Original Article

Targeting Oxidative Stress and Neuro-Inflammation by *Gastrodia elata* Improves Disease Outcomes in a Rat Model of Epilepsy

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Abstract

Background: Oxidative stress and inflammation play an important role in the development of seizures. *Gastrodia elata* (GE) is a Chinese plant used for treating epileptic convulsions. **Objectives:** To determine the effects of GE on oxidative stress, inflammation, and behavioral changes after pentylenetetrazole (PTZ)-induced seizure in rats. **Materials and Methods:** Twenty-five adult Wistar albino male rats, six weeks old, weighing between 150 and 250 g, were housed at the animal house of the College of Medicine, University of Babylon. Rats were divided into: Group 1: Control group, Group 2: PTZ (40 mg/kg), Group 3: PTZ (40 mg/kg) and GE (883.56 mg/kg), Group 4: PTZ (40 mg/kg) and zonisamide (100 mg/kg), Group 5: Zonisamide (100 mg/kg). The rats were sacrificed by decapitation. Brain tissue homogenate was prepared for the estimation of biochemical parameters: glutathione (GSH), malondialdehyde (MDA), interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), total antioxidant capacity (T-AOC), and behavioral tests (latency, severity, and duration of fits). **Results:** In Group 2, GSH and T-AOC levels were significantly decreased, while MDA levels were significantly increased, compared to Group 1. TNF-α, IL-1, and IL-6 were significantly increased in Group 2 in comparison with Group 1. T-AOC and GSH were significantly increased in Groups 3, 4, and 5 compared to Group 2 with a significant reduction in MDA levels. IL-6, IL-1, and TNF-α were significantly decreased in Groups 3, 4, and 5. Duration and severity of fits were significantly decreased, and latency of fits was significantly increased in Groups 3, 4, and 5. **Conclusions:** GE had antioxidant and anti-inflammatory effects, and it ameliorated the severity, decreased the duration and increased the latency of fits.

Keywords: Epilepsy, *Gastrodia elata*, lacosamide, oxidative stress, zonisamide

Introduction

Epilepsy is one of the neurological disorders that causes unprovoked, recurrent seizures that affect people of all ages.[1] Epilepsy is diagnosed when the patient has two or more seizures with no other identifiable causes. Although the exact causes of seizures are not known, hyperexcitability, oxidative stress, cortical stimulation, and genetic factors are among the contributing pathogenic factors.[2] In 2020, around 50 million people worldwide have epilepsy,[3] so it is one of the most common neurological diseases, globally an estimated 5 million people are diagnosed each year with epilepsy. Nearly, 80% of epileptic people live in low- and middle-income countries because three-quarters of these people do not receive the treatment they need.[4] Epilepsy is controllable with medication in about 70% of cases, and epilepsy is more common in males than females. The total annual cost of epilepsy in 2019–2020 was $ 12.3 billion.[5] Seizures can affect any process of brain coordination because they are caused by abnormal

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activity in the brain. Temporary confusion, stiff muscles, a staring spell, loss of consciousness, and uncontrollable jerking of arms and legs are all symptoms of epilepsy, and symptoms vary according to the type of seizures.[6]

It has recently been shown that inflammation plays a significant role in the epilepsy pathogenesis that results from status epilepticus (SE), stroke, and other lesions to the brain. The inflammatory phase contributed significantly to epilepsy associated with temporal lobe epilepsy (TLE) and cortical malformation,[7] with increased neuronal deficiency, gliosis, and microgliosis, contributing intensely to epileptogenesis.[8] The homeostatic imbalance between antioxidants and oxidants may generate seizures through free radicals' effects on mitochondrial DNA damage and lipid peroxidation. These findings might have contributed to decreasing the seizure threshold.[9]

Antioxidant plays a major role in maintaining the balance between antioxidant and oxidant compounds, which can be defined as molecules that are capable of preventing or decreasing the macromolecules' oxidation.[10] Enzymes and non-enzymes such as superoxide dismutase and Glutathione (GSH), respectively, were demonstrated to have an important function in free radicals scavenging in the body. Based on the mechanism of action, antiepileptic drugs (AEDs) are divided into three broad classes: those that modulate voltage-gated ion channels, those that improve GABA-mediated inhibitory neurotransmission, and those that attenuate glutamate-mediated excitatory neurotransmission.[11] Zonisamide new AEDs are used for epilepsy. In addition to its mechanism, zonisamide was found to have anti-inflammatory and antioxidant effects. *Gastrodia elata* (GE), a famous Chinese herbal plant, has been conventionally utilized to treat many different situations involving epilepsy, headache, stroke, and others (Chines Pharmacopeia Commission, 2015). GE could reverse the increase of inflammatory cytokine levels in brain tissues, so it may have potential value in the alleviation of inflammation accompanied by seizures. GE might be able to protect against oxidative stress by inhibiting glutamate, impact of GE as an antiepileptic which was by downregulation of the overexpression of para-mammalian target of rapamycin (P-mTOR) pathway. Therefore, GE has biological activities like anti-inflammatory, antioxidant, and anticonvulsant.

Materials and Methods

Animals

Forty adult male albino rats aged between 10 and 12 weeks, weighing 250–300 g, were enrolled. These rats were habituated under animal house conditions at 25°C, a room humidity of 60–65%, and the environment was maintained in 14 h light-dark cycles provided with a standard commercial diet and water.

Chemicals and reagents

Pentylenetetrazol was purchased from Santa Cruz (United States). Zonisamide was procured from Sun Pharmaceutical (India). Formaldehyde was procured from AL-Jubail (KSA). Phosphate buffer saline was procured from HiMedia (India). ELISA kits for rat interleukin-1 (IL-1), rat IL-6, and rat tumor necrosis factor-α (TNF-α) are used in the sandwich-ELISA principle. Total antioxidant capacity (T-AOC) is measured by enzyme method. GSH ELISA kits were procured from Elabscience Biotechnology (United States) used the competitive-ELASA principle. Lipid peroxidation kits were purchased from Bilişim Destek Hizmetleri (Turkey) and measured by thiobarbituric acid reactive substances.

Preparation of plant

The GE rhizome (Tian Ma) powder was dissolved in distilled water for intragastric (oral) administration.

Study design and experimental protocols

Animals: The selected rats were divided randomly into five groups with five rats in each Group as follows:

Group 1: Control group: In this group, the rats were exposed to the same condition and each rat received i.p, i.g normal saline only. Group 2: Rats received pentylenetetrazole (PTZ) 40 mg/kg each 48 h for 15 days. Group 3: Rats received GE 883.56 mg/kg and 40 mg/kg PTZ, Group 4: Rats received zonisamide 100 mg/kg and PTZ 40 mg/kg, Group 5: Rats received zonisamide 100 mg/kg.

Behavioral evaluation by Racine's scale

To analyze the behavioral features, a modified version of Racine's scale has been utilized; seizure activity has been observed for 30 min; post-PTZ has been administered. Behavioral features, including convulsion, duration, and latency, severity or intensity phases, were recorded for 30 min post-each dosage of PTZ, and the following measures from Racine's scale were observed and documented in Table 1.

Table 1: Modified Racine's scale[12]

Stages	Seizure intensity
0	No response
1	Hyperactivity, restlessness, and vibrissae twitching
2	Head nodding, head clonus, and myoclonic jerks
3	Unilateral or bilateral limb clonus
4	Forelimb clonic seizures
5	Generalized clonic seizures with falling
6	Hind limb extensor
7	Death

Preparation of sample and decapitation of rat brain

Isolation of the brain

On day 15 after PTZ-kindling, the animals were sacrificed by decapitation 24 h after the last treatment. The brains were removed after dissection of the skull from the foramen magnum posteriorly. The olfactory bulbs and cerebellums were cut, and the brain was removed gently from the skull. The mid and forebrain were dissected out, washed with phosphate-buffered saline solution, weighed, kept in sterilized Eppendorf tubes, and frozen on dry ice, then kept in a deep freeze at −20°C.

Statistical analysis

All experiments were performed using three technical replicates (i.e., $n = 3$ per group). Results were reported as mean ± standard deviation (SD). Statistical comparisons were undertaken using either one-way or two-way analysis of variance test (ANOVA) (with Dunnett's correction for multiple comparisons) using statistical package for the social sciences (SPSS) version 23.0 (IBM Company, Chicago, IL, USA). Differences were considered significant at $P \leq 0.05$.

Ethical approval

The study protocol was reviewed and approved by a local ethics committee according to document number 403, on July 6, 2022.

Results

Oxidative stress biomarker

GSH: The effects of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on GSH level (μg/mL) in the rat's brain after every other day for PTZ group and 15 days' exposure for the other groups, as explained in Figure 1.

Malondialdehyde (MDA) biomarker: The effects of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on MDA level (μmol/L) in the rat's brain after every other day for PTZ group and 15 days' exposure for the other groups, as explained in Figure 2.

AOC biomarker: Effects of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on T-AOC level (μmol/g) in the rat's brain after every other day for PTZ group and 15 days' exposure for the other groups, as explained in Figure 3.

Inflammation biomarkers

TNF-α biomarker: Effects of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on TNF-α level (μg/L) in rats' brains after every other day for PTZ group and 15 days' exposure for the other groups, as shown in Figure 4.

IL-6 biomarker: Effects of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on IL-6 level (μg/L) in rats' brains

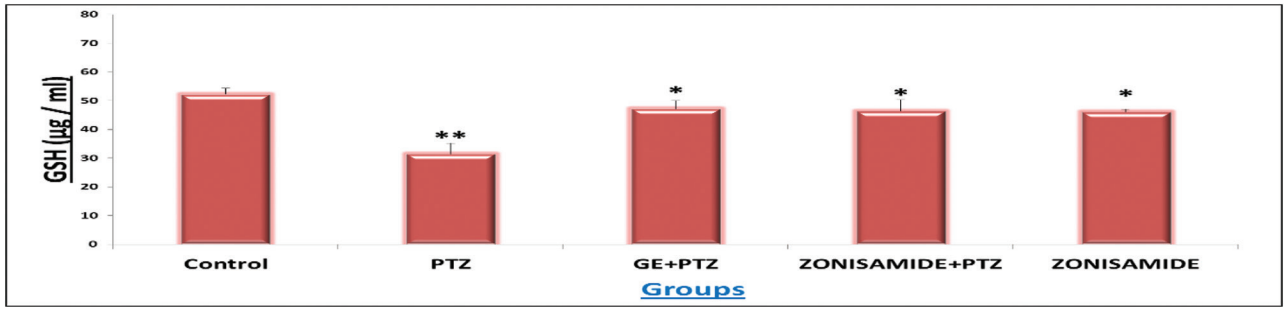

Figure 1: Impact of PTZ (40 mg/kg), *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide on GSH level (μg/mL) in rat's brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

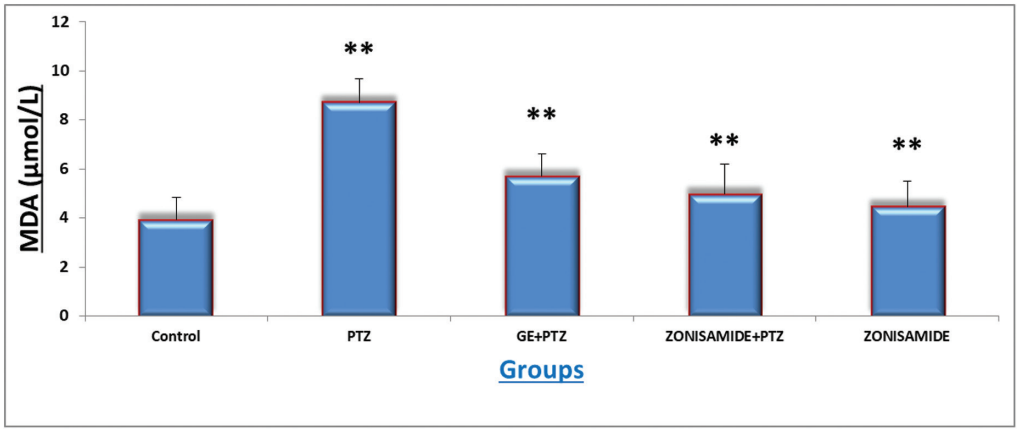

Figure 2: Impact of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o., administration of zonisamide on MDA level (μmol/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

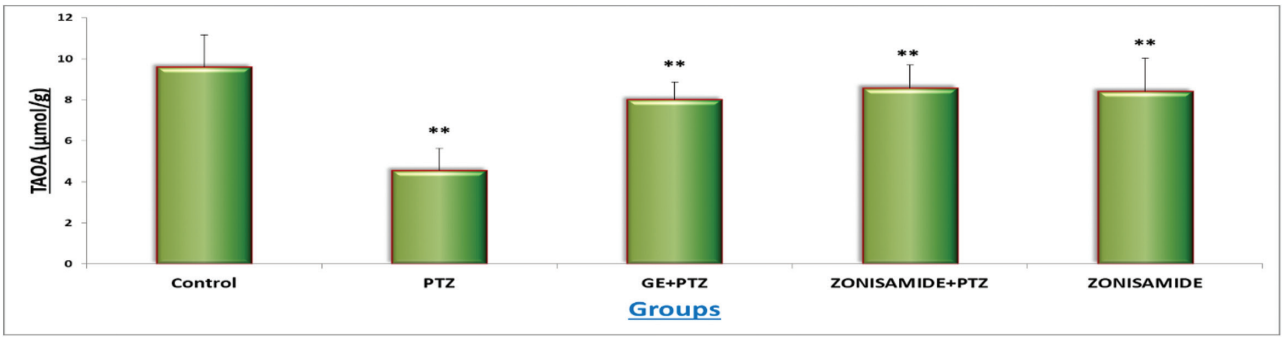

Figure 3: Impact of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide, on TAO-C level (μmol/g) in rat's brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

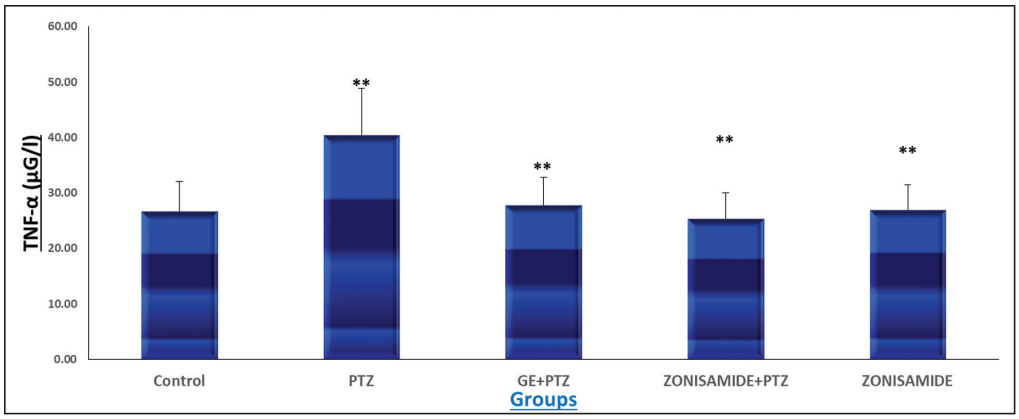

Figure 4: Impact of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide, on TNF-α level (μg/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

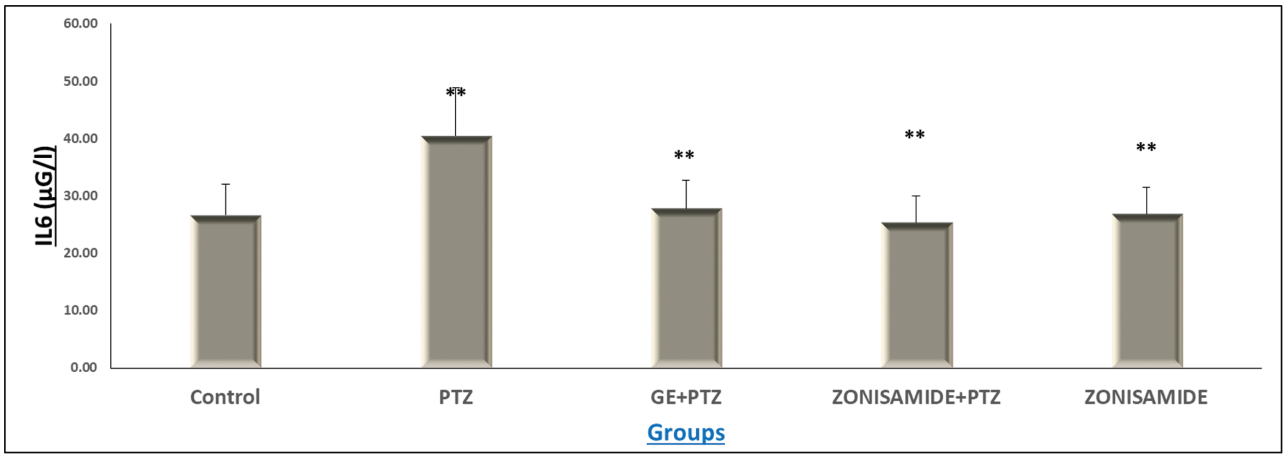

Figure 5: Impact of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o, oral administration of zonisamide on IL-6 level (μg/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD). (*$P < 0.05$), (**$P < 0.001$).

after every other day for PTZ group and 15 days' exposure for the other groups, as shown in Figure 5.

IL-1 biomarker: Effects of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on IL-1 level (µg/L) in rats' brains after every other day for PTZ group and 15 days' exposure for the other groups, as shown in Figure 6.

Behavioral test results

Latency: Impact of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on latency (min) in rats' brains after every other day for the PTZ group and 15 days' exposure for the other groups, as shown in Figure 7.

Duration: Impact of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on duration (min) in rat's brain after every

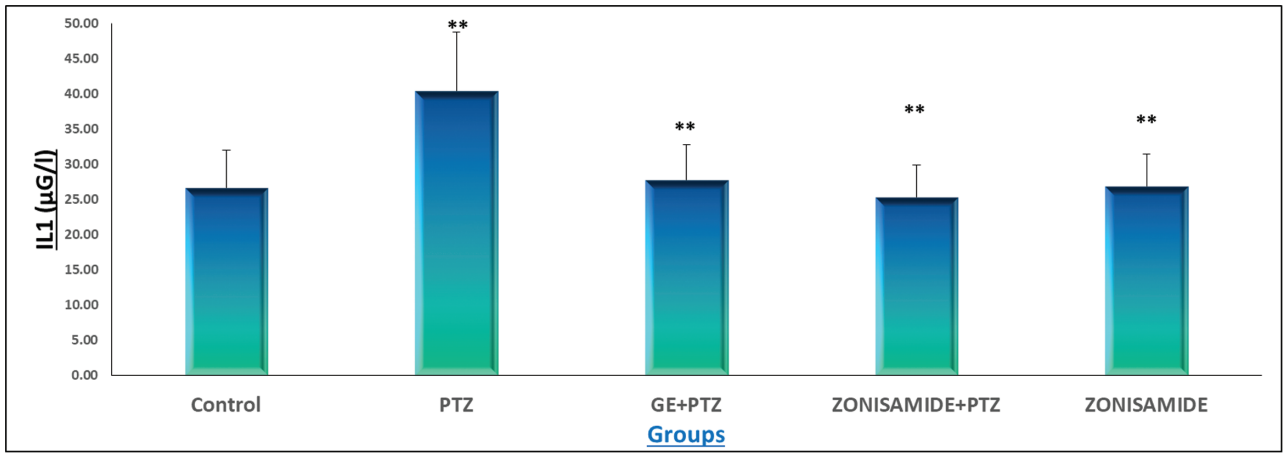

Figure 6: Impacts of PTZ (40 mg/kg), i.p, every other day, *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide, on IL-1 level (µg/L) in rats' brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

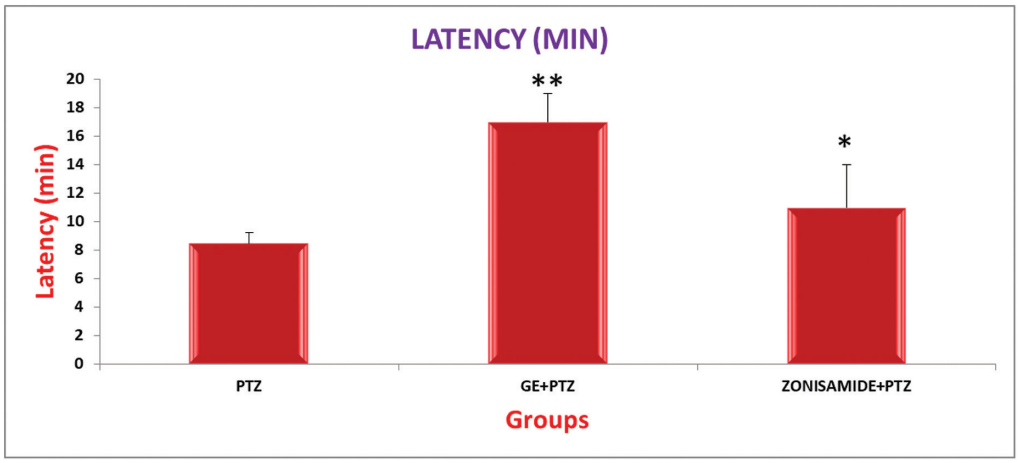

Figure 7: Impacts of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide, on latency (min) in rats' brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

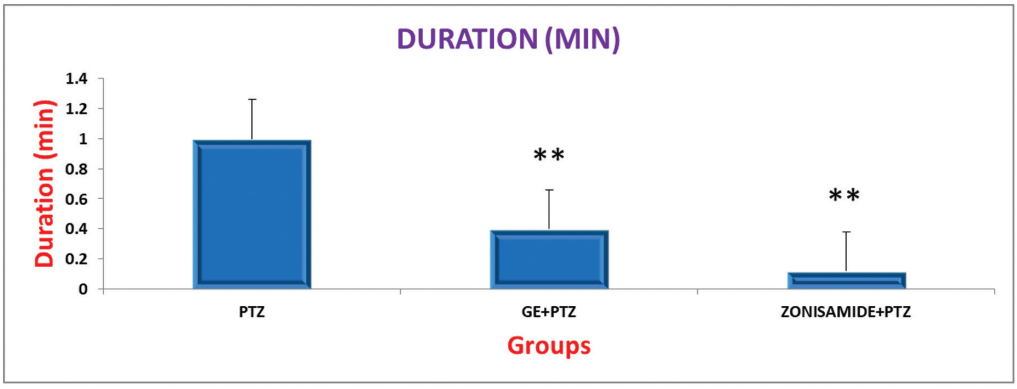

Figure 8: Impacts of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide, on the duration of fits. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

other day for the PTZ group and 15 days' exposure for other groups, as shown in Figure 8.

Severity: Impact of PTZ, GE + PTZ, zonisamide + PTZ, and zonisamide on severity (fits/min) in rats' brains after every other day for the PTZ group and 15 days' exposure for the other groups, as shown in Figure 9.

Discussion

The findings of the current study demonstrated that the level of GSH decreased with PTZ-kindling (Group 2) compared with the control group (Group 1). PTZ-kindling was found to cause neuronal cell death due to the increased activity of glutamatergic transmitters that

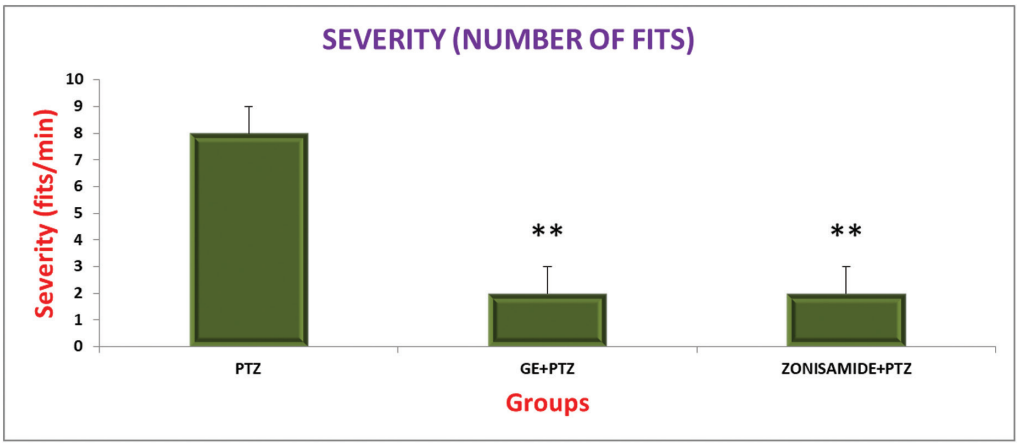

Figure 9: Impacts of PTZ (40 mg/kg), i.p, *Gastrodia elata*, (883.56 mg/kg), p.o., oral administration of zonisamide on severity (fits/min) in rats' brains. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

generate free radicals, so this mechanism might have played a crucial role. PTZ-kindling increased glutamate release and NMDA receptor activation, leading to increased oxidative stress. GE was found to upregulate the innate antioxidant GSH level and inhibit the decrease in GSH level.[13] GE in the present study acts through maintaining endogenous antioxidant GSH levels, cell survival reduced lactate dehydrogenase (LDH) leakage, and increased endogenous antioxidant GSH level in the injury, model.[14]

Zonisamide (ZNS) drug was found to have an antioxidant effect and might be able to increase the level of GSH in astroglial, C6 cells (glial cell line in the rat brain) but not in dopaminergic neurons, ZNS increased GSH by enhancing the astroglial cysteine transport system and/or proliferation of astroglial via S100β (multifunctional protein) secretion or production. ZNS was found to act as a neuroprotectant against oxidative stress.[15] ZNS + PTZ (Group 4) was found to increase GSH level compared with Group 2.

Regarding the effects on MDA biomarkers, PTZ-kindling model in rats was found to increase the MDA level and raise lipid peroxidation, combined with a decrease in antioxidant activity, compared to the control group. These results were also detected in many previous investigations, implying the oxidative stress function in the pathophysiology of epilepsy.[16]

Due to high-lipid content and the brain's limited antioxidant capacity, it is found to be more susceptible to oxidative damage. Excessive production of free radicals can cause cell component damage like lipid peroxidation caused by OH-free radical.[17] In the current investigation, PTZ-kindling model (Group 2) was found to increase the MDA level in comparison with the control group (Group 1). PTZ increased the production of reactive oxygen species (ROS) by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which causes an increase in MDA level[18] which might be the major cause of oxidative stress and subsequently neuronal damage. In the current study, GE was found to suppress the PTZ-induced seizure progression via modulating oxidative stress in Group 3, GE significantly decreased the level of MDA compared to PTZ-kindling (Group 2). GE might be able to protect against oxidative stress by inhibiting glutamate and also preventing glutamate-induced (Ca^{2+}) influx, thereby blocking the activation of the calmodulin-dependent Kinase II.[19] On this basis, these results suggest that GE might act as free radical scavengers and to some extent has neuroprotective effects. Zonisamide + PTZ (Group 4) presented the effect of zonisamide in reducing oxidative stress via a significant decrease in the level of MDA compared with (Group 2) PTZ-kindling. These findings were compatible with several studies that have shown the mechanism of the antiepileptic effect of ZNS which may involve the protection of neurons from damage by free radicals via scavenging hydroxyl toxic ROS generated from the decomposition of H_2O_2 and nitric oxide radicals.[20]

Regarding the effect on T-AOC biomarker, PTZ-kindling, Group 2, was found to increase the oxidative stress in the rat's brain through the decrease in antioxidant GSH, and an increase in MDA level, which means PTZ decreased T-AOC because T-AOC is a measure of the whole amount of oxidative stress state. T-AOC increased with pretreatment and treatment in Groups 3 and 4, respectively, and also increased with AED and ZNS in Group 5 as the same explanation discussed before for GSH and MDA levels.

Rats with artificially induced seizures have been shown to have a strong inflammatory response in the regions of the brain involved in the propagation and initiation of epileptic activity. PTZ (Group 2) in the present study demonstrated a significant increase in pro-inflammatory cytokines IL-1β, IL-6, and TNF-α compared to the rats in (Group 1). This result was consistent with previous studies that showed the IL-1β, IL-6, and TNF-α were expressed

at very low levels in the brain of normal animals, but their pro-inflammatory cytokines were rapidly upregulated after initiation of seizures during (≤30 min) decreasing to basal levels within (48–72 h) from the seizure onset.[21]

PTZ-kindling led to a rise in IL-1, IL-6, and TNF-α through increased cytokine production in the brain observed in microglia and astrocytes.[22] Rats kindling might possibly exacerbate the effects of the amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-receptor that facilitated the excitotoxicity; based on the amount of time, the tissue was exposed to the kindling and the extracellular amount of these cytokines throughout the injury.[23] GE in Group 3 had produced a reduction in the IL-1β, IL-6, and TNF-α levels to various degrees compared with the PTZ-kindling (Group 2). These results have agreed with previous studies that highlighted the administration of GE could reverse the increase of inflammatory cytokine levels in brain tissues of rats with PTZ-induced seizures, so GE may have potential value in the alleviation of inflammation accompanied by seizure.[24] Zonisamide + PTZ (Group 4) ZNS was found to have an anti-inflammatory effect in the present study as it decreased the cytokine levels significantly compared to the Group 2 of PTZ-kindling rats. These results were consistent with other previous studies that presented the effect of ZNS in attenuating the inflammatory cascade associated with the progression of seizures. ZNS can be further considered as a neuroprotective agent in epileptic seizures.[25]

Regarding the behavioral evaluation, in comparison with the control, the PTZ-kindling (Group 2) presented with a much lower amount of time to onset of seizures, indicating it has a shorter latency period. This conclusion was consistent with the findings of prior investigations that had demonstrated an instantaneous beginning of seizures after the effective injection of PTZ.[26]

The duration and severity of seizures with PTZ-kindling (Group 2) were increased significantly compared to the control (Group 1). The PTZ-induced seizure was caused by antagonizing the gamma-aminobutyric (GABA)–A receptor.[27] PTZ suppressed the inhibitory synapses' functions and subsequently caused an increase in neuronal activity. This antagonism leads to generalized seizures in animals. Upon treatment with GE (Group 3), it has been found that the rats exhibited a significant prolongation in the latency period of seizure initiation compared to PTZ (Group 2) and also improvement in mean seizure severity and shorter duration of seizures.

Current results were compatible with studies that revealed GE treatment prevented a large proportion of rats from developing seizures, delayed the onset time, reversed the decrease in GABA level in the synaptic cleft by inhibiting its degradation, and decreased the glutamate (Glu) activity.[28]

Zonisamide + PTZ (Group 4) in the present study found that ZNS caused prolongation in the latency period and a decrease in duration and severity compared to (Group 2) PTZ-kindling rats. This result aligned with several previous studies that presented the role of ZNS drug in the treatment of epilepsy as a first-line therapy for refractory epilepsy by helping serotonergic and dopaminergic neurotransmission through blocking voltage-dependent Na^+ channels, lowering voltage-dependent T-type inward Ca^{+2} currents, and reducing Ca^+ dependent K^+ triggered the release of glutamate intracellularly.[29]

Conclusions

The present study revealed that PTZ can attenuate basal antioxidant mechanisms and increase inflammatory cell infiltration in male rats' brain, and it demonstrated that GE can minimize the pro-oxidant effect and decrease inflammation. Behavioral changes with GE were increased latency, decreased severity, and decreased duration of fits.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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