Niger. J. Physiol. Sci. 38 (December 2023): 187 – 193
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Quercetin Exerts Anticonvulsant Effect through Mitigation of Neuroinflammatory Response in Pentylenetetrazole-induced Seizure in Mice

Adeoluwa, O.A.[1], Adeoluwa, G.O.[1], Adeniyi, F.R.[2], Oni, J.O.[1], Akinluyi, E.T.[1], Olojede, S.O.[3] and Bakre, A.G.[4]

[1] *Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti.*

[2] *Department of Pharmacology and Toxicology, College of Pharmacy, Afe Babalola University, Ado Ekiti.*

[3] *Department of Human Biology, Faculty of Medicine and Health Sciences, Walter Sisulu University, Nelson Mandela Drive, Mthatha, South Africa.*

[4]*Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan. Ibadan, Nigeria*

Summary: Epilepsy is a chronic disease of the brain characterized by seizures. The currently available anticonvulsants only treat symptoms with serious adverse drug reactions. Therefore, there is need for new therapeutic intervention that will prevent epileptogenesis with greater therapeutic success. Quercetin (QT) is a flavonoid with known neuroprotective and anti-inflammatory properties. The study aimed to investigate its effects against pentylenetetrazole (PTZ)-induced seizures. Animals were divided into four groups (n = 10). Group 1(control) only received vehicle (10 mL/kg), group 2 received vehicle, groups 3 and 4 received QT 12.5 mg/kg and 25 mg/kg respectively. Sixty minutes after treatments, animals in groups 2 to 4 were injected with sub-convulsive dose of pentylenetetrazole (35 mg/kg, i.p.) on every alternate day (48±2h) for 21 days. The mice were observed for 30 minutes after each PTZ injection for seizure activity. Brain samples were collected for biochemical assays. Administration of PTZ caused significant increase in the intensity of seizures, neuronal degeneration and level of proinflammatory cytokines in animals compared to control. These behavioural alterations were attenuated significantly by QT (12.5 and 25 mg/kg). The PTZ-induced increase in IL-12, TNF-α and IFN-ɣ were significantly reduced by pre-treatment with the QT (12.5 and 25 mg/kg, p.o). Quercetin also reduced neuronal loss compared to control. Quercetin attenuates seizures in kindled mice and reduces neuroinflammation and neurodegeneration. This neuroprotective effect may be attributed to its ability to inhibit inflammatory mediators in the brain.

Keywords*: Epilepsy, neuroinflammation, kindling, neurodegeneration, epileptogenesis*

**Authors for correspondence: ag.bakre@mail.ui.edu.ng , Tel: +234-8081062365*

Manuscript received- March, 2023; Accepted- July, 2023

DOI: https://doi.org/10.54548/njps.v38i2.7

INTRODUCTION

Epilepsy is a neurological condition with a prevalence of more than 65 million people worldwide (Pearson-Smith and Patel, 2017). Hallmarks of epilepsy include recurrent seizures (Sankaraneni and Lachhwani, 2015), spontaneous, abnormal and extreme neuronal firing in the central nervous system (Muke *et al*., 2018). Refractoriness to available neuroleptics is one of the significant challenges affecting the effective management of epilepsy (de Souza *et al*., 2019). In addition, an associated cognitive impairment following prolonged administration of these neuroleptics has been reported in 50% of epileptic patients (Mehla *et al*., 2010). The current neuroleptics only focus on reducing the symptoms of epilepsy (Muke *et al*., 2018) without necessarily preventing the onset of epileptic seizures. It is important to develop novel therapeutic agents that will delay epileptogenesis and prolong the latency of epileptic attacks (Muke *et al*., 2018, Samokhina and Samokhin, 2018).

The mechanisms underpinning the pathophysiology of epilepsy are very complex and multifactorial. Increasing knowledge emanating from clinical and experimental studies has suggested and given credence to inflammatory processes as a crucial underlying factor in epilepsy pathology (Riazi *et al*., 2010, Choi *et al*., 2009, Vezzani *et al*., 2011, Vezzani and Baram, 2007). Previous studies have shown the antiepileptic property of steroids and anti-inflammatory agents in some drug-resistant epilepsies (Tavakoli *et al*., 2023, Wheless *et al*., 2007, Wirrell *et al*., 2005, Riikonen, 2004). Similarly, a high level of inflammatory mediators has been demonstrated in febrile convulsion (Dinarello, 2004). The inflammatory hypothesis is further reinforced by the high incidence of epilepsies in an autoimmune system in some patients suffering from

seizures (Dalmau *et al*., 2008, Vincent and Bien, 2008, Bien *et al*., 2007).

Evidence has emerged that inflammation might be a causative factor and the aftermath of epilepsy (Vezzani *et al*., 2011). Numerous inflammatory markers were detected in the brain tissue of patients suffering from refractory epilepsy (Vezzani *et al*., 2011). The occurrence of neuroinflammation in epilepsy that was not associated with immune dysfunction indicates the role of chronic inflammation in the manifestation of epilepsies (Vezzani *et al*., 2011). Evidence of mediators like pro-inflammatory cytokines and microglial as key players in epilepsy has been documented (Ahmadian *et al*., 2019, Salgado *et al*., 2018, Cerri *et al*., 2017, Gales and Prayson, 2017). In an animal model of seizure, activation of microglia has been reportedly significant in elevating tumour necrosis factor-alpha (TNF-α) levels, interleukin-1 beta (IL-1β) levels and other inflammatory biomarkers (Jeon *et al*., 2008, Chen *et al*., 2004, Thompson *et al*., 2004, Gupta *et al*., 2003). Past studies have documented elevated nitric oxide levels concerning pentylenetetrazole (PTZ) kindling in laboratory animals (Ahmadian *et al*., 2019, Haj-Mirzaian *et al*., 2019, Amiri *et al*., 2016, Watanabe *et al*., 2013).

The PTZ kindling model is a chronic epilepsy model, which involves continuous administration of low doses of a convulsant resulting in seizures (Dehkordi *et al*., 2023, Dhir, 2012, McNamara, 1984). It mimics clinical epilepsy and the various psychiatric and neurological changes and symptoms associated with this neurological disorder (Kaur *et al*., 2016). It helps scientists study the pathophysiology and molecular pathways related to the onset and duration of epileptic seizures, which are very important when screening new potential antiepileptic drugs (Shimada and Yamagata, 2018). The three phases of this model involve the pre-kindling phase, the kindling phase and the post-kindling phase (Samokhina and Samokhin, 2018). Continuous administration of PTZ (20 to 40 mg/kg, i.p.) can trigger seizures in laboratory animals such as rats and mice (Dhir, 2012). The PTZ acts by blocking and suppressing gamma amino butyric acid (GABA) mediated neurotransmission at GABAA receptors at alpha 1, 2, 3 and 5 (Samokhina and Samokhin, 2018). The experimental animal population can record various phases of convulsions like myoclonic seizures, straub's tail, clonus, tonic-clonic jerks, and death. Furthermore, the kindling model provides an avenue for researchers to study cognitive deficits associated with epilepsy (Pourmotabbed *et al*., 2011). This model mentioned above is a chronic epilepsy model which can result in neuronal loss and degeneration in the CA1, CA3 and dentate gyrus of the hippocampus with the amygdala and entorhinal cortex (Mehla *et al*., 2010), subsequently resulting in memory impairment.

Phytochemicals and some herbal products with anti-inflammatory properties have been able to ameliorate epileptic symptoms and seizures in recent times (Ahmadian *et al*., 2019, Shimada *et al*., 2014). Quercetin is a flavonoid that possesses neuroprotective properties (Nassiri-Asl *et al*., 2016, Chakraborty *et al*., 2014). The antioxidant and anti-inflammatory effects of quercetin may serve as prime targets in preventing neurodegeneration in epilepsy. Several studies have documented quercetin's anti-inflammatory and antioxidant properties (Li *et al*., 2016b, Li *et al*., 2016a, Zheng *et al*., 2016). Studies have shown an antiseizure effect of quercetin-containing extracts in animal models of epilepsy (Can and Özkay, 2012, Guo *et al*., 2011, Manigauha and Patel, 2010). Although, effect of quercetin against picrotoxin and PTZ-induced convulsion has been demonstrated in several studies, antiepileptic property of quercetin against PTZ-induced seizures and neurodegeneration in kindled mice has not been investigated. Therefore, we evaluated the neuroprotective potential of quercetin against seizures and neurodegeneration in mice using the pentylenetetrazole kindling model.

MATERIALS AND METHODS

Experimental animals: Forty male mice weighing between (22-25g) were purchased from the Central Animal House. The animals were kept in a standard environment with unlimited access to a standard rodent pellet diet and water ad libitum. They were kept in a room temperature-controlled environment with 12hr/12hr (light/dark) cycle, 40-70% relative humidity, with water and standard rodent chow ad libitum. Ethical approval was sought from the Animal Care and Use Research Ethics Committee for the experimental procedures. It was carried out in line with the NIH's care and animal use guidelines.

Drugs and Chemicals: Pentylenetetrazole (Sigma Aldrich), quercetin (Sigma Aldrich), silver nitrate, (Sigma Aldrich) potassium dichromate (Sigma Aldrich).

PTZ-induced kindling model: Kindling was induced using a well-established method (Taiwe *et al*., 2016, Kiasalari *et al*., 2013). In brief, a sub-convulsive dose of PTZ (35 mg/kg, i.p.) was administered every alternate day, and seizure behaviours were monitored for 30 minutes after each PTZ injection. Subsequently, the animals were monitored for 30 min after PTZ administration for seizure activity and scored using a scoring scale of 0 to 6: stage 0: no response; stage 1: hyperactivity, vibrissae twitching; stage 2: head nodding, head clonus and myoclonic jerk; stage 3: unilateral forelimb clonus; stage 4: rearing with bilateral forelimb clonus; stage 5: generalised tonic-clonic seizure with loss of writing reflex, stage 6: lethality (Malhotra and Gupta, 1997, Racine, 1972). The number of myoclonic jerks, the duration and the latencies to myoclonic jerks, generalised tonic-clonic seizures and lethality were recorded. The animals were considered to be kindled after exhibiting at least three consecutive maximum seizures stages.

Experimental procedure: The neuroprotective effect of quercetin on pentylenetetrazole-induced epileptogenesis was evaluated using a known method (Taiwe *et al*., 2016, Kiasalari *et al*., 2013). The animals were randomly divided into four (4) groups of 10 each. Animals in group 1, served as normal control, received vehicle (10 mL/kg, p.o.), group 2 were given vehicle which served as negative control. Mice in groups 3 and 4 were treated with the selected doses of quercetin (12.5 mg/kg and 25 mg/kg, p.o.). Sixty minutes after treatments, animals in groups 2 to 4 were given pentylenetetrazole (35 mg/kg, i.p.) on every alternate day (48± 2 h) for 21 days (total of 10 PTZ injection). The mice were monitored for 30 minutes after each PTZ administration. Several grades of seizures were scored using

a scoring scale according to Racine (1972) as modified by Malhotra and Gupta (1997). The total number of myoclonic seizures, the duration and the latencies of myoclonic seizures, tonic-clonic seizures and lethality were noted.

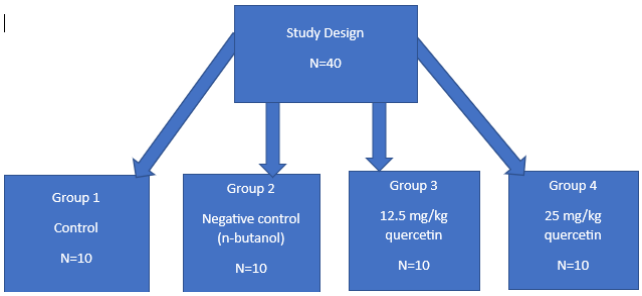

Figure A:
This figure depicts the study design which entails the grouping, total number of animals and agents administered.

Preparation of brain tissues for biochemical studies: After behavioural studies on day 22, mice in respective groups were euthanised with cervical dislocation, and the brain samples were harvested immediately, weighed and cleaned with cold 0.90% saline. Afterwards, the hippocampus was dissected out and homogenised with phosphate buffer (0.1M, PH 7.4) and centrifuged at 10,000 rpm at 4o C for 10 minutes. Thereafter, the supernatant was separated for the various biochemical tests.

Measurement of brain levels of pro-inflammatory cytokines with ELISA techniques: The ELISA technique is a widely used method for measuring the levels of specific proteins or molecules in a sample. In this case, the levels of interferon-gamma (IFN-ɣ), interleukin-12 and tumour necrosis factor-α (TNF- α) were measured in brain tissue samples according to the respective manufacturers' instructions. To quantify the concentration of the cytokines in the brain tissue samples, a standard curve was first generated using known concentrations of the cytokines and their respective optical densities (OD) values obtained at 450nm. The concentrations of cytokines in the brain tissue samples were then extrapolated from this standard curve based on their OD values. These concentrations were expressed as pg/mL.

Golgi Staining Procedure: On the last day of treatment, animals were perfused and their brains fixed with 10% formo-saline. The brain tissues were later stained using Golgi staining techniques.
Brain samples were fixed in 4% paraformaldehyde for 1 day (24 hours). The tissue blocks were thereafter immersed in 3% potassium dichromate for 7 days in darkness. Solution was changed daily. The tissues were later immersed into 2% silver nitrate solution for 72 hours at room temperature in darkness. Before putting tissue blocks to a solution containing silver nitrate. The excess solution was absorbed by using an absorbent paper. The silver nitrate solution was changed several times until brown precipitate stopped appearing. The various brain sections were cut into distilled water using a vibratome which is 60 µm thick. They were mounted on foist plus slides and dried under normal room temperature for 10 minutes. Dehydration through 95% alcohol then 100% alcohol was ensured. Finally, the section was cleared in xylene, covered with slide and snapped.

Statistical analysis: Values were expressed as mean ± SD. All data were analyzed using one-way analysis of variance (ANOVA). The Tukey's post hoc test was carried out in order to spot inter-group differences, the comparison groups being the normal and negative controls. The statistical differences were considered at $p < 0.05$.

RESULTS

Effect of Quercetin on PTZ-induced kindling behaviour in mice: Seizure grades were analysed to know the possible anti-epileptogenic effect of quercetin in the PTZ-induced kindling model. The PTZ-treated group exhibited a significant ($P< 0.05$) increase in seizure intensity from 2nd injection to 9th injection compared to the control. However, quercetin at both doses significantly ($P<0.05$) reduced seizure intensity (stage) compared to the saline+PTZ group in 5th, 6th, 7th, 8th and 9th injections.

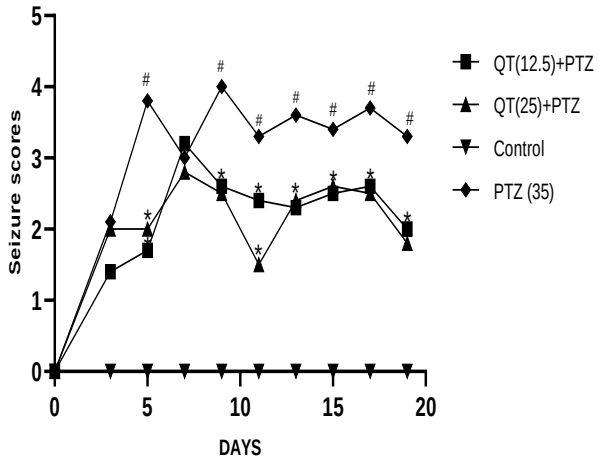

Figure 1:
Effect of quercetin pretreatment (QT 12.5 or 25 mg/kg p.o.) on the mean kindling score as assessed every other day from day1 to day15 of the study. #p < 0.05 in relation to vehicle + saline-treated group. *p < 0.05 in relation to vehicle + PTZ-treated group (group 2) (ANOVA followed by Tukey's test). Group 1: Control (vehicle); group 2: vehicle + PTZ (35 mg/kg); group 3: QT (12.5) + PTZ; group 4: QT (25) + PTZ

Effect of Quercetin on PTZ-induced central IL-12 production: The one-way ANOVA test showed significance elevation [$F (3, 20) = 20.20$, $P = 0.0001$] in brain level of IL-12 between the treated groups. Post hoc analysis further reveal that PTZ significantly ($P< 0.05$) elevated IL-12 level (Fig.c2) compared to the control (VEH). However, pretreatment with Q (12.5 mg/kg and 25 mg/kg, p.o.) significantly decreased hippocampal IL-12 levels in comparison with the PTZ-treated group (VEH + PTZ) (Fig 2).

Effect of quercetin on PTZ-induced central IFN-ɣ production: The one-way ANOVA test showed a significant difference [$F (3, 20) = 9.858$, $P=0.0003$] in the brain level of IFN-ɣ between the treated groups. Post hoc analysis further reveals that PTZ (30 mg/kg i.p.)

significantly (P< 0.05) increased IFN-ɣ level (Fig.3) compared to the control (VEH). However, pretreatment with Q (12.5 mg/kg and 25 mg/kg, p.o.) significantly decreased IFN-ɣ level in relation to the PTZ-treated group (VEH+PTZ) (Fig. 3).

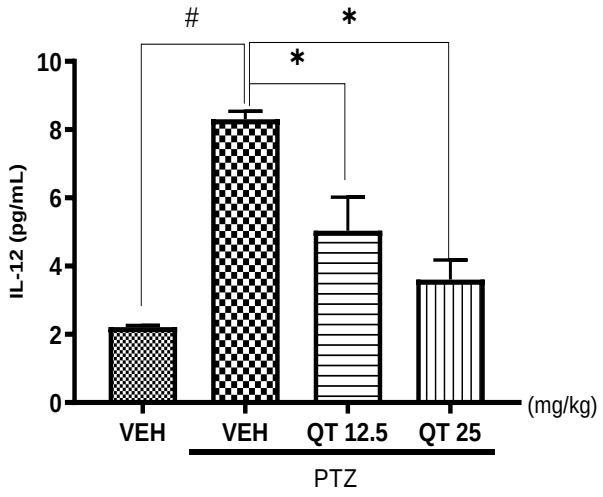

Figure 2:
Effect of quercetin on the brain level of IL-12 in rats subjected to the PTZ model of epilepsy. Data represent mean ± SD (n=6). #p < 0.05 when compared with control group (VEH); ⁎p < 0.05, when compared with PTZ control group (VEH + PTZ); one-way ANOVA with Tukey's multiple comparison

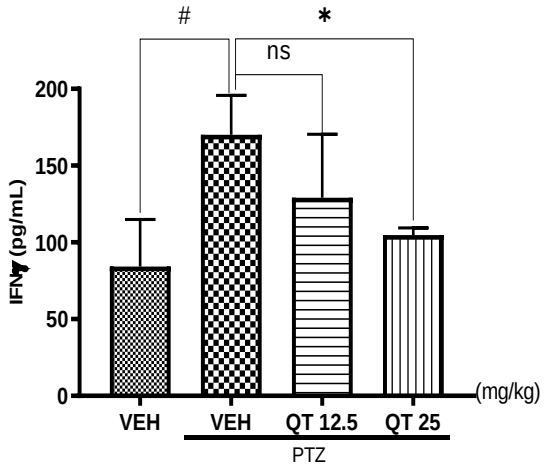

Figure 3:
Effect of quercetin on the brain level of IFN-γ in mice subjected to PTZ model of epilepsy. Data represent mean ± SD (n= 6). # p < 0.05 when compared with control group (VEH); ⁎p < 0.05, when compared with PTZ control group (VEH + PTZ); one-way ANOVA followed by Tukey's multiple comparison.

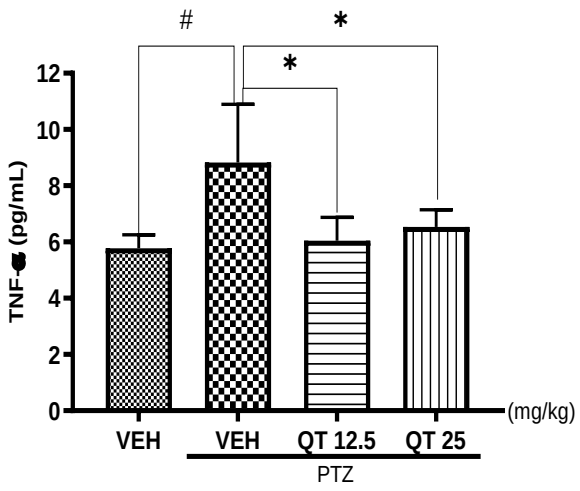

Figure 4:
Effect of quercetin on the brain level of TNF-α in mice subjected to the PTZ model of epilepsy. Data represent mean ± SD (n=5). #p < 0.05 in comparison with the control group (VEH); ⁎p < 0.05, when compared with PTZ control group (VEH + PTZ); one-way ANOVA followed by Tukey's multiple comparison.

Neuroprotective effect of the extract on PTZ-induced neuronal damage: Histology of the hippocampus CA1 revealed evident neurodegeneration of dendrite and neuronal cells in the groups exposed to PTZ. Well-defined pyramidal neurons given rise to a single apical dendrite with many terminal branches and well-arborized basal dendrites were observed in the CA1 of the control animals. The pyramidal cells and dendrites were significantly depleted in the group exposed to the PTZ group compared to the control. However, this depletion was significantly reduced by the treatment with quercetin at 12.5 mg/kg and 25 mg/kg (Plate 1) compared to the group treated with PTZ.

DISCUSSION

Epilepsy is a neurological condition affecting about 30 million people worldwide, out of which 30% of patients no longer respond to conventional medical treatment (Meng and Yao, 2020, Singh and Trevick, 2016, Laxer *et al*., 2014). Some of the features of this neurological disorder include recurrent seizures, an imbalance between inhibitory and excitatory neurotransmitters, and neuronal damage, amongst others (Rana and Musto, 2018). Recent and emerging evidence has linked the pathogenesis of epilepsy with neuroinflammation, which is connected with elevated pro-inflammatory cytokine levels, microglial activation and neurodegeneration (Rana and Musto, 2018).

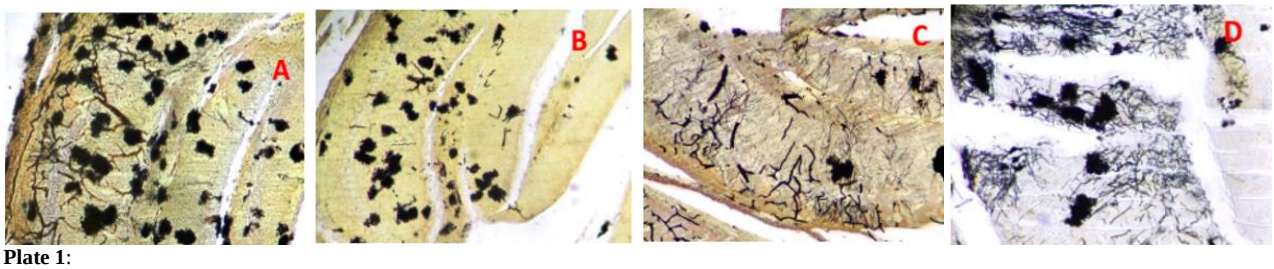

Plate 1:
Photomicrograph of a brain section stained by Golgi cox techniques showing CA1 of the PTZ-treated animals. The control group (A) shows the presence of moderate neuronal processes, while the PTZ group (B) shows depleted neuronal processes. Treatment with QT 12.5 mg/kg (C) and QT 25 mg/kg (D) prevents neuronal loss and shows the presence of mild neuronal processes. Magnification: (X100).

In this study, PTZ kindling epilepsy method was employed to evaluate the neuroprotective property of quercetin in a kindled mouse. Kindling is a chronic epilepsy model, which involves the monotonous administration of sub-convulsive doses of a convulsant resulting in seizures (Dhir, 2012, McNamara, 1984). This model has demonstrated face and construct validities in replicating and mimicking clinical epilepsy and the various psychiatric and neurological changes and symptoms associated with seizure disorders. It makes it possible to study the underlying molecular mechanisms involved in the pathogenesis of epilepsy (Shimada and Yamagata, 2018). Continuous and alternate administration of PTZ induces kindling in laboratory animals, such as rats and mice (Dhir, 2012), by blocking and suppressing GABA-mediated neurotransmission at GABAA receptors (Samokhina and Samokhin, 2018). This chronic model of epilepsy has resulted in neuronal loss and degeneration in the CA1, CA3 and dentate gyrus of the hippocampus and the amygdala (Mehla *et al*., 2010), resulting in memory impairment. Hence, it provides an avenue to study cognitive deficits associated with epilepsy (Pourmotabbed *et al*., 2011) and neuronal decline and morphological changes in the hippocampus and dentate gyrus (Samokhina and Samokhin, 2018).

The findings of this study show that repetitive administration of PTZ sub-convulsive dose consistently elevated the severity of seizures and myoclonic jerks in the PTZ-kindled mice. Conversely, treatment with quercetin significantly reduced the severity of PTZ-induced seizures and myoclonic jerks in mice. While the PTZ induced seizures have been attributed to inhibition of GABA and activation of NMDA (N-methyl-D-aspartate) receptors increasing calcium ion influx into the neurons (Akyuz *et al*., 2021) quercetin on the other hand has been reported to modulates excessive glutamate neurotransmission by acting as an NMDA antagonist, thus blocking the influx of calcium ions into the neurons and preventing abnormal neuronal excitability (Choudhary *et al*., 2011, Subash and Subramanian, 2009). In addition, flavonoids have been shown to modulate GABAA chloride ion channels because of their similar structures with benzodiazepines (Nieoczym *et al*., 2014, Choudhary *et al*., 2011). In managing temporal lobe epilepsy, GABAA receptors are crucial targets (Schipper *et al*., 2016). Therefore, the modulatory action of quercetin as a flavonoid at this receptive site may also account for its antiepileptic effect observed in this study.

There is emerging evidence of an inflammatory undertone in epilepsy pathophysiology. The high incidence of seizures in autoimmune disorders underscores the significance of inflammation in the pathophysiology of epilepsy (Dalmau *et al*., 2008, Vincent and Bien, 2008, Bien *et al*., 2007). In an animal model of seizure, chemoconvulsants like PTZ and kainic acid have been reported to increase and up-regulate pro-inflammatory cytokine via activation of microglia in the brain (Semple *et al*., 2020, Rawat *et al*., 2019, Jeon *et al*., 2008). Consistently, in this study, chronic administration of PTZ has markedly elevated the IL-12, IF-gamma and TNF-alpha levels in the brain of PTZ-treated mice. Epilepsy has been associated with increased interferon-gamma levels (Lach *et al*., 2022). Similarly, inflammatory biomarkers have reportedly been high in febrile convulsion (Dinarello, 2004). From our findings, treatment with quercetin, however, significantly reduced PTZ-induced pro-inflammatory cytokines (i.e. IL-12, TNF-α, IF-gamma). As an anti-inflammatory agent, quercetin has dramatically suppressed the levels of interleukin-12 and interferon-gamma in the brain (Akyuz *et al*., 2021). Previous studies have shown the antiepileptic property of steroids and anti-inflammatory agents in some drug-resistant epilepsies (Wheless *et al*., 2007, Wirrell *et al*., 2005, Riikonen, 2004). The TNF-alpha is a pro-inflammatory cytokine implicated in epileptogenesis, which up-regulates glutamate microglial release by increasing Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, thus amplifying glutaminergic excitatory neurotransmission (Rana and Musto, 2018, Galic *et al*., 2012, Takeuchi *et al*., 2006). In addition, TNF alpha downregulates GABA inhibitory neurotransmission, thus triggering abnormal neuronal excitability and epileptogenesis (Rana and Musto, 2018, Stellwagen *et al*., 2005). In addition, TNF-alpha levels were elevated in PTZ-treated animals. In the study, we observed that treatment with quercetin significantly reduced the level of TNF alpha compared to the group of animals that were exposed to PTZ alone.

Furthermore, PTZ triggered the neurodegeneration of dendrites and neuronal cells in the hippocampus CA1 region and dentate gyrus of mice that were exposed to PTZ alone. Quercetin reversed the depletion of dendrites and pyramidal cells in the hippocampus CA1 region and dentate gyrus of mice. This neuroprotective mechanism of quercetin may be attributed to its antioxidant property, ability to downregulate inflammatory mediators and reduce oxidative stress (Wu *et al*., 2020, Rishitha and Muthuraman, 2018), thus protecting the brain from neurodegeneration (Sefil *et al*., 2014).

In conclusion, quercetin was able to suppress PTZ-induced seizures and significantly declined interferon-gamma and interleukin 12 brain levels. In addition, quercetin was able to reverse PTZ-induced neurodegeneration due to its anti-inflammatory, antioxidant and neuroprotective properties of quercetin. Nevertheless, a thorough understanding of the molecular patterns surrounding quercetin's antiepileptic and neuroprotective effects is vital for developing new therapeutic remedies that can ameliorate epilepsy.

Acknowledgements

The authors appreciate the technical support from the technical unit of Department of Pharmacology and Therapeutics, Afe Babalola University Ado Ekiti

REFERENCES

Ahmadian, S. R., Ghasemi-Kasman, M., Pouramir, M. & Sadeghi, F. (2019): Arbutin attenuates cognitive impairment and inflammatory response in pentylenetetrazol-induced kindling model of epilepsy. *Neuropharmacology*, 146, 117-127.

Akyuz, E., Paudel, Y. N., Polat, A. K., Dundar, H. E. & Angelopoulou, E. (2021): Enlightening the neuroprotective effect of quercetin in epilepsy: From mechanism to therapeutic opportunities. *Epilepsy & Behavior*, 115, 107701.

Amiri, S., Haj-Mirzaian, A., Amini-Khoei, H., Shirzadian, A., Rahimi-Balaei, M., Razmi, A., Bergen, H., Rastegar, M., Kordjazy, N. & Haj-Mirzaian, A. (2016): Lithium attenuates the proconvulsant effect of adolescent social isolation stress via involvement of the nitrergic system. *Epilepsy & Behavior*, 61, 6-13.

Bien, C., Urbach, H., Schramm, J., Soeder, B., Becker, A., Voltz, R., Vincent, A. & Elger, C. (2007): Limbic encephalitis as a

precipitating event in adult-onset temporal lobe epilepsy. *Neurology*, 69, 1236-1244.

Can, Ö. D. & Özkay, Ü. D. (2012): Effects of Hypericum montbretti extract on the central nervous system and involvement of GABA (A)/benzodiazepine receptors in its pharmacological activity. *Phytotherapy Research*, 26, 1695-1700.

Cerri, C., Caleo, M. & Bozzi, Y. (2017): Chemokines as new inflammatory players in the pathogenesis of epilepsy. *Epilepsy Research*, 136, 77-83.

Chakraborty, J., Singh, R., Dutta, D., Naskar, A., Rajamma, U. & Mohanakumar, K. P. (2014): Quercetin improves behavioral deficiencies, restores astrocytes and microglia, and reduces serotonin metabolism in 3-nitropropionic acid-induced rat model of Huntington's disease. *CNS neuroscience & therapeutics*, 20, 10-19.

Chen, Z., Duan, R.-S., Concha, Q. H., Wu, Q., Mix, E., Winblad, B., Ljunggren, H.-G. & Zhu, J. 2004. IL-12p35 deficiency alleviates kainic acid-induced hippocampal neurodegeneration in C57BL/6 mice. *Neurobiology of disease*, 17, 171-178.

Choi, J., Nordli, D. R., Alden, T. D., Dipatri, A., Laux, L., Kelley, K., Rosenow, J., Schuele, S. U., Rajaram, V. & Koh, S. 2009. Cellular injury and neuroinflammation in children with chronic intractable epilepsy. *Journal of neuroinflammation*, 6, 1-14.

Choudhary, N., Bijjem, K. R. V. & Kalia, A. N. 2011. Antiepileptic potential of flavonoids fraction from the leaves of Anisomeles malabarica. *Journal of Ethnopharmacology*, 135, 238-242.

Dalmau, J., Gleichman, A. J., Hughes, E. G., Rossi, J. E., Peng, X., Lai, M., Dessain, S. K., Rosenfeld, M. R., Balice-Gordon, R. & Lynch, D. R. 2008. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *The Lancet Neurology*, 7, 1091-1098.

De Souza, A. G., Chaves Filho, A. J. M., Oliveira, J. V. S., De Souza, D. A. A., Lopes, I. S., De Carvalho, M. A. J., De Lima, K. A., Sousa, F. C. F., Vasconcelos, S. M. M. & Macedo, D. 2019. Prevention of pentylenetetrazole-induced kindling and behavioral comorbidities in mice by levetiracetam combined with the GLP-1 agonist liraglutide: involvement of brain antioxidant and BDNF upregulating properties. Biomedicine & Pharmacotherapy, 109, 429-439.

Dehkordi, H. T., Bijad, E., Saghaei, E., Korrani, M. S. & Amini-Khoei, H. 2023. Chronic stress but not acute stress decreases the seizure threshold in PTZ-induced seizure in mice: role of inflammatory response and oxidative stress. Naunyn-Schmiedeberg's Archives of Pharmacology, 396, 973-982.

Dhir, A. 2012. Pentylenetetrazol (PTZ) kindling model of epilepsy. *Current protocols in neuroscience*, 58, 9.37. 1-9.37. 12.

Dinarello, C. A. 2004. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *Journal of endotoxin research*, 10, 201-222.

Gales, J. M. & Prayson, R. A. 2017. Chronic inflammation in refractory hippocampal sclerosis-related temporal lobe epilepsy. *Annals of Diagnostic Pathology*, 30, 12-16.

Galic, M. A., Riazi, K. & Pittman, Q. J. 2012. Cytokines and brain excitability. *Frontiers in neuroendocrinology*, 33, 116-125.

Guo, J., Xue, C., Duan, J.-A., Qian, D., Tang, Y. & You, Y. 2011. Anticonvulsant, antidepressant-like activity of Abelmoschus manihot ethanol extract and its potential active components in vivo. *Phytomedicine*, 18, 1250-1254.

Gupta, Y., Kumar, M. V. & Srivastava, A. 2003. Effect of Centella asiatica on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Pharmacology Biochemistry and Behavior, 74, 579-585.

Haj-Mirzaian, A., Ramezanzadeh, K., Tafazolimoghadam, A., Kazemi, K., Nikbakhsh, R., Nikbakhsh, R., Amini-Khoei, H., Afshari, K., Haddadi, N.-S. & Shakiba, S. 2019. Protective effect of minocycline on LPS-induced mitochondrial dysfunction and decreased seizure threshold through nitric oxide pathway. *European journal of pharmacology*, 858, 172446.

Jeon, G. S., Park, S. K., Park, S. W., Kim, D. W., Chung, C. K. & Cho, S. S. 2008. Glial expression of interleukin-18 and its receptor after excitotoxic damage in the mouse hippocampus. *Neurochemical research*, 33, 179-184.

Kaur, D., Pahwa, P. & Goel, R. K. 2016. Protective effect of nerolidol against pentylenetetrazol-induced kindling, oxidative stress and associated behavioral comorbidities in mice. *Neurochemical research*, 41, 2859-2867.

Kiasalari, Z., Khalili, M., Roghani, M., Heidari, H. & Azizi, Y. 2013. Antiepileptic and antioxidant effect of hydroalcoholic extract of ferula assa foetida gum on pentylentetrazole-induced kindling in male mice. *Basic and clinical neuroscience*, 4, 299.

Lach, P., Klus, W., Zajdel, K., Szeleszczuk, A., Komorowska, E., Burda, K. & Kurowski, P. 2022. Neuroinflammation in Epilepsy—Diagnostics and Therapeutic Perspectives. Current Pharmacology Reports, 8, 31-35.

Laxer, K. D., Trinka, E., Hirsch, L. J., Cendes, F., Langfitt, J., Delanty, N., Resnick, T. & Benbadis, S. R. 2014. The consequences of refractory epilepsy and its treatment. *Epilepsy & behavior*, 37, 59-70.

Li, Yao, Han, Yang, J., Chaudhry, M. T., Wang, S., Liu, H. & Yin, Y. 2016a. Quercetin, inflammation and immunity. *Nutrients*, 8, 167.

Li, X., Wang, H., Gao, Y., Li, L., Tang, C., Wen, G., Zhou, Y., Zhou, M., Mao, L. & Fan, Y. 2016b. Protective effects of quercetin on mitochondrial biogenesis in experimental traumatic brain injury via the Nrf2 signaling pathway. *PloS one*, 11, e0164237.

Malhotra, J. & Gupta, Y. K. 1997. Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats. *British journal of pharmacology*, 120, 282-288.

Manigauha, A. & Patel, S. 2010. Pentylenetetrazole induced convulsion in RATS. *International journal of pharma and bio sciences*, 1, 2.

Mcnamara, J. O. 1984. Kindling: an animal model of complex partial epilepsy. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 16, S72-S76.

Mehla, J., Reeta, K., Gupta, P. & Gupta, Y. K. 2010. Protective effect of curcumin against seizures and cognitive impairment in a pentylenetetrazole-kindled epileptic rat model. *Life sciences*, 87, 596-603.

Meng, F. & Yao, L. 2020. The role of inflammation in epileptogenesis. *Acta Epileptologica*, 2, 1-19.

Muke, S., Kaikini, A., Peshattiwar, V., Bagle, S., Dighe, V. & Sathaye, S. 2018. Neuroprotective effect of coumarin nasal formulation: kindling model assessment of epilepsy. *Frontiers in pharmacology*, 9, 992.

Nassiri-Asl, M., Hajiali, F., Taghiloo, M., Abbasi, E., Mohseni, F. & Yousefi, F. 2016. Comparison between the effects of quercetin on seizure threshold in acute and chronic seizure models. *Toxicology and industrial health*, 32, 936-944.

Nieoczym, D., Socała, K., Raszewski, G. & Wlaź, P. 2014. Effect of quercetin and rutin in some acute seizure models in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 54, 50-58.

Pearson-Smith, J. N. & Patel, M. 2017. Metabolic dysfunction and oxidative stress in epilepsy. *International Journal of Molecular Sciences*, 18, 2365.

Pourmotabbed, A., Nedaei, S., Cheraghi, M., Moradian, S., Touhidi, A., Aeinfar, M., Seyfi, Z. & Pourmotabbed, T. 2011. Effect of prenatal pentylenetetrazol-induced kindling on learning and memory of male offspring. *Neuroscience*, 172, 205-211.

Racine, R. J. 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalography and clinical neurophysiology*, 32, 281-294.

Rana, A. & Musto, A. E. 2018. The role of inflammation in the development of epilepsy. *Journal of neuroinflammation*, 15, 1-12.

Rawat, C., Kukal, S., Dahiya, U. R. & Kukreti, R. 2019. Cyclooxygenase-2 (COX-2) inhibitors: future therapeutic strategies for epilepsy management. *Journal of neuroinflammation*, 16, 1-15.

RIAZI, K., GALIC, M. A. & PITTMAN, Q. J. 2010. Contributions of peripheral inflammation to seizure susceptibility: cytokines and brain excitability. *Epilepsy research*, 89, 34-42.

Riikonen, R. 2004. Topical review: infantile spasms: therapy and outcome. *Journal of child neurology*, 19, 401-404.

Rishitha, N. & Muthuraman, A. 2018. Therapeutic evaluation of solid lipid nanoparticle of quercetin in pentylenetetrazole induced cognitive impairment of zebrafish. *Life sciences*, 199, 80-87.

Salgado, P. R. R., Da Fonsêca, D. V., De Melo, C. G. F., Leite, F. C., Alves, A. F., Ferreira, P. B., Piuvezam, M. R., De Sousa, D. P. & De Almeida, R. N. 2018. Comparison of behavioral, neuroprotective, and proinflammatory cytokine modulating effects exercised by (+)-cis-EC and (−)-cis-EC stereoisomers in a PTZ-induced kindling test in mice. *Fundamental & Clinical Pharmacology*, 32, 507-515.

Samokhina, E. & Samokhin, A. 2018. Neuropathological profile of the pentylenetetrazol (PTZ) kindling model. *International Journal of Neuroscience*, 128, 1086-1096.

Sankaraneni, R. & Lachhwani, D. 2015. Antiepileptic drugs—a review. *Pediatric annals*, 44, e36-e42.

Schipper, S., Aalbers, M., Rijkers, K., Swijsen, A., Rigo, J., Hoogland, G. & Vles, J. 2016. Tonic GABAA receptors as potential target for the treatment of temporal lobe epilepsy. *Molecular neurobiology*, 53, 5252-5265.

Sefil, F., Kahraman, I., Dokuyucu, R., Gokce, H., Ozturk, A., Tutuk, O., Aydin, M., Ozkan, U. & Pinar, N. 2014. Ameliorating effect of quercetin on acute pentylenetetrazole induced seizures in rats. *International Journal of Clinical and Experimental Medicine*, 7, 2471.

Semple, B. D., Dill, L. K. & O'brien, T. J. 2020. Immune challenges and seizures: how do early life insults influence epileptogenesis? *Frontiers in Pharmacology*, 11, 2.

Shimada, T., Takemiya, T., Sugiura, H. & Yamagata, K. 2014. Role of inflammatory mediators in the pathogenesis of epilepsy. *Mediators of inflammation*, 2014.

Shimada, T. & Yamagata, K. 2018. Pentylenetetrazole-induced kindling mouse model. JoVE (Journal of Visualized Experiments), e56573.

Singh, A. & Trevick, S. 2016. The epidemiology of global epilepsy. *Neurologic clinics*, 34, 837-847.

Stellwagen, D., Beattie, E. C., Seo, J. Y. & Malenka, R. C. 2005. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-α. *Journal of Neuroscience*, 25, 3219-3228.

Subash, S. & Subramanian, P. 2009. Morin a flavonoid exerts antioxidant potential in chronic hyperammonemic rats: a biochemical and histopathological study. *Molecular and cellular biochemistry*, 327, 153-161.

Taiwe, G. S., Tchoya, T. B., Menanga, J. R., Dabole, B. & De Waard, M. 2016. Anticonvulsant activity of an active fraction extracted from Crinum jagus L.(Amaryllidaceae), and its possible effects on fully kindled seizures, depression-like behaviour and oxidative stress in experimental rodent models. *Journal of ethnopharmacology*, 194, 421-433.

Takeuchi, H., Jin, S., Wang, J., Zhang, G., Kawanokuchi, J., Kuno, R., Sonobe, Y., Mizuno, T. & Suzumura, A. 2006. Tumor necrosis factor-α induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *Journal of Biological Chemistry*, 281, 21362-21368.

Tavakoli, Z., Dehkordi, H. T., Lorigooini, Z., Rahimi-Madiseh, M., Korani, M. S. & Amini-Khoei, H. 2023. Anticonvulsant effect of quercetin in pentylenetetrazole (PTZ)-induced seizures in male mice: The role of anti-neuroinflammatory and anti-oxidative stress. *International Immunopharmacology*, 116, 109772.

Thompson, C., Gary, D., Mattson, M., Mackenzie, A. & Robertson, G. S. 2004. Kainic acid-induced naip expression in the hippocampus is blocked in mice lacking TNF receptors. *Molecular brain research*, 123, 126-131.

Vezzani, A. & Baram, T. Z. 2007. New roles for interleukin-1 Beta in the mechanisms of epilepsy. *Epilepsy currents*, 7, 45-50.

Vezzani, A., French, J., Bartfai, T. & Baram, T. Z. 2011. The role of inflammation in epilepsy. *Nature reviews neurology*, 7, 31-40.

Vincent, A. & Bien, C. G. 2008. Anti-NMDA-receptor encephalitis: a cause of psychiatric, seizure, and movement disorders in young adults. *The Lancet Neurology*, 7, 1074-1075.

Watanabe, M., Miyai, A., Danjo, S., Nakamura, Y. & Itoh, K. 2013. The threshold of pentylenetetrazole-induced convulsive seizures, but not that of nonconvulsive seizures, is controlled by the nitric oxide levels in murine brains. *Experimental neurology*, 247, 645-652.

Wheless, J. W., Clarke, D. F., Arzimanoglou, A. & Carpenter, D. 2007. Treatment of pediatric epilepsy: European expert opinion, 2007. *Epileptic disorders*, 9, 353-412.

Wirrell, E., Farrell, K. & Whiting, S. 2005. The epileptic encephalopathies of infancy and childhood. *Canadian journal of neurological sciences*, 32, 409-418.

Wu, D., Zheng, Z., Fan, S., Wen, X., Han, X., Wang, S., Wang, Y., Zhang, Z., Shan, Q. & Li, M. 2020. Ameliorating effect of quercetin on epilepsy by inhibition of inflammation in glial cells. *Experimental and Therapeutic Medicine*, 20, 854-859.

Zheng, J., Wu, J., Chen, J., Liu, J., Lu, Y., Huang, C., Hu, G., Wang, X. & Zeng, Y. 2016. Therapeutic effects of quercetin on early inflammation in hypertriglyceridemia-related acute pancreatitis and its mechanism. P*ancreatology*, 16, 200-210.