Therefore, improving seizure control and reducing or changing the anti-epileptic drugs can solve these problems, but these problems will not be solved in most cases. In this work, we looked at the effects of pioglitazone, a Peroxisome Proliferator-Activated Receptor agonist used to treat type 2 diabetes, on pilocarpine-induced seizures in mice. The Racine scale was used to classify pilocarpine-induced convulsions. After that, all of the animals were beheaded, and the brain and hippocampus were dissected. Finally, biochemical techniques were used to determine the levels of Malondialdehyde and Catalase activity, as well as Superoxide Dismutase and Glutathione Reductase in the hippocampus. The results of this investigation suggest that pioglitazone's antioxidant action may play a key role in its neuroprotective properties against pilocarpine-induced seizure neuronal damage.

Keywords: seizure, pioglitazone, pilocarpine, oxidative stress, anticonvulsants.

Resumo

A epilepsia é um dos distúrbios neurológicos mais comuns que afetam a maioria dos aspectos sociais, econômicos e biológicos da vida humana. A maioria dos pacientes com epilepsia tem convulsões não controladas e apresenta efeitos colaterais de medicamentos. Pacientes com epilepsia, geralmente, têm problemas de atenção, memória e velocidade de processamento de informações, ocasionados por convulsões, causas subjacentes ou anticonvulsivantes. Portanto, melhorar o controle das crises e reduzir ou alterar as drogas antiepilépticas pode resolver esses problemas, mas, na maioria dos casos, eles não serão resolvidos. Neste trabalho, analisamos os efeitos da pioglitazona, um agonista do receptor ativado por proliferador de peroxissoma usado para tratar diabetes tipo 2, em convulsões induzidas por pilocarpina em camundongos. A escala de Racine foi usada para classificar as convulsões induzidas pela pilocarpina. Em seguida, todos os animais foram decapitados, e o cérebro e o hipocampo foram dissecados. Finalmente, técnicas bioquímicas foram utilizadas para determinar os níveis de atividade do malondialdeído e da catalase, bem como da superóxido dismutase e glutationa redutase no hipocampo. Os resultados desta investigação sugerem que a ação antioxidante da pioglitazona pode desempenhar um papel fundamental em suas propriedades neuroprotetoras contra o dano neuronal convulsivo induzido pela pilocarpina.

Palavras-chave: convulsão, pioglitazona, pilocarpina, estresse oxidativo, anticonvulsivantes.

1. Introduction

Epilepsy is a central nervous system (CNS) illness that causes abnormal brain activity, resulting in seizures, odd behavior, and occasionally loss of consciousness. The brain's electrical activity is periodically disturbed, resulting in some degree of temporary brain dysfunction (Camporeze et al., 2018; Liu et al., 2018, 2019; Malik and Willnow, 2019). People of various ages, genders, and ethnicities can be affected by epilepsy. The terms "seizures" and "epilepsy" are not interchangeable (Dhrivastava et al., 2019). Seizures are one of the epilepsy symptoms, although not everyone who has one has epilepsy (Beghi, 2020). Seizures are a single occurrence, but epilepsy is a neurological condition marked by two or more seizures. Seizures include a wide range of symptoms. Uncontrollable bodily movements are not always associated with seizures. During a seizure, some persons with epilepsy stare at a single location for a few seconds, while others move their arms or legs abnormally (Mahamud et al., 2018; Zelano et al., 2020; Karoly et al., 2021). Seizures are classified according to how they begin and which portion of the brain they affect (Pack, 2019). The majority of seizures last between 30 and two minutes. A medical emergency is a seizure that lasts more than five minutes (DeLorenzo et al., 1999; Shinnar et al., 2001). In industrialized countries, epilepsy affects 40-70 people per 100,000, but in poor countries, it affects 100-190 people per 100,000 (Keykhosravi et al., 2019). When Alfred Hartmann developed phenobarbital in 1912, pharmacologic epilepsy therapy became widespread around the turn of the century (Villalba, 2017; Rostamian et al., 2021). Phenytoin, valproate, and carbamazepine were among the antiepileptic medications found during the next few decades (Wat et al., 2019). Anticonvulsant drugs are required by more than 60% of persons with epilepsy (Thijs et al., 2019). Cognitive problems, ataxia, and drowsiness are all adverse effects of these medicines. Even after the use of existing treatments, seizures are still occurring in 30% of individuals (Brunbech and Sabers, 2002; Zaccara et al., 2008). As a result, new anticonvulsant drugs with reduced side effects and excellent effectiveness are urgently needed. Antioxidants, such as melatonin, added to antiepileptic medicines have been demonstrated in clinical studies to lessen epilepsy-related neurological problems (Naziroğlu, 2015; Rocha et al., 2018; Dinka, 2020; Wałowski, 2021). Due to a change in sources of energy, like the use of fat-derived ketone bodies, ketogenic diets lower mitochondrial reactive oxygen/nitrogen species (ROS/RNS) (Zhou et al., 2021).

Seizures, Alzheimer's disease, stroke, and migraine are the most common neurological ailments related to epilepsy (Farrell et al., 2017; Azimi and Asgarpanah, 2021; Alharbi, 2021). According to research, seizures have been demonstrated to attack neurons by causing oxidative stress and generating free radicals (Patel, 2002, 2004; Lin et al., 2020). Status epilepticus can lead to energy loss due to mitochondrial respiratory chain malfunction (Young and Dragunow, 1994). The results may exacerbate oxidative stress and lead to hippocampal neuronal death (Freitas et al., 2005; Liu et al., 2010). As a result, taking antioxidants may lower the chance of seizures causing brain damage. Oxidative stress produced by employing pilocarpine in large dosages harms the hippocampus's GABAergic (gamma-aminobutyric acidergic) neurons, which finally leads to status epilepticus is one way to induce seizure in rodents (Freitas et al., 2004; Alharbi, 2021). Pioglitazone, an agonist of the Peroxisome Proliferator-Activated Receptor -γ nuclear receptor, improves insulin receptor sensitivity and is used to treat type 2 diabetes (Yap et al., 2020). According to research, Pioglitazone possesses antioxidant properties and shields neurons from oxidative stress-related damage (Nicolakakis et al., 2008; Khasabova et al., 2019). Pioglitazone strengthens the antioxidant defense system by scavenging free radicals (Mahamud et al., 2018). Thus, the pilocarpine-induced seizures in mice under the pioglitazone's anticonvulsant and antioxidant effects were investigated in the current study.

2. Materials and Methods

Epilepsy is a persistent brain condition that impacts roughly 1% of the global population. It can have a detrimental influence on a patient's safety, relationships, employment, and life quality. In roughly 70% of instances, seizures may be controlled with anti-epileptic medicines, whereas 30% of patients remain resistant. It is consequently necessary to find new anti-epileptic targets. In this study, 28 adult male mice (about six weeks old) weighing 20±2 grams were obtained. Throughout the research, the mice had unrestricted access to water and food. They were housed in separate cages at 22-24oC room temperature with a 12-hour light/dark cycle. This research was carried out in conformity with institutional, national, and international regulations and standards for animal testing (OPRR, 1986; OLAW, 2002; Couto and Cates, 2019). The following chemicals were bought from Sigma Aldrich: pilocarpine hydrochloride (P6503), xylazine hydrochloride (X1251), ketamine hydrochloride (K113), and pioglitazone hydrochloride (E6910). In this investigation 80 mg/kg of pioglitazone was dissolved in 0.1% carboxymethyl cellulos (w/v) and given to the mice orally (Rajaba et al., 2014). To induce seizure, 400 mg/kg of pilocarpine (a single dose) was administered intraperitoneally (IP) (Figure 1) (Alharbi, 2021).

Figure 1. The medication was injected intraperitoneally into the mice.

The animals were also given Xylazine or Ketamine IP to induce anesthesia. The mice were separated into the following classes:

1. 240 minutes after the oral dose of carboxymethyl cellulos 0.1%, normal saline was injected (*Control group*);
2. 240 minutes following the 80 mg/kg oral dose of pioglitazone, normal saline was injected (*Pioglitazone group*);
3. 240 minutes after an oral dose of carboxymethyl cellulos 0.1%, pilocarpine was administered at 390 mg/kg (*Pioglitazone group*);
4. 240 minutes after 80 mg/kg of pioglitazone was given orally, pilocarpine was administered at 390 mg/kg (*Treatment group*).

For 60 minutes following the pilocarpine injection, the animals' convulsive behavior was recorded on camera. Based on a modified Racine's scale (Cela et al., 2019), the strength of the convulsions was recorded as no response (Phase 0); vibrissae twitching, restlessness, and hyperactivity (Phase 1); myoclonic jerks, clonus, and head nodding (Phase 2); bilateral or unilateral limb clonus (Phase 3); clonic seizures of forelimbs (Phase 4); and generalized tonic-clonic seizures and falling (Phase 5).

After determining the intensity of seizures, animals were killed by guillotine under deep levels of ketamine-induced anaesthesia. The hippocampus was detached from the brain, homogenized in 1.5% KCl solution weighed, and blotted dry as soon as the brain was removed from the cranium. The homogenates subsequently centrifuged to yield hippocampal PMF (Post-Mitochondrial Fluid), which was used in the biochemical experiment. The quantity of proteins in the hippocampus was measured using bovine serum albumin as a reference (Sultan, 2013).

Employing the Thiobarbituric Acid technique, the Malondialdehyde level in the mice's hippocampus was evaluated to assess lipid peroxidation.

One ml Thiobarbituric Acid and three ml phosphoric acid (3%) were added to a centrifuge tube containing 0.5 ml hippocampal sample. In a boiling water bath, the solution was boiled for 45 minutes. Then, a spectrophotometer was used to measure the absorbance of the n-butanol (organic layer) at 539 nm. With 1, 1, 3, 3-tetramethoxypropane as the standard, a standard curve was produced. Malondialdehyde nanomoles contained in one gram of hippocampus were used to represent the results (Naderi et al., 2017, 2020). The Catalase activity was measured using the Claiborne technique. The reaction solution for this technique was made up of 1.89 mL phosphate buffer (pH=7.3, 0.12 M), 1.1 mL H_2O_2 (0.02 M), and 0.049 mL PMF. Afterwards, at 240 nm the absorbance was measured, and the findings were represented (Rashid et al., 2014; Kandemir et al., 2017).

The capacity of such an enzyme to block the degradation of NBT (Nitroblue-tetrazolium) was used to measure Superoxide Dismutase activity. The sample (0.1 mL) was mixed in with the reagent solution made up of 1.5 mM NBT, 0.3 mM NaCN, 0.1M EDTA, and 0.067 M KHPO$_4$ (pH 7.8). For 10 minutes the reaction mixture was incubated after adding 0.12 mM riboflavin to the samples. At 560 nm the absorbance was measured using a spectrophotometer. The enzyme unit/mg protein was used to quantify the data. The quantity of enzyme necessary to achieve a 50%

inhibition was one unit (Zhang et al., 2016). The activity of glutathione reductase was measured using the method of Carlberg and Manevrick (Shakeel et al., 2017). The reagent mixture consisted of 0.1 mL 10% PMS, 0.1 mL NADPH (0.1 mM), 0.05 mL GSH (1 mM), 0.1 mL EDTA (0.5 mM), and 1.65 mL phosphate buffer (pH=7.3, 0.12 M). NADPH consumption was used as a metric for determining enzyme activity. Nmol NADPH oxidized/min/mg/protein was used to represent the data (Sandhir and Gill, 1995), and at 340 nm, the absorbance was measured.

For the data analysis, we utilized GraphPad prism 8. In groups of seven items, all data was presented as Means±SEM. We further employed a one-way analysis of variance (ANOVA) to analyze the mean differences. Statistical significance was defined as a P<0.05.

3. Results and Discussion

Plasma glucose levels were evaluated at baseline and after 14 days of daily therapy with either dimethyl sulfoxide (DMSO) or pioglitazone + DMSO, but before pilocarpine injection, to see if pioglitazone altered plasma glucose levels and neuronal excitability in experimental mice. Pioglitazone and DMSO had no effect on blood glucose levels (Figure 2).

Mice were given daily doses of pioglitazone or DMSO to see if activating Peroxisome Proliferator-Activated Receptor-γ might reduce the severity of seizures. The mice were given pilocarpine after 14 days to produce seizures and excitotoxicity. Pioglitazone had no effect on the latency period of acute seizures in any of the groups (Figure 3).

The study found that neither 80 mg/kg pioglitazone nor the control group caused the mice to have seizures. After injection, 400 mg/kg pilocarpine caused to phase 1-5 seizures. The injection of pioglitazone 4 hours prior to administering pilocarpine substantially exacerbated the beginning of phases 1 to 4 of seizure, according to observations seen between pilocarpine and pioglitazone groups (P≤0.01-0.001). Furthermore, pioglitazone stopped pilocarpine-induced seizures from progressing to phase 5 (Table 1).
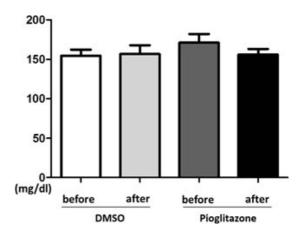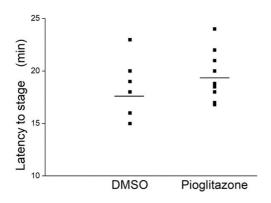

Figure 2. Pioglitazone treatment for two weeks had no effect on mice's blood glucose levels.
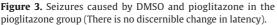
Table 1. Pioglitazone's effect on convulsion latency in seizures induced by pilocarpine in mice.

Groups	Means±SEM				
	Phase 5	Phase 4	Phase 3	Phase 2	Phase 1
Treatment (Pioglitazone + Pilocarpine)	-	392.6±12.612	216±22.971	113.4±7.733	92.8±15.19
Pioglitazone	-	-	-	-	-
Pilocarpine	254±13.95	218.6±19.241	95.6±3.744	88.1±8.785	41.6±9.17
Control	-	-	-	-	-

Table 2. After a seizure induced by pilocarpine in mice, the pioglitazone effects on the Catalase, Superoxide Dismutase, and Glutathione Reductase in the hippocampus are presented.

Groups	Means±SEM		
	Catalase activity	Superoxide Dismutase activity	Glutathione Reductase activity
Treatment (Pioglitazone + Pilocarpine)	12.6±0.86	58.12±8.73	45.32±5.73
Pioglitazone	13.73±2.31	60.13±18.23	54.84±6.32
Pilocarpine	6.23±0.83	27.23.6±7.53	25.63±7.32
Control	13.76±1.86	60.06±17.32	51.13±8.12

Figure 3. Seizures caused by DMSO and pioglitazone in the pioglitazone group (There is no discernible change in latency).
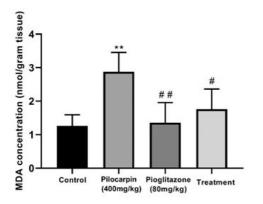

Figure 4. After a seizure induced by pilocarpine, the pioglitazone effect on Malondialdehyde (MDA) levels in the hippocampus of mice. **P<0.01 compared to the control group. #P<0.05 compared to the pilocarpine group. ##P<0.01 compared to the pilocarpine group.

The levels of lipid peroxidation in the hippocampus of mice were considerably increased by pilocarpine-induced seizures, resulting in a much-elevated level of Malondialdehyde than the control group (P<0.01; Figure 4). However, the level of Malondialdehyde in pioglitazone-treated mice was considerably lower than in pilocarpine-treated mice. As a result, after pilocarpine-induced seizures in mice, pioglitazone injection dramatically reduced the degree of lipid peroxidation (Figure 4).

When compared to the control group, the pilocarpine-induced seizure reduced Glutathione Reductase (P<0.001), Superoxide Dismutase (P<0.05), and Catalase activity (P<0.01) in the hippocampus of mice (Table 2). Furthermore, as compared to the pilocarpine group, 80 mg/kg pioglitazone given 4 hours preceding 400 mg/kg pilocarpine injection substantially elevated Glutathione Reductase (P<0.001), Superoxide Dismutase (P<0.05), and Catalase activity (P<0.05) (Table 2). Finally, as compared with the control group, the injection of pioglitazone had no influence on the function of these enzymes in the hippocampus of mice (P>0.05) (Table 2).

4. Conclusion

In the current study, seizure severity was assessed based on the Racine scale. According to our results, the injection of pioglitazone 4 hours preceding injecting pilocarpine accelerated the beginning of phases 1 to 4 of pilocarpine-induced seizures. According to previous research, the excessive formation of free oxygen radicals has been linked to neuronal injury in mice suffering from pilocarpine-induced seizures. Increased generation of free oxygen radicals after a pilocarpine-induced seizure causes lipid peroxidation and the formation of

Malondialdehyde in the hippocampus of mice. Pilocarpine administration also elevated Malondialdehyde levels in the hippocampus of mice while decreasing the Glutathione Reductase, Superoxide Dismutase, and Catalase enzymes' antioxidant activity. Throughout this investigation, the latency of the commencement of seizure caused by pilocarpine was dramatically delayed after pre-treatment with pioglitazone. Furthermore, it prevented pilocarpine-induced status epilepticus. In the hippocampus, pioglitazone simultaneously enhanced antioxidant defenses and reduced oxidative stress. Because pioglitazone decreased oxidative threat posed by pilocarpine toxicity, the results of this paper imply that it has anticonvulsant properties.

References

ALHARBI, K.S., 2021. Anticonvulsant effects of desvenlafaxine on modulating brain monoamine and oxidative stress in mice. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, e246194. PMid:34468514.

AZIMI, G. and ASGARPANAH, J., 2021. Chemical composition of *Zhumeria majdae* essential oil and its effects on the expression of morphine withdrawal syndrome and tolerance to the anticonvulsant effect of morphine on pentylenetetrazole-induced seizures in mice. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 4, pp. 881-886. http://dx.doi.org/10.1590/1519-6984.228825. PMid:33053122.

BEGHI, E., 2020. The epidemiology of epilepsy. *Neuroepidemiology*, vol. 54, no. 2, suppl. 2, pp. 185-191. http://dx.doi.org/10.1159/000503831. PMid:31852003.

BRUNBECH, L. and SABERS, A., 2002. Effect of antiepileptic drugs on cognitive function in individuals with epilepsy: a comparative review of newer versus older agents. *Drugs*, vol. 62, no. 4, pp. 593-604. http://dx.doi.org/10.2165/00003495-200262040-00004. PMid:11893228.

CAMPOREZE, B., MANICA, B.A., BONAFÉ, G.A., FERREIRA, J.J.C., DINIZ, A.L., DE OLIVEIRA, C.T.P., DE AGUIAR, P.H.P. and ORTEGA, M.M., 2018. Optogenetics: the new molecular approach to control functions of neural cells in epilepsy, depression and tumors of the central nervous system. *American Journal of Cancer Research*, vol. 8, no. 10, pp. 1900-1918. PMid:30416844.

CELA, E., MCFARLAN, A.R., CHUNG, A.J., WANG, T., CHIERZI, S., MURAI, K.K. and SJÖSTRÖM, P.J., 2019. An optogenetic kindling model of neocortical epilepsy. *Scientific Reports*, vol. 9, no. 1, pp. 5236. http://dx.doi.org/10.1038/s41598-019-41533-2. PMid:30918286.

COUTO, M. and CATES, C., 2019. Laboratory guidelines for animal care. *Methods in Molecular Biology*, vol. 1920, pp. 407-430. http://dx.doi.org/10.1007/978-1-4939-9009-2_25. PMid:30737706.

DELORENZO, R.J., GARNETT, L.K., TOWNE, A.R., WATERHOUSE, E.J., BOGGS, J.G., MORTON, L., CHOUDHRY, M.A., BARNES, T. and KO, D., 1999. Comparison of status epilepticus with prolonged seizure episodes lasting from 10 to 29 minutes. *Epilepsia*, vol. 40, no. 2, pp. 164-169. http://dx.doi.org/10.1111/j.1528-1157.1999.tb02070.x. PMid:9952262.

DHRIVASTAVA, A.K., SHRIVASTAV, A., SHRIVASTAV, M., GUPTA, A., PRAKASH, S., FATIMA, A. and SRIVASTAV, A., 2019. Epilepsy: the next generation drugs (a review). *Journal of Drug Delivery and Therapeutics*, vol. 9, no. 1, pp. 286-292. http://dx.doi.org/10.22270/jddt.v9i1.2279.

DINKA, M.O., 2020. Groundwater quality composition and its suitability for drinking in long-term irrigated area. *Journal of Water and Land Development*, vol. 44, no. 1-3, pp. 43-54.

FARRELL, J.S., WOLFF, M.D. and TESKEY, G.C., 2017. Neurodegeneration and pathology in epilepsy: clinical and basic perspectives. *Advances in Neurobiology*, vol. 15, pp. 317-334. PMid:28674987.

FREITAS, R.M., SOUSA, F.C.F., VASCONCELOS, S.M.M., VIANA, G.S.B. and FONTELES, M.M.F., 2004. Pilocarpine-induced status epilepticus in rats: lipid peroxidation level, nitrite formation, GABAergic and glutamatergic receptor alterations in the hippocampus, striatum and frontal cortex. *Pharmacology, Biochemistry, and Behavior*, vol. 78, no. 2, pp. 327-332. http://dx.doi.org/10.1016/j.pbb.2004.04.004. PMid:15219774.

FREITAS, R.M., VASCONCELOS, S.M., SOUZA, F.C., VIANA, G.S. and FONTELES, M.M., 2005. Oxidative stress in the hippocampus after pilocarpine-induced status epilepticus in Wistar rats. *The FEBS Journal*, vol. 272, no. 6, pp. 1307-1312. http://dx.doi.org/10.1111/j.1742-4658.2004.04537.x. PMid:15752349.

KANDEMIR, F.M., KUCUKLER, S., ELDUTAR, E., CAGLAYAN, C. and GÜLÇIN, İ., 2017. Chrysin protects rat kidney from paracetamol-induced oxidative stress, inflammation, apoptosis, and autophagy: a multi-biomarker approach. *Scientia Pharmaceutica*, vol. 85, no. 1, pp. 4. http://dx.doi.org/10.3390/scipharm85010004. PMid:28134775.

KAROLY, P.J., RAO, V.R., GREGG, N.M., WORRELL, G.A., BERNARD, C., COOK, M.J. and BAUD, M.O., 2021. Cycles in epilepsy. *Nature Reviews. Neurology*, vol. 17, no. 5, pp. 267-284. http://dx.doi.org/10.1038/s41582-021-00464-1. PMid:33723459.

KEYKHOSRAVI, E., SAEIDI, M. and KIANI, M.A., 2019. A brief overview of epilepsy with emphasis on children. *International Journal of Pediatrics*, vol. 7, no. 11, pp. 10387-10395.

KHASABOVA, I.A., KHASABOV, S.G., OLSON, J.K., UHELSKI, M.L., KIM, A.H., ALBINO-RAMÍREZ, A.M., WAGNER, C.L., SEYBOLD, V.S. and SIMONE, D.A., 2019. Pioglitazone, a PPARγ agonist, reduces cisplatin-evoked neuropathic pain by protecting against oxidative stress. *Pain*, vol. 160, no. 3, pp. 688-701. http://dx.doi.org/10.1097/j.pain.0000000000001448. PMid:30507781.

LIN, T.-K., CHEN, S.-D., LIN, K.-J. and CHUANG, Y.-C., 2020. Seizure-induced oxidative stress in status epilepticus: is antioxidant beneficial? *Antioxidants*, vol. 9, no. 11, pp. 1029. http://dx.doi.org/10.3390/antiox9111029. PMid:33105652.

LIU, J., WANG, A., LI, L., HUANG, Y., XUE, P. and HAO, A., 2010. Oxidative stress mediates hippocampal neuron death in rats after lithium–pilocarpine-induced status epilepticus. *Seizure*, vol. 19, no. 3, pp. 165-172. http://dx.doi.org/10.1016/j.seizure.2010.01.010. PMid:20149694.

LIU, Y., GAO, J., PENG, M., MENG, H., MA, H., CAI, P., XU, Y., ZHAO, Q. and SI, G., 2018. A review on central nervous system effects of gastrodin. *Frontiers in Pharmacology*, vol. 9, pp. 24. http://dx.doi.org/10.3389/fphar.2018.00024. PMid:29456504.

LIU, Y.-J., CHEN, J., LI, X., ZHOU, X., HU, Y.-M., CHU, S.-F., PENG, Y. and CHEN, N.-H., 2019. Research progress on adenosine in central nervous system diseases. *CNS Neuroscience & Therapeutics*, vol. 25, no. 9, pp. 899-910. http://dx.doi.org/10.1111/cns.13190. PMid:31334608.

MAHAMUD, Z., BURMAN, J. and ZELANO, J., 2018. Risk of epilepsy after a single seizure in multiple sclerosis. *European Journal of Neurology*, vol. 25, no. 6, pp. 854-860. http://dx.doi.org/10.1111/ene.13618. PMid:29512931.

MALIK, A.R. and WILLNOW, T.E., 2019. Excitatory amino acid transporters in physiology and disorders of the central nervous system. *International Journal of Molecular Sciences*, vol. 20,

no. 22, pp. 5671. http://dx.doi.org/10.3390/ijms20225671. PMid:31726793.

NADERI, Y., PANAHI, Y., BARRETO, G.E. and SAHEBKAR, A., 2020. Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review. *Neural Regeneration Research*, vol. 15, no. 5, pp. 773-782. http://dx.doi.org/10.4103/1673-5374.268898. PMid:31719236.

NADERI, Y., SABETKASAEI, M., PARVARDEH, S. and ZANJANI, T.M., 2017. Neuroprotective effect of minocycline on cognitive impairments induced by transient cerebral ischemia/reperfusion through its anti-inflammatory and anti-oxidant properties in male rat. *Brain Research Bulletin*, vol. 131, pp. 207-213. http://dx.doi.org/10.1016/j.brainresbull.2017.04.010. PMid:28454931.

NAZIROĞLU, M., 2015. Role of melatonin on calcium signaling and mitochondrial oxidativestress in epilepsy: focus on TRP channels. *Turkish Journal of Biology*, vol. 39, no. 6, pp. 813-821. http://dx.doi.org/10.3906/biy-1505-43.

NICOLAKAKIS, N., ABOULKASSIM, T., ONGALI, B., LECRUX, C., FERNANDES, P., ROSA-NETO, P., TONG, X.-K. and HAMEL, E., 2008. Complete rescue of cerebrovascular function in aged Alzheimer's disease transgenic mice by antioxidants and pioglitazone, a peroxisome proliferator-activated receptor γ agonist. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, vol. 28, no. 37, pp. 9287-9296. http://dx.doi.org/10.1523/JNEUROSCI.3348-08.2008. PMid:18784309.

OFFICE FOR PROTECTION FROM RESEARCH RISKS – OPRR, 1986. *Public Health Service policy on humane care and use of laboratory animals.* Bethesda: National Institutes of Health.

OFFICE OF LABORATORY ANIMAL WELFARE – OLAW, 2002. *Institutional animal care and use committee guidebook.* Bethesda: OLAW.

PACK, A.M., 2019. Epilepsy overview and revised classification of seizures and epilepsies. *CONTINUUM: Lifelong Learning in Neurology*, vol. 25, no. 2, pp. 306-321. PMid:30921011.

PATEL, M., 2004. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. *Free Radical Biology & Medicine*, vol. 37, no. 12, pp. 1951-1962. http://dx.doi.org/10.1016/j.freeradbiomed.2004.08.021. PMid:15544915.

PATEL, M.N., 2002. Oxidative stress, mitochondrial dysfunction, and epilepsy. *Free Radical Research*, vol. 36, no. 11, pp. 1139-1146. http://dx.doi.org/10.1080/1071576021000016391. PMid:12592665.

RAJABA, A., OSTADHADI, S., RASTEGAR, H. and DEHPOUR, A., 2014. Anti-pruritic activity of pioglitazone on serotonin-induced scratching in mice: possible involvement of PPAR-gamma receptor and nitric oxide. *European Journal of Pharmacology*, vol. 744, pp. 103-107. http://dx.doi.org/10.1016/j.ejphar.2014.10.002. PMid:25310911.

RASHID, S., ALI, N., NAFEES, S., HASAN, S.K. and SULTANA, S., 2014. Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food and Chemical Toxicology*, vol. 66, pp. 185-193. http://dx.doi.org/10.1016/j.fct.2014.01.026. PMid:24486618.

ROCHA, A.K.A., CIPOLLA-NETO, J. and AMADO, D., 2018. Epilepsy: neuroprotective, anti-inflammatory, and anticonvulsant effects of melatonin. In: L. CORREIA and G. MAYERS, eds. *Melatonin: medical uses and role in health and disease.* New York: Nova Science Publishers, chap. 8.

ROSTAMIAN, S., KESHAVARZ HEDAYATI, S., KHOSRAVIANI, S., AALI, E. and NADERI, Y., 2021. Anticonvulsive and antioxidant effects of pioglitazone on pilocarpine-induced seizures in mice. *Iranian Journal of Toxicology*, vol. 15, no. 4, pp. 271-278. http://dx.doi.org/10.32598/IJT.15.4.833.1.

SANDHIR, R. and GILL, K.D., 1995. Effect of lead on lipid peroxidation in liver of rats. *Biological Trace Element Research*, vol. 48, no. 1, pp. 91-97. http://dx.doi.org/10.1007/BF02789081. PMid:7626375.

SHAKEEL, S., REHMAN, M.U., TABASSUM, N. and AMIN, U., 2017. Effect of naringenin (a naturally occurring flavanone) against pilocarpine-induced status epilepticus and oxidative stress in mice. *Pharmacognosy Magazine*, vol. 13, no. 49, suppl. 1, pp. 154. http://dx.doi.org/10.4103/0973-1296.203977.

SHINNAR, S., BERG, A.T., MOSHE, S.L. and SHINNAR, R., 2001. How long do new-onset seizures in children last? *Annals of Neurology*, vol. 49, no. 5, pp. 659-664. http://dx.doi.org/10.1002/ana.1018. PMid:11357957.

SULTAN, F.A., 2013. Dissection of different areas from mouse hippocampus. *Bio-Protocol*, vol. 3, no. 21, e955. http://dx.doi.org/10.21769/BioProtoc.955. PMid:27390757.

THIJS, R.D., SURGES, R., O'BRIEN, T.J. and SANDER, J.W., 2019. Epilepsy in adults. *Lancet*, vol. 393, no. 10172, pp. 689-701. http://dx.doi.org/10.1016/S0140-6736(18)32596-0. PMid:30686584.

VILLALBA, S.V., 2017. *The role of pharmacogenetics in the treatment of neurocardiac dysfunction in two mouse models of epilepsy.* Shreveport: Louisiana State University. PhD Thesis in Philosophy.

WAŁOWSKI, G., 2021. Development of biogas and biorafinery systems in Polish rural communities. *Journal of Water and Land Development*, vol. 49, pp. 156-168.

WAT, R., MAMMI, M., PAREDES, J., HAINES, J., ALASMARI, M., LIEW, A., LU, V.M., ARNAOUT, O., SMITH, T.R., GORMLEY, W.B., AGLIO, L.S., MEKARY, R.A. and ZAIDI, H., 2019. The effectiveness of antiepileptic medications as prophylaxis of early seizure in patients with traumatic brain injury compared with placebo or no treatment: a systematic review and meta-analysis. *World Neurosurgery*, vol. 122, pp. 433-440. http://dx.doi.org/10.1016/j.wneu.2018.11.076. PMid:30465951.

YAP, K.H., YEE, G.S., CANDASAMY, M., TAN, S.C., MD, S., ABDUL MAJEED, A.B. and BHATTAMISRA, S.K., 2020. Catalpol ameliorates insulin sensitivity and mitochondrial respiration in skeletal muscle of type-2 diabetic mice through insulin signaling pathway and AMPK/SIRT1/PGC-1α/PPAR-γ activation. *Biomolecules*, vol. 10, no. 10, pp. 1360. http://dx.doi.org/10.3390/biom10101360. PMid:32987623.

YOUNG, D. and DRAGUNOW, M., 1994. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. *Neuroscience*, vol. 58, no. 2, pp. 245-261. http://dx.doi.org/10.1016/0306-4522(94)90032-9. PMid:8152537.

ZACCARA, G., GANGEMI, P.F. and CINCOTTA, M., 2008. Central nervous system adverse effects of new antiepileptic drugs: a meta-analysis of placebo-controlled studies. *Seizure*, vol. 17, no. 5, pp. 405-421. http://dx.doi.org/10.1016/j.seizure.2007.12.003. PMid:18262442.

ZELANO, J., HOLTKAMP, M., AGARWAL, N., LATTANZI, S., TRINKA, E. and BRIGO, F., 2020. How to diagnose and treat post-stroke seizures and epilepsy. *Epileptic Disorders*, vol. 22, no. 3, pp. 252-263. http://dx.doi.org/10.1684/epd.2020.1159. PMid:32597766.

ZHANG, C., BRUINS, M.E., YANG, Z., LIU, S. and RAO, P., 2016. A new formula to calculate activity of superoxide dismutase in indirect assays. *Analytical Biochemistry*, vol. 503, pp. 65-67. http://dx.doi.org/10.1016/j.ab.2016.03.014. PMid:27033009.

ZHOU, X., JOSHI, S., PATIL, S., KHARE, T. and KUMAR, V., 2021. Reactive oxygen, nitrogen, carbonyl and sulfur species and their roles in plant abiotic stress responses and tolerance. *Journal of Plant Growth Regulation*, vol. 41, pp. 119-142.