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# Potential Antiepileptic Activity of *Gastrodia Elata*: The Concept of Antioxidant and Anti-Inflammatory Properties in Rats Model

A thesis Submitted to the Council of the College of Medicine, the University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master in Pharmacology \ Pharmacology and Toxicology

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”بسم الله الرحمن الرحيم“

# قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (25) وَيَسِّرْ لِي أَمْرِي (26) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (27) يَفْقَهُوا قَوْلِي (28)

صدق الله العليَ العظيم

(سورة طه آيات 25,26,27,28)

# Supervision Certificate

We certify that this thesis entitled (**Potential Antiepileptic Activity of *Gastrodia Elata*: The concept of antioxidant and anti-inflammatory properties in Rats Model)** was prepared by (**Noor Alhuda Mohammed Khumair Alebadi)** under our supervision at the department of pharmacology. College of medicine, university of Babylon (Iraq) in partial fulfillment of the requirements for the master degree of sciences in Pharmacology and Toxicology.

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# Examining Committee Certificate

We certify that we have read this thesis entitled "**Potential Antiepileptic Activity of *Gastrodia Elata*: The concept of antioxidant and anti-inflammatory properties in Rats Model**" and as an examining committee examined the student "**Noor Alhuda Mohammed Khumair**" in its contents and that in our opinion it meets the standard of a thesis for the degree of Master, in Pharmacology and Toxicology.

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# ***Dedication***

*I must to express my deepest thanks to my great MOM and DAD ever and for my beautiful sisters and brothers especially my big brother Ali …*

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*Noor Alhuda Alebadi*

## Summary

Epilepsy as a neurological disorder and one of the most common brain disease which associated with one or two unprovoked seizures (fits) that occurred in less than 24 hours apart. Epilepsy occurs at all ages. Six axes of etiological epilepsy were classified by international league against epilepsy(ILAE) which were: structural, genetic, infectious, metabolic, immunological and unknown etiologies.

Epilepsy is not a fixed neurological condition, but it is a continuous, progressive, excitatory disorder during the life span. Recently, there is a critical demand of getting better-tolerated antiepileptic drugs (AEDs) that are effective as a neuroprotective, and / or anticonvulsants. Nowadays, there is emerging evidence towards the relationship between oxidative stress, and / or inflammation and epilepsy.

*Gastrodia elata* (GE) is a famous traditional Chinese herb from the family orchidaceae which has been used for centuries. This study aimed to explore the effect of GE as antiepileptic, antioxidant, anti–inflammatory.

Forty male albino rats had been divided into eight groups; Group1 (control) was neither exposed to pentylenetetrazole (PTZ) nor received any treatment while group 2 was exposed to PTZ (40mg/kg), i.p. every other day for 15 days, group 3 received GE, 883.56mg/kg for 14 days then induction of seizure by 40mg/kg PTZ, then GE, 883.56 mg / kg, for 5 days after kindling. Group 4 were exposed to GE 883.56mg/kg and PTZ 40mg / kg. Group 5 received lacosamide 50mg/kg orally and PTZ 40mg /kg. Group6 received lacosamide 50mg /kg orally. Group 7 received zonisamide 100mg /kg, orally, and group 8 received zonisamide 100mg/kg and PTZ 40mg/kg.

The anticonvulsant effect of GE and selected AEDs were evaluated by measuring the latency, severity, and duration of fits using Racine's scale. In group 2, GSH and TAOC were significantly ($p$ <0.05) decreased in comparison group 1,

however, MDA, NO, IL-1, IL-6, and TNF-α were highly significantly ($p$ <0.001) increased. In group3 to group8, GSH and TAOA were highly significantly ($p$ <0.001) increased, but, MDA, NO, IL-1, IL-6, and TNF-α were highly significantly ($p$ <0.001) decreased in comparison to group2. In group 2, duration and severity of fits were highly significantly ($p$ <0.001) increased, however, latency was significantly ($p$ <0.05) decreased compared to group1 (control group). In groups 3, 4, 5, and 8, duration and severity were highly significantly ($p$ <0.001) decreased, furthermore, latency was highly significantly ($p$ <0.001) increased in comparison to group 2.

Kindling with PTZ has been found to display significant morphological alterations and sever inflammation in histopathological study. GE has been found to ameliorate the histopathological damage and mild inflammation area of brain compared to group2. Lacosamide was found to decrease the inflammation in group 5 and also zonisamide was have the effect in reduce inflammation as in group8 compared to PTZ group2.

In conclusion, *Gastrodia elata* have an anticonvulsant, antioxidant, and anti-inflammatory effect as depicted by behavioral, biochemical tests and histopathological results which demonstrated that *Gastrodia elata* increased antioxidant biomarkers and decreased anti-inflammatory parameters. Moreover, *Gastrodia elata* decreased the severity and duration of fits moreover, increased the latency just like what the selected AEDs (lacosamide and zonisamide) done.

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# List of abbreviations

| Abbreviation | Meaning |
|---|---|
| AEDs | Antiepileptic drugs |
| AMPs | Adenosine monophosphate |
| ATP | Adenosine triphosphate |
| BBB | Blood brain barrier |
| CAT | Catalase |
| CNS | Central nervous system |
| CRMP2 | Colapsin response mediator protein2 |
| CYP3A4 | Cytochrome P450 3A4 |
| D.W | Distilled water |
| DNA | Deoxyribonucleic Acid |
| EEG | Encephalogram |
| EP | Eppendorf |
| ETC | Electron transport chain |
| GABA | Gamma-Aminobutyric Acid |
| GE | *Gastrodia Elata* |
| GLT-1 | glutamate transporter |
| GLAST | Glutamate aspartate transporter |
| GPx | Glutathione peroxidase |
| GR | Glutathione reductase |

| | |
|---|---|
| GSH | Glutathione |
| H2O2 | Hydrogen peroxide |
| HIC | high income countries |
| HRP | Horse radish peroxidase |
| hrs. | Hours |
| p.o. | Orally |
| i.p. | Intraperitoneal |
| IL | Interleukin |
| ILAE | International League Against Epilepsy |
| LCM | Lacosamide |
| LMIC | Low and middle income countries |
| MDA | Malondialdehyde |
| RNA | ribonucleic acid |
| mRNA | Messenger RNA |
| MS | Multiple sclerosis |
| mtDNA | Mitochondrial DNA |
| mtROS | Mitochondrial reactive oxygen species |
| MTS | Medial temporal sclerosis |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NLRP3 | nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 |
| NO | Nitric oxide |
| NrF2 | nuclear factor erythroid 2–related factor 2 |
| $O_2^{\bullet-}$ | Superoxide anion |
| OD | Optical density |

| | |
|---|---|
| OH· | Hydroxyl |
| ONOO- | Peroxynitrate anion |
| PTZ | Pentylenetetrazole |
| RNS | reactive nitrogen species |
| SDS | Sodium dodecyl sulfate |
| SE | Statues epilepticus |
| SMAP | Sufamoyl acetyl phenol |
| SOD | Superoxide dismutase |
| SSADH | Succinic semialdehyde dehydrogenase |
| SSAR | Succinic semialdehyde reductase |
| TLR4 | Toll Like Receptor 4 |
| T Max | Time maximum |
| TAOC | Total antioxidant Capacity |
| TB | Tuberculosis |
| TLE | Temporal Lobe Epilepsy |
| TNF- α | Tumor Necrosis factor Alpha |
| VGSCs | Voltage gated sodium channels |
| WHO | World health organization |
| ZNS | Zonisamide |

## 1.1. Introduction

Epilepsy is one of the neurological disorders that cause unprovoked, recurrent seizures which affected people of all ages (Moshé *et al.*, 2015). Epilepsy diagnosed when the patient has two or more seizures with no other identifiable causes. Although the exact etiological causes of seizures are not known, hyperexcitability, oxidative stress, cortical stimulation, genetic factors are among the causing pathogenic factors (Walsh *et al.*, 2017). At 2020, around 50 million people worldwide have epilepsy (Shampa *et al.*, 2021), so it is one of the most common neurological disease, 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 quarter of this people do not received the treatment that they need (WHO:2019).

Epilepsy are controllable with medication is about 70% of cases (Eadie, 2012), epilepsy is more common in males than females (Neligan *et al.*, 2012). In the United Kingdom UK, 40-60 % of death estimated is prevented possibly. The total annual cost of epilepsy in 2019-2020 was $ 12.3 billion, it results in direct economic costs of nearly $1 billion in United states. Epilepsy costs around 15.5 billion euros in Europe, in India estimated that epilepsy result in costs of US $ 1.7 billion (Wilden and Cohen-Gadol, 2012).

This cost of epilepsy including lost productivity and medical expenditures. seizures can affect any process of brain coordinates because it is caused by abnormal activity in the brain, temporary confusion, stiff muscles, a staring spell, loss of consciousness, uncontrollable jerking arms and legs movements, are all symptoms of epilepsy, symptoms vary according to the type of seizures (Erika Klein, 2019).

The International League Against Epilepsy (ILAE) in 1989 classified the

epilepsies in to four types in 2017 this classification refined and recommendation into four categories and subcategories appropriate with technologic and scientific advances into: known cause (genetic mostly), symptomatic (pathologic abnormalities or grass anatomic), provoked (systemic or environment), cryptogenic (cause has not been identified).

It has recently been shown that inflammation plays a significant role in the epilepsy pathogenesis that results from status epilepticus (SE), stroke, and other brain injury. Inflammatory significantly contributes to epilepsy related with cortical malformation and temporal lobe epilepsy (TLE) neuronal defect, intense elevated in gliosis and microgliosis contribute to epileptogenesis (Alyu and Dikmen, 2017). Inflammation insult may be started in central nervous system or it can travel via the bloodstream to the brain from the systemic circulation via BBB damaging.

Homeostatic imbalance between antioxidant and oxidants may be generated seizure through free radical's effects on mitochondrial DNA damage and lipid peroxidation. These findings might have contributed to decrease the seizure threshold (Borowicz-Reutt and Czuczwar, 2020).

Antioxidant enzyme and non- enzyme such as SOD and GSH respectively were demonstrated to have an important function in free radicals scavenging in the body. Based on the mechanism of action, antiepileptic medicines (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 (Juan G. Ochoa *et al.*, 2022).

Another treatment includes surgery to remove a small part of brain that causing the seizures, implant a small electrical device inside the body that can help control seizures. Lacosamide and zonisamide, AEDs, used for

epilepsy. In addition to their mechanism, they found to have anti-inflammatory and anti-oxidant effects.

*Gastrodiae elata (GE)*, a famous Chinese herbal plant, which was conventionally utilized to treat many different situations involving epilepsy, headache, stroke and others (chines pharmacopeia commission, 2015).

*Gastrodia elata* has biological activities like anticonvulsant, antioxidant, anti-inflammatory because of its bioactive components, moreover, phytochemical constituents extracted from *Gastrodia elata* may be considered to integrated in the programs development of new anticonvulsant drug (Matias *et al.*, 2016).

## Aims of the Study

The present study aimed to:

1. Measure the antiepileptic impact of *Gastrodia elata* via it is effects on latency, duration and severity of seizures in male rat model of seizure.

2. Explore the effects of *Gastrodia elata* on the selected oxidative insult parameters, reduced glutathione, malondialdehyde, nitric oxide and total antioxidant capacity in male rat's model of seizure.

3. Investigate the effects of *Gastrodia elata* on the expression of inflammatory biomarkers, Interleukin-1, Interleukin -6, and tumor necrosis factor-α, in male rats after seizure induction by pentylenetetrazole.

4. Highlight the effect of selected antiepileptic drugs, lacosamide and zonisamide on inflammatory and oxidative stress biomarkers in male rat's model of seizure.

## 1.2. Epilepsy

Epilepsy defined as the brain disorder that's characterized by recurrent seizures that might be associated with cognitive, psychological, neurobiological and social consequences (Falco-Walter *et al.*, 2018). This definition was in 2005 by the international league Against Epilepsy (ILAE).

On other hand epileptic seizure refers to a transient occurrence of abnormal repetitive skeletal muscles contraction according to abnormal increasing in neuronal activity in the brain although these definitions stay conceptual but they are difficult to use in clinical setting of real life. So, the definition of epileptic seizure was expanded to include practical definition: Epilepsy one of the brain illness, which is the most common neurological disorders with at least two bursts or unprovoked seizures (fits) happened in less than 24 hours apart (Fisher *et al.,* 2014). Epilepsy occurs at all ages and affects around 50 million people worldwide annually according to (WHO).

### 1.2.1. Prevalence and Incidence of Epilepsy

Epilepsy one of the most common neurological disease in the world and occurs in people of all ages and races, social classes and geographic of locations. In meta-analysis and systematical studies of the incidence, it has been found that the rate of epilepsy was 61.4 per 100.000 patients / years (Fiest *et al.,* 2017). Higher incidence of epilepsy in low and middle income countries (LMIC) the incidence in these countries 139.0 / year. However, the incidence of this disease in high income countries (HIC) was 48.9/year. This difference can be explained by the population in LMIC subject to perinatal risk parameters, CNS infections with greater rates and TB infection (Beghi *et al.,* 2014).

Epilepsy seems to be more common in certain nations than others, and this is mostly due to differences in the regional distribution of danger and etiologic variables (Fiest *et al.,* 2017), prevalence of epilepsy was 7.60 per

1.000 population (remission cases) or lifetime prevalence while the prevalence of active epilepsy was 6.38 per 1.000. Epilepsy is somewhat more common and prevalent in males than in women, both in terms of incidence and prevalence (Beghi, 2020).

### 1.2.2. Epilepsy and Seizures Classification

The first classification of a seizure officially by ILAE since 1989 (Brodie *et al.,* 2018). The classification can help to use specific drug that is effective to a certain type of seizure and also to identify the comorbidities and prognosis related with a specific type of seizure. (Scheffer, 2017). The framework of the classification consists of four components in the new classification of ILAE in 2017 (Fisher *et al.*, 2017) as explain in fig.1.2.

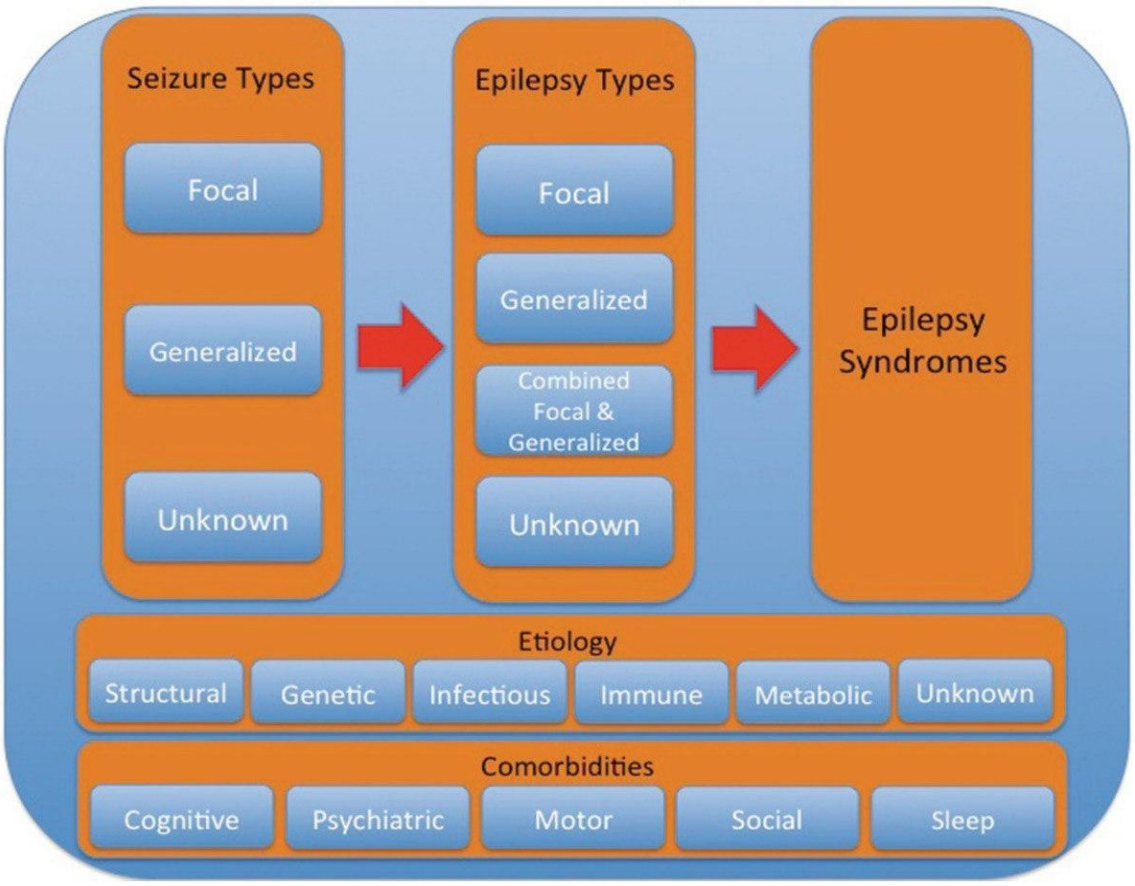



Figure 1.1: ILAE 2017 Classification of epilepsies.

### 1.2.3. Seizure Classification

Seizure classification by ILAE based on these features:

1. The seizure origin in the brain.
2. The awareness degree during seizure.
3. Movement level of the body.

According to **the first feature**, a seizure either focal onset or generalized onset. **The second features** refer to either awareness impaired or awareness intact (know his self and environment) and **the third feature** to mean that a seizure may be motor or non-motor (Auvin *et al.*, 2018).

Focal seizure happens when there is abnormal in electrical discharge originates from one side of the brain. In focal seizure, awareness may be present or absent and may be related to jerk in single limb and can progress to include both limbs. Nevertheless, generalized seizures mean that there are abnormal electrical activities begin simultaneously from the rights and left side of the brain then transported or released to the other neuronal networks of the brain diagnosed by EEG or clinical manifestation (Brodie *et al.*, 2018) as show in fig.1.3.

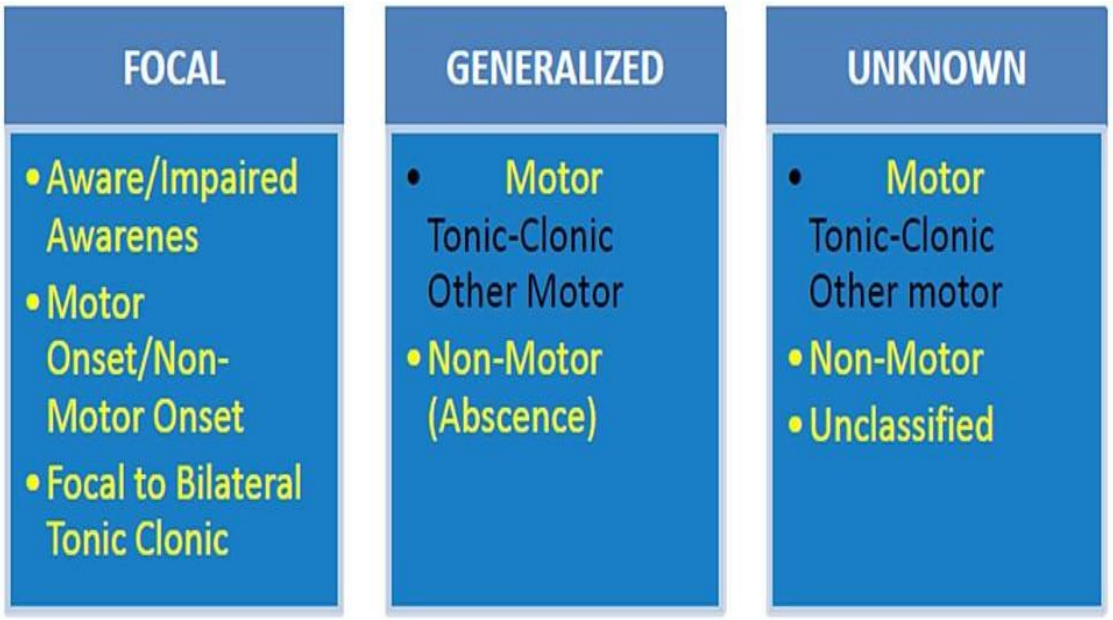



**Figure 1.2. ILAE 2017 Classification of Seizure Types.**

### 1.2.4. Etiologies of Epilepsy

New feature of epilepsy classification in 2017 has been introduced by combining etiological components with co-morbidities in epilepsy (ILAE 2017). Six axes of etiological epilepsy were classified by ILAE which are: structural, genetic, infectious, metabolic, immunological and unknown etiologies. Comorbidities by other hand must be case by case when classifying or diagnosing epilepsy including sleep disorders, motor deficits, behavior and psychiatric abnormalities as highlight in fig 1.4.

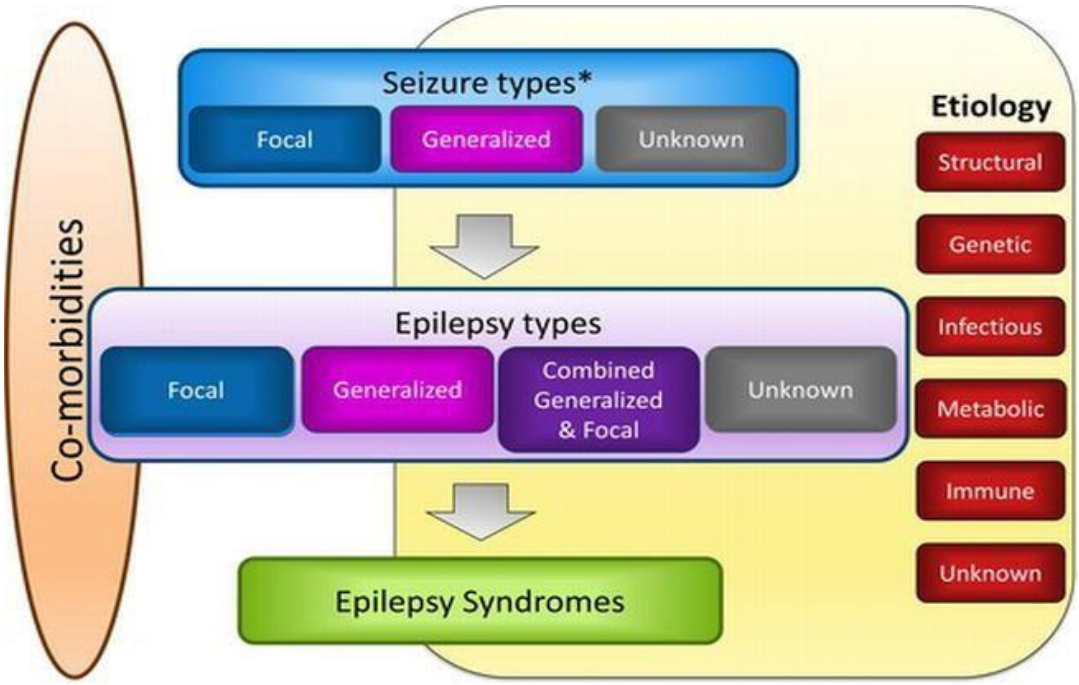



Figure.1.3. ILAE frameworks for the classification of epilepsies, (Scheffer *et al.*, 2017)

#### 1.2.4.1. Structural Etiology

Structural etiology was either primitive (congenital malformations) or acquired (trauma, infections, hypoxia, and stroke) (Scheffer *et al.*, 2017). Structural abnormalities are diagnosis by using magnetic resonance images (MRI) or computerized tomography (CT) scanning.

### 1.2.4.2. Genetic Etiology

By which a certain disease exists and causes gene variation to be the explained by epileptogenicity. More than eighty percent of people diagnosed with Dravet syndrome have a mutation in a subunit of the voltage-gated sodium channel. Dravet syndrome seems to be an instance of a hereditary form of epilepsy (Falco-Walte *et al.,* 2018).

### 1.2.4.3. Infectious Etiologies

Infectious etiologies are the most common curable causes of epilepsy in Sub – Saharan Africa (Carrizosa & Kakooza, 2017). Common examples of this type of etiological epilepsy, include tuberculosis TB, cerebral malaria, neurocysticerocosis, sub-acute sclerosing panencephalitis (SSPE), cerebral toxoplasmosis, cytomegalovirus and congenital infections.

### 1.2.4.4. Metabolic Etiologies

The main cause of this type of etiological epilepsy occurs as a result of a metabolic derangement. Clinical entities such as uremia, amino-acido-pathies, porphyria or pyridoxine dependent seizures. Metabolic epilepsies may be associated with genetic defect and a few can be acquired for example cerebral folate deficiency (Scheffer *et al.,* 2017).

### 1.2.4.5. Immune Etiologies

The immunological epilepsies were caused by an autoimmune syndrome that was seen in both children and adults. This disorder was related with the signs of autoimmune in the central nervous system. For instance, anti_ NMDA (N-methyl -D-aspartate) receptor encephalitis. (Johnson *et al.,* 2017).

### 1.2.4.6. Unknown Etiologies

For many patients with epilepsy with no clear exact reason. The diagnosis and management in such cases were just built on EEG and clinical findings. Etiology of this kind of epilepsy differs from country to another according to the common realistic causes with local health systems (Scheffer *et al.*, 2017).

## 1.2.5. The Epilepsy Neurobiology

### 1.2.5.1. Molecular Mechanism of Epilepsy

Literally, the mechanisms explained epilepsy to be idiopathic that occurs because of genetic or developmental disorders or malformations or acquired that developed after progression of changes in response to injury or an acute insult. Nearly 50 % of epilepsy patient present with acquired epilepsy.

The effects of acquired epilepsy are far more widespread than those of developing epilepsies, which are mostly mitochondrial or ion channel disorders (Martinc *et al.,* 2012). There are a number of genetic parameters that could be continuing to increase neuronal activity in idiopathic epilepsies. If there is a mutation in ligand gated ion channels or neurotransmitter receptors, synapses, or the mitochondrial respiratory chain complex, this could alter the brain's regular excitability and lead to epileptic seizures (Engel *et al.*, 2022).

**Three phases in the development of acquired epilepsy (**Pitkanen & sutula, 2002)

1- Acute phase.

2- Latent phase.

3- Chronic phase.

1. **The acute phase (insult)**

In acquired epilepsy, the epileptogenesis process can be started by many types of brain injury for instance, trauma, infections, tumors, stroke, status epilepticus, hypoxia, and febrile convulsion in children, these diseases and injuries can rise the occurrence of acquired epilepsy (Delorenzo *et al.,* 2010).

The most important etiological factors in acquired epilepsy are cerebrovascular accident (CVA), brain trauma and status epilepticus (Delorenzo *et al.,* 2010). Although certain differences in the factors nature that cause acquired epilepsy, they are associated with the mechanism that cause brain injury i.e. excitotoxicity occurred by increase of glutamate concentration in extracellular this lead to continuous stimulation of α – amino – 3 – hydroxyl – 5 – methylisoxazol – 4 – propionic acid (AMPA) receptors, greater increase in intracellular calcium ion, recruitment of N-methyl- D- aspirate (NMDA) receptor, and activation of signaling pathways of calcium ion that cause death of neuron (Delorenzo *et al.,* 2010).

**2. Latent Phase or (Epileptogenesis)**

During this phase, changes in the physiology of the brain result in the development of epilepsy. These processes might be lasting months or years in which seizures didn't occur (Pitkanen *et al.*, 2009). In addition, commonly epilepsy not a remaining disorder but developing through the life span (scharfman *et al.*,2007).

There is currently a poorly understanding about the pathophysiological processes involved in the epileptogenesis process. (Herman, 2006), but are thought to consider neuronal cell death that occur as a response to the initial injury of brain as the primary causative factor to provoke epilepsy.

The certain role of cell death is still under dissection. Cell death may be either the cause or a sequence of seizures or may be both. Cell death may be either the cause or a sequence of seizures or may be both. This method of cell death has been validated by the observation that the surgical removal of the damaged hippocampus may alleviate the symptoms and signs of temporal lobe epilepsy (TLE) (Delorenzo *et al.,* 2010).

Initial cerebral injury findings in a series of biochemical, physiological and structural variations involving BBB disruption in high prevalence following all

cerebral lesion that lead to post injury epilepsy like stroke, brain infection, traumatic brain injury, increase intracranial pressure and necrosis (Schevon *et al.*, 2019).

In traumatic epilepsy for example, it was supposed, which intracerebral hemorrhage caused localized damage of red blood cells (RBC) and release iron ions from hemoglobin which can be resulting in production of free radical's species of reactive-oxygen (ROS) that damaged neuronal cells (Willmore *et al.*, 2009). Excessive release of glutamate neurotransmitter is an important part of epileptogenesis after brain injury. This resulted in excitotoxicity and excessively depolarized sharply increase in concentration of calcium ions intracellularly and cell death (Aroniadou *et al*., 2008).

High calcium concentration also causes changes in the function of inhibitory GABA - A receptor, protein expression, alteration in gene transcription and neurogenesis (Blair *et al*., 2004).

Hence, glutamate excitotoxicity and an elevated calcium ion concentration, in addition to a number of other pathophysiological consequences, play a definite part in the evolution of spontaneous recurring seizures.

**3. Chronic Phase or Recurrent Epilepsy**

At this phase, one observes the failing of fixed enzymes such as aconitase, complex I, and oxidized/reduced glutathione (GSH/GSSG) redox status. This failure might precipitate oxidative damage caused by exposed to $O^2$ and H2O2 constantly during the chronic epileptogenesis phase (Rowley and Patel ,2013).

These chemical alterations may lead to cellular damage, which may play a role in the development of persistent epilepsy.

## 1.3. Oxidative Stress

Oxidative stress term refers to that there was an imbalance between elimination and generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Ramalingam *et al.,* 2013). When ROS produced in nigh quantities are able to cause oxidative damage (Sharifi-Rad *et al.,* 2020). ROS and RNS alter the genetic apparatus function by oxidizing proteins, nucleic acids, and lipids, that are major component for cells and lead to death of cells (Hajam *et al.,* 2022).

The most affected organ by ROS and RNS is a brain because it is very rich in poly-unsaturated fatty acids, and needs a high concentration of oxygen which reflects acceleration in mitochondrial enzymatic reactions.

This considered the most important cause to release the free radicals, presence of ROS required defense system to protect the organs from insults of the ROS and RNS, superoxide dismutase (SOD) ,catalase (CAT) and reduced glutathione (GSH) are the most important endogenous antioxidant defense mechanisms , these antioxidants represent the critical key role in free radicals scavenging by which catalase enzyme presented in mitochondria and responsible for catalyzing the decomposition of hydrogen peroxide (H2O2) to water and oxygen O2 (Rodrigues *et al*., 2013).

It was found that high concentration of H2O2 in tissues causes hippocampal cells damage during the acute phase of epilepsy (Tâmara *et al.,* 2015).

Peroxynitrate anion (ONOO-) is highly reactive molecule that generates from reaction between super peroxide radical and nitric oxide which is capable of inducing lipid oxidation and inactivate sulfhydryl–bearing enzymes, and consequently depleting the sulfhydryl protein content (Rodrigues *et al.,* 2013), (ONOO-) is a very cytotoxic and acts as an important role in neurodegenerative and neurological disorders (Dato *et al.,* 2013).

Super oxide dismutase (SOD) enzyme acts as a key role in detoxification of superoxide anion $O_2^-$ via catalyzing it to $O_2$ and $H_2O$ to prevent cell damage by oxidative stress (Kiasalari, 2013).

Nitric oxide (NO) is another molecule that involved in oxidative stress insult by which NO reacts with other biological molecules leading to induce oxidative insult and consequently neuronal toxicity (Marques, 2013).

Glutathione (GSH) is the pivotal intracellular non-protein thiol scavenger of ROS (Aseervatham *et al*., 2014) which acts as an antioxidant and plays a vital role in prevention of cell damage by oxidative stress. Seizure and neuronal survival can be regulated by modification of endogenous GSH level intracellularly (Liu *et al*., 2012).

Antioxidants play an important role in protection of cell from damaging by oxidative insult molecules and have therapeutic role in neurodegenerative and neurological diseases (Olalekan Bukunmi *et al*., 2022). Medicinal plants nowadays have been given attention as a protective agent that might be used against oxidative stress and subsequent neurological disorders like parkinsonism and epilepsy (Xie, 2012).

### 1.3.1. Oxidative Stress and Epilepsy

The brain considered a very susceptible part to oxidative damage because of it is high polyunsaturated fatty acids, increasing metabolic demands and oxygen consumption.

Hypermetabolism of the brain was found to be a crucial characteristic of seizures disorders, an increase in the consumption of brain to oxygen and glucose are observed through the activity of seizure in experimental models and clinical trials (Fabisiak and Patel, 2022).

Dysfunction of interictal metabolism caused by metabolic stresses that was demonstrated by hypometabolism of glucose in chronic epilepsy and deficits mitochondrial metabolism occurs by inhibition of the complexes enzyme

ofmitochondria (Fabisiak & Patel, 2022).

It was presented that the mitochondrial reacted oxygen species (mtROS) resulted in a severe damage to mitochondrial electrons transporting chains ETC complex I and a decrease in mitochondria-oxidations-phosphorylation subsequently (Rowley *et at.*, 2015), which might be able to increase ROS synthesis and initiation of epilepsy by damage to lipid, protein and nuclear molecules by oxidation process and alleviated of the redox status.

**1.3.1.1. Protein Oxidation**

Exposure of protein to ROS might resulted in alteration in primary, secondary, and tertiary structures, activity and function of proteins. After this, the protein will experience spontaneous fragmentation and an increased vulnerability to proteolysis

Damage to proteins caused by oxidation may be measured in terms of the quantity of carbonyl present; this marker is abundant in certain parts of the brain and becomes more prevalent with advancing age, neurological conditions, and neurodegenerative illnesses (Simon Waldbaum and Manisha Patel, 2010).

The most common proteins that are affected by ROS are aconitases, alpha ketoglutarate dehydrogenases (α-KGDH), and mitochondrial complex I (CI) these proteins affected by ROS through the epileptogenic period or by post–translational modifications (Rowley and Patel, 2013).

The endogenous aconitase activity measurement demonstrated both an extent of oxidative damage to proteins and index of $O_2\cdot^-$ levels.

Inactivation of aconitase may have been brought on by the emission of iron as well as the formation of $H_2O_2$ generation and consequently from $HO\cdot^-$ release which resulted additional oxidative stress.

This cycle could be inhibited through the application of an iron-chelating agent, that also supplied neuroprotective effects in the experimentally

induced (Simon Waldbaum and Manisha Patel, 2010), complex I (CI) acts as an important key role in the beginning of the electron cascade in the mitochondrial electron transport chain (ETC).

A large number of studies have shown that an oxidative stress-induced post-translational modification, which was interfered by catalytic oxidation of Complex I in exploratory epilepsy of the temporal lobe (TLE), was responsible for the condition (Ryan *et al.,* 2012). Complex I (CI), which serves as both a target and a source of oxygen radicals, is responsible for the extra mitochondrial damage and formation of reactive oxygen species (ROS).

Oxidative damage and fluctuating local ATP content both had an influence on glutamate transporters, glutamate synthesis, and Na+/K+ ATPase, all of which were thought to be related with hyperexcitability in neuron (Rowley and Patel, 2013).

Glutamate transporter (GLT_1) and glutamate aspartate transporters (GLAST) are controlled by variations in the amount of extracellular ATP that occurs throughout activating of the extracellular signal-regulated protein kinase (ERK) in the hippocampus area (Frizzo *et at.*, 2007).

Damage to glutamate transporters, which may be caused by oxidative stress, can result in a reduction in the function of the uptake system.

Reduced expression of both (GLT-1 and GLAST), which was coordinated with mitochondrial oxidative stress, that might cause in an increasing the intensity of experimental seizures susceptibility, this finding was also demonstrated in clinical investigations of epileptic patients (Meldrum *et al.,* 2007).

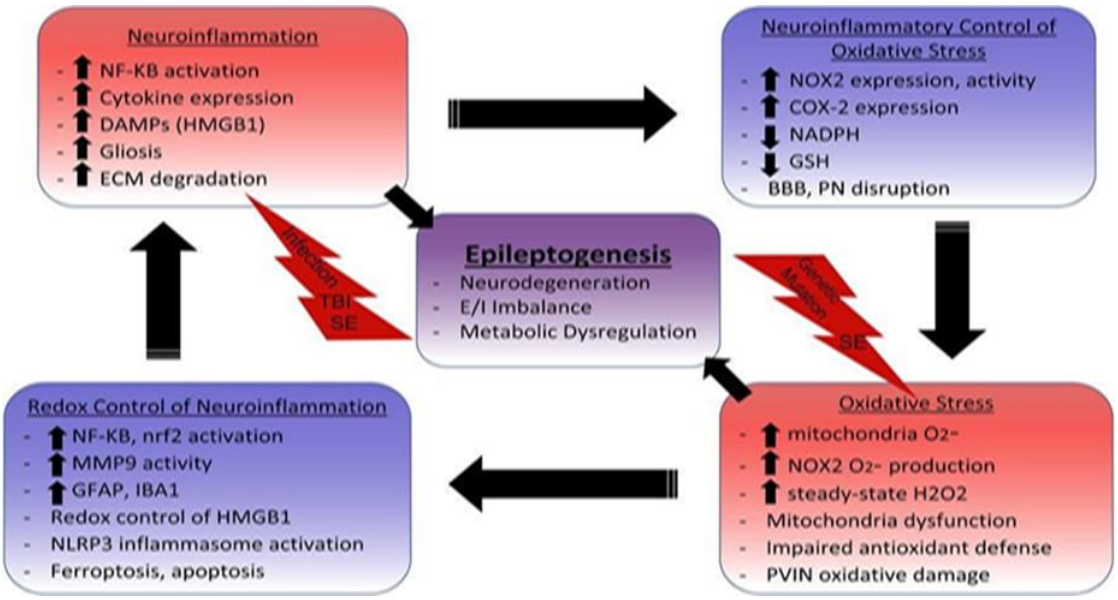



**Fig. 1.4.** Neuroinflammation and Oxidative Stress Cycle in Epilepsy (Fabisiak T. and Patel M, 2022),(NF-κB) Nuclear factor kappa B, (ECM)extracellular matrix, DAMPs) Damage-associated molecular patterns, (GFAP) Glial fibrillary acidic protein, (Nox2) cytochrome b (558), (HMGB1) High mobility group box.

### 1.3.1.2. Lipid Peroxidation or Oxidative Damage to Lipid

The abstraction of hydrogen from polyunsaturated fatty acids by $OH\cdot^{-}$ transformed from $H_2O_2$ and $O\cdot^{-}2$ by the catalytic action of metals transition lead to lipid peroxidation (Shehta, *et al.*, 2022). The products resulted from lipid peroxidation and oxidative stress highly affected on brain and cause brain and mitochondrial injury because they are very highly sasuptabile to lipid peroxidation due to high content of polyunsaturated phospholipids that demonstrated at the major site of reactive oxygen species productions (Simon Waldbaum & Manisha Patel, 2010).

Malondialdehyde (MDA), F2 isoprostanes (Isops), 4 – hydroxy 2 (E) nonenal (4 – HNE), and isofurans (isops) are all by-products of the peroxidation of polyunsaturated fatty acids and are considered to be the primary markers of lipid peroxidation. Two hours after receiving the

chemconvulsant (pilocarpin), MDA levels rise (Tejada *et al.*, 2007). In the epilepsy kindling model, it has been shown that MDA and 4 – HNE levels rise in both hemispheres 24 hours following the conclusion of the most recent episode (Frantseva *et al.*, 2000).

Vitamin E and glutathione were able to stop the increase in lipid peroxides and the death of hippocampus neurons. Nevertheless, they have not proven successful in preventing the onset of seizures throughout the kindling process.

It was hypothesized that seizures were caused by lipid peroxidation, and it is also possible that it was included in the initial stages of epileptogenesis fig. 1.5.

#### 1.3.1.3. Oxidative Damage to Mt DNA

Recently, oxidative damage to mtDNA has presented as potential cause of epileptogenesis. mtDNA very susceptible to oxidative damage because of; mtDNA does not have histones which own a crucial role in regulation of chromatin and act on preservative the strands of DNA in compact form, and also its location is closed to the of mitochondrial inner membrane which where the release of ROS (Rowley & Patel, 2013). Increased of ROS production may cause mtDNA damage that causes decrease in activities of electron transporter chain mitochondrial complexes I, III, IV and V.

### 1.3.2. Seizure-Induced Alteration in Antioxidant Defenses and Redox Status

#### 1.3.2.1. Seizure – induced changes in antioxidant defenses

The antioxidant defenses are complex endogenous and integrated system which have the ability to protect the living cells from the destruction by the impacts of ROS. Oxidative damage occurs when antioxidant defense cannot damage ROS leading to accumulates of it particularly, in mitochondria (Tsu-Kung Lin, *et al.*, 2020).

Seizure alterations were found to be associated with antioxidant

defenses, however, an increase or a decrease in antioxidant level remains controversial.

Super oxide dismutase activity in pilocarpine model in the hippocampus site has been cause decrease at 24 hours and through the chronic phase of epilepsy while the glutathione peroxidase (GPx) activity is increased (Tejada *et al.,* 2007). CAT, SOD, and GPx activities has also been reported to be increased two hours after induction of seizure by pilocarpine (Tejada *et al*., 2007).

Numerous authors demonstrated that the hippocampal activities of CAT, but just not SOD, had been lowered after twenty-four hours after the injection of pilocarpine. This suggested that the hippocampus need not employ SOD as an antioxidant, but rather utilizes CAT and GSH very frequently in its place (Freitas *et al*., 2005).

CAT and SOD activity enhanced in both piriform cortex as well as hippocampus at 48 hours within a week of 5 days of kainate administering was decreased (Simon Waldbaum & Manisha Patel, 2010), in contrast, CAT and SOD activity increased in both piriform cortex and hippocampus at 48 hrs. after 5 days of kainate administration considering the disparity in the evidence from different models of epilepsy. Basically, the compensation of antioxidant issue in response to the seizure activity was still unsolved.

### 1.3.2.2. Seizure – Induced Alteration in NrF2 Regulated Redox Status

The nuclear factor erythroid 2–related factor 2 (Nrf2) in a wide variety of tissues, the redox-sensitive transcription parameter Nrf2 seems to be a key component that controls anti-oxidant defenses and the integrity of cell membranes (Baired, 2011).

Every living cell is dynamically controlled by Nrf2 and has an innate potential to scavenge reactive oxygen species (ROS).

Nuclear factor erythroid 2–related factor 2 is mainly regulating the phase II cellular antioxidant response, Nrf2 shown in recent studies many actions such as improve mitochondrial function, an anti – inflammatory action, influence autophagy and confirm xenobiotic detoxification so, modulating in Nrf2 might be helped in treatment and prevention many of disorders and chronic diseases, such as epilepsy, neurodegenerative diseases, multiple sclerosis, chronic kidney disease and diabetes mellitus type 2 (Martin *et al.*, 2015).

Super oxide dismutase and CAT are enzymes that are responsible for the neutralization of ROS. Enzymes which are involved in the formation and biosynthesis of GSH are under the transcriptional control of Nrf2.

Glutathione is recycled from glutathione disulfide (GSSG) by glutathione reductase (GR) and oxidation NADPH, GSH / GSSG ratio is widely used in biological systems as an oxidative stress biomarker.

A decrease in GSH and GSSG levels may cause alteration in GSH synthesis and /or transport.

Decrease GSH / GSSG ratios may induce changes in mitochondrial enzyme activities, structural damage to the membranes of mitochondria and mitochondrial dysfunction that might affect the excitability of neurons (Faisal Nuhu *et al.*, 2020).

## 1.4. Inflammation – Epilepsy Relationship

In recent years, researchers have shown that inflammation plays a significant role as an insult in the development of epilepsy caused by stroke and other brain traumas. New research suggested that inflammatory processes may have had a role in both epilepsies of the temporal lobe and epilepsy associated with cortical abnormalities (Iqra Mukhtar *et al.*, 2020). The neuronal dysfunction, microgliosis, and gliosis, together with the heightened intensity and neuro-inflammatory activity, all lead to

epileptogenesis (Alyu and Dikmen, 2017).

Inflammation might well have originated in the central nervous system (CNS), or it may have entered the CNS from the circulatory system through disruption to the blood brain barrier (BBB), extensive research has been conducted on neuro-inflammation and epileptic seizures, with a focus on hippocampal foci as opposed to extra-hippocampal epileptic foci.

This is owing to the well-documented pathological, physiological, and anatomical issues that are encountered in hippocampal atrophy and sclerosis. On the other hand, there is evidence that links neuroinflammation with the loss of neuronal cells and the accumulation of gliosis in the extra-hippocampal area (Iqra Mukhtar *et al.*, 2020).

It is believed that the acute phase of neuroinflammation contributes to and exacerbates the condition of chronic neuroinflammation Butler *et al.*, 2016).

For instance, thosepatients with multiple sclerosis (MS) consider to be a higher chance to have epilepsy, and this risk is raised with the development of intracortical lesions that induce widespread massive cortical inflammation.

### 1.4.1. Role of Pro-Inflammatory Pathways in Epileptogenesis

A developing body of indication highlighted the role of chronic inflammation which induced pro-inflammatory signals that may lead to pathological status such as loss of neurons, impaired blood brain barrier and hyperexcitability of the nerve cells, these factors contribute in epileptogenesis (Iqra Mukhtar, *et al*., 2020)

The activation of astrocytes and microglia considered as a common principal pro – inflammatory signals in the medial temporal sclerosis (MTS) and as a consequence temporal lobe epilepsy (TLE) (Vezzani *et al.*, 2011) fig.1.5.

### 1.4.2. Cytokines as Inflammatory Mediators

Cytokines are proteins which modulate inflammatory processes, glial cells are responsible for primarily production of cytokines and also could be produced by neurons during inflammation of the brain (Alyu & Dikmen, 2017). Cytokines pro –inflammatory ;1L-1β, IL–2 and IL– 6, concentrated within the brain in low quantities typically and increased after seizures (Scorza *et al*., 2017).

Clinical investigation has shown that increased in cytokines levels of IL–1β, IL–6 and tumor necrosis factor–α (TNF–α) in cerebrospinal fluid in association with epilepsy (Rana *et al*., 2018).

Cytokines and cytokines receptors involve numerous pathways of inflammatory that cause neuronal hyperexcitability and harmful synaptic changes.

#### 1.4.2.1. Interleukin – 1β

Interleukine–1 is a pro – inflammatory cytokine presented in activated astrocytes and microglia which enhances glutamate release from astrocytes and decrease it's re–uptake, with increasing the availability of glutamate in neuronal synapses this might be a real cause of neuronal hyperexcitability (Alyu & Dikmen, 2017), It's possible that IL-1 causes seizures by causing an upregulation of NMDA receptors on post-synaptic cells. This is accomplished by activating GluN2B, which is a subunit of the NMDA receptor. Utilizing models of epilepsy, the researchers discovered that the generation of GluN2B mRNA increased throughout the 24 hours following seizures. Additionally, they discovered that an alteration in NMDA receptors might well cause impaired plasticity in the synaptic transmission of excitatory glutamate, which ultimately led to seizures (Postnikova *et al*., 2017).

Recent studies have shown that unregulated levels of IL–1β decreased physiological synaptic plasticity and may lead to potential dysfunction of neuron. (Han *et al.*, 2016).

The research by Roseti *et al.*, 2015 revealed that the pathogenic rise in amounts of IL-1 in temporal lobe epilepsy (TLE) impaired GABA mediated neurotransmission by up to 30% and caused seizures due to the hyperexcitability of the neurons (Roseti *et al*,2015).

**1.4.2.2. Interleukin-6**

Interleukin -6 is a cytokine pro-inflammatory that promotes inflammation and is normally detected in only small amounts in the central nervous system. There is a correlation between stimulation of astrocytes and microglia in an increased in IL-6 generation (Gruol, 2015).

Elevated of cytokines levels such as IL-1β, TNF-α, IFN-gamma, and IL-17 might be result in up regulated in IL-6 (Erta *et al*., 2012).

Moreover, up regulation of IL-6 decreased hippocampal neurogenesis although increasing gliosis which might contribute in epileptogenesis (Levin & Godukhin, 2017).

A previous study focused on prenatal exposure to IL–6 can cause high risk of neurodegeneration of hippocampal neurons lead to change in both function and hippocampal morphology which resulted in hippocampal remodeling and seizure induction (Rana *et al*., 2018).

Polyinosinic- Polycylidylic acid (PIC) in pregnancy induced maternal immune activation – (MIA) leading to hippocampal hyperexcitability and significantly leads to rapid development of epileptogenesis in the offspring via elevated pro – inflammatory IL–6 and IL-1β in their hippocampus (Pineda *et al.*, 2013).

Such pieces of evidence further support the implication of IL-6 role in epileptogenesis. Moreover, despite it is clear that IL–6 contributed in neuronal inflammation that might induce epilepsy, further studies required

to consider this cytokine, IL-6, as a disease modifying therapy (Rana & Musto, 2018).

**1.4.2.3. Tumor Necrosis Factor-α (TNF_α)**

It is crucial inflammatory cytokine, contributes in proliferation, differentiation and extravasation of immune-competent cells in the central nervous system via systemic inflammation (Sonar and Lal, 2015).

Tumor necrosis factor-α released from astrocytes and activated microglia, it has been found that a low level of glutamate in glial cells lead to TNF-α to up regulate synapses and retain an adequate certain level of neuronal excitatory input (Iqra Mukhtar, 2020).

A critical study has indicated that TNF-α regulated N-Cadherin which is an adhesion molecule that is responsible for organization and formation of excitatory, as well as, inhibitory synapses (Rana, *et al.,* 2018). Furthermore, the up regulation of glutaminase and microglial gap junctions, it increases microglia glutamate release, TNF-α fount to up regulate amino methyl propionic acid (AMPA) receptors via glutamatergic transmission. So, increased AMPA receptors permit the over uptake of calcium, causing neurotoxicity (Galic *et al.,* 2012). TNF-α was not found only to increase the glutamate receptors numbers, but also increase GABA mediated endocytosis, diminished the inhibitory drive leading to persistent changes in excitability (Iqra Mukhtar, 2020).

In spite of the significant part that TNF- plays in the development of epilepsy, the anti-TNF- medication that is now being used to treat epilepsy is the subject of much debate due to the possible hazards that it poses to infections and the growth of cancer. Additionally, the TNF receptor (TNFR) and TNF family certain ligands which have emerged as promising pharmacological targets. In patients with TLE, a significant upregulation of TNF -α related apoptosis inducing ligand (TRAIL) expression increased to certain levels and cell death (Alyu and Dikmen, 2017).

## 1.5. Pharmacological Treatment of Epilepsy

There are now more than 20 AEDs that have been approved for use in the treatment of epilepsy; however, studies have shown that these treatments were only ever successful in treating 60–70% of patients, regardless of whether they are administered alone or in combination. In spite of AEDs monotherapy is the treatment of choice, there are instances in which polytherapy may be effective when individual medicines have been unsuccessful (David Y Ko.*et al.*, 2022).

Thus, there is an urgent need for new, better- tolerated AEDs that are effective against refractory epilepsy (Brigo *et al.*, 2012).

In terms of their mechanism of action, antiepileptic drugs fundamentally bring back the equilibrium between the excitation and inhibition of neuronal activity. Modulation of voltage-gated ion channels, increase of gamma-aminobutyric acids (GABA)-meditation inhibitory neurotransmission, and regression of glutamate-mediated excitatory neuronal activity are the three primary kinds of mechanisms that have been identified. Table 1.1 highlights the primary pharmacological targets that may be targeted by antiepileptic drugs that are available now a days.

**Table.1.1** A listing of recognized AEDs, the year they were first introduced and the principal mechanism (s) of action. Data is from Schmidt and Schachter (2014).

| AED | Year | Presumed mechanism of action |
|---|---|---|
| **Potassium bromide** | 1857 | GABA potentiation |
| **Phenobarbital** | 1912 | GABA potentiation |
| **Phenytoin** | 1938 | $Na^+$ channel blockade |
| **Primidone** | 1954 | GABA potentiation |
| **Ethosuximide** | 1958 | T-type $Ca^{2+}$ channel blockade |
| **Diazepam** | 1963 | GABA potentiation |
| **Carbamazepine** | 1964 | $Na^+$ channel blockade |
| **Valproate** | 1967 | Multiple mechanisms: GABA potentiation, glutamate (NMDA) inhibition, $Na^+$ channel and T-type $Ca^{2+}$ channel blockade |
| **Clonazepam** | 1968 | GABA potentiation |
| **Clobazam** | 1975 | GABA potentiation |
| **Vigabatrin** | 1989 | GABA potentiation |
| **Lamotrigine** | 1990 | $Na^+$ channel blockade |
| **Oxcarbazepine** | 1990 | $Na^+$ channel blockade |
| **Gabapentin** | 1993 | $Ca^{2+}$ channel blockade **(**$\alpha_2$ subunit) |
| **Topiramate** | 1995 | Multiple mechanisms: GABA potentiation, glutamate (AMPA) inhibition, $Na^+$ channel and $Ca^{2+}$ channel blockade |
| **Levetiracetam** | 2000 | SV2A modulation |
| **Zonisamide** | 2000 | $Na^+$ and T-type $Ca^{2+}$ channel blockade |
| **Stiripentol** | 2002 | GABA potentiation |
| **Pregabalin** | 2004 | $Ca^{2+}$ channel blockade ($\alpha_2$subunit) |
| **Rufinamide** | 2004 | $Na^+$ channel blockade |
| **Lacosamide** | 2008 | $Na^+$ channel blockade (enhanced slow inactivation) |
| **Eslicarbazepine** | 2009 | $Na^+$ channel blockade (enhanced slow inactivation) |
| **Perampanel** | 2012 | Glutamate (AMPA) inhibition |

## 1.6. Anti-Epileptic Drugs (AEDs)

### 1.6.1. Lacosamide

Lacosamide (LCM) is a third-generation anti-epileptic drug approved for treating focal epilepsy and primary generalized seizure. Lacosamide (R–2–acetamido-N-benzyl–3–methoxy propionamidle) which is a functional molecule amino acid believed to have a dual mode of action. Lacosamide assumed to be selectively enhanced the slow inactivation of voltage gated sodium channels (VGSCs) (Chunsong Yang *et al.*, 2021) and interacted with collapsin response mediator protein 2 (CRMP2). The slow inactivation curve shifting by lacosamide to more hyperpolarize potential selectivity confirmed the slow activation of (VGSCs). Furthermore, this promoted for additional membrane potentials to enter the state of slow inactivation of prolonging depolarization (Beydoun *et al.*, 2009).

Lacosamide interacts with CRMP2, however, the effect of CRMP2 did not explain the mechanism of seizures control, a single study presented that hippocampus with drug resistant TLE patients depicted that there was a reduce in CRMP2 levels compared with control patients without epilepsy (Amanda M. do Canto *et al.*, 2021). Although it was not fully understood that how CRMP2 interacting with lacosamide may be beneficial in refractory seizures patients.

#### 1.6.1.1. Pharmacokinetics

After oral administration lacosamide is absorbed rapidly and completely with negligible effect of first pass metabolism (Andreia Carona *et al* ., 2021). Approximately, 100% bioavailability, regardless to food. Intravenous (i.v.) administration of lacosamide showed bioequivalence when given by infusion over 30 or 60 min. peak serum concentration after oral administration of

lacosamide occur in 1-4 hrs. with a half-life of plasma approximately is 13 hrs. (Cawello, 2015). Inactive metabolite of lacosamide is, o-desmethyl metabolite, which has not any pharmacological activity. Nearly 30 % of the o-desmetlyl metabolite and 40% of unchanged of lacosamide are eliminated by renal excretion. Risk of drug-drug interactions of lacosamide is very low, because it has minimal protein binding (<15 %).

### 1.6.1.2. Adverse Effects

Table 1.2. (Krauss *et al*, 2010) include the main adverse effects of lacosamide.

| **Adverse effects** | **Subjects (%)** |
|---|---|
| Abnormal coordination | 5.3 to >10 |
| Ataxia | 13 |
| Blurred vision | >10 |
| Diplopia | 9–12 |
| Dizziness | 6–35 |
| Fatigue | 5.6–14 |
| Headache | 7–16 |
| Nausea | 6.8–14 |
| Nystagmus | 6 to >10 |
| Somnolence | >10 |
| Tremor | >10 |

Lacosamid has no effect on other AEDs serum levels such as carbamazepine, levetriacetam, topiramte, valproate, zonisamiale, lamotrigine, gabapentin and phenytoin (Kellinghaus, 2009). Moreover, it does not have any effect on pharmacokinetics of digoxin, oral contraceptive, metformin, and omeprazole.

#### 1.6.1.3. Contraindications/Precaution

Lacosamide has no documented contraindications, it like other AEDs may increase the risk of suicidal thoughts (Package insert, 2009), it must be withdrawn over at least 1 week to minimize the potential increase of seizure frequency.

#### 1.6.1.4. Dosage and Administration

Lacosamide dose should start with 50 mg, twice daily. Increase the dose weekly by 100mg/day, and to be given in two doses up to 200 or 400 mg as total daily dose. Patients whose creatinine clearance is less than 30 ml/min and who have significant renal impairment should take no more than 300 mg per day, according to the suggested max dosage (Harris & Murphy, 2011). Patient with mild or moderate hepatic impairment, the daily recommended dose is 300 mg. Safety and efficacy of lacosamide has not been established for under 17 years old patients. Available doses of lacosamide are 50 mg, 100 mg, 150mg and 200mg tablets. Furthermore, 200mg /20mL also available as a single dose vial.

### 1.6.2. Zonisamide

Zonisamide is a novel antiepileptic medication with a broad spectrum that has shown to be very successful in the treatment of patients with refractory partial seizures (sackellares *et al.*, 2004).

### 1.6.2.1. Mechanisms of action

1. The blocking of certain voltage-gated ion channels: which may be the mechanism by which the antiepileptic impacts on generalized tonic-clonic and partial seizures might come from the emerge of sodium gated channels. Zonisamide was shown to reduce high-frequency repeated firing in an in vitro research, and this effect was attributed to its ability to inhibit voltage-gated sodium channe. In addition to this, zonisamide is effective in blocking low-voltage-gated T-type calcium channels (Kadian and Kumar, 2023).

2. Enhancement of GABA release: Studies shown that zonisamide enhanced the GABA release. So, the elevated brain levels of GABA and increase in GABA-mediated inhibition were highly related to an anti-seizure activity of this drug. In addition, zonisamide can elevate the GABA level in extracellular via the up-regulation of the neuronal glutamate transporter (Kanner AM, *et al.,* 2018).

3. Inhibition of the neurotransmission of glutamate: Preclinical research examined the impact of zonisamide on neuro-transmission as well as the calcium levels inside cells. They demonstrated that zonisamide was able to reduce calcium-dependent and potassium-triggered extracellular glutamate emission in the hippocampus (Li *et al.,* 2020). As a consequence of this, there was a reduction in the frequency of seizures, and there is a possibility that this had a significant impact on the amelioration of epileptogenesis.

### 1.6.2.2. Zonisamide role in Scavenging of Free Radical and Neuro-protective Effects

Recent research has shown a connection between epilepsy and damage caused by free radicals (Ueno *et al.,* 2018). Antiepileptic effects of of

zonisamide may give a protection of neurons from the detrimental impact of free radicals through scavenging hydroxyl in a dose-dependent manner. This protection might well be achieved by scavenging hydroxyl. In addition, it declined the formation of lipid peroxide in rats with epileptogenic foci induced by iron (Ueno *et al.*, 2018).

Zonisamide also inhibited the excessive release of glutamate following ischemic injury that has been shown to protect against neuronal damage which induced by glutamate increasing in gerbils.

Zonisamide has another neuro-protective affect regardless to its activity as anticonvulsant which was noted by (Hayakawa *et al*., 1994), that showed a reduction in hypoxic-ischemic damage in neonatal rat's brain through using of zonisamide treatment, however, the mechanism behind this effect is still unknown.

### 1.6.2.3. Pharmacokinetics

After oral administration zonisamide absorbed completely and rapidly. 2-6 hrs. the zonisamide time to maximal concentration (T- max) after oral administration dose of (200-400 mg), food intake delayed the (T- max) to 4-6 hrs. with stable maintenance dose, usually steady state achieved within 14 days (Liparoti *et al.*, 2022).

Zonisanide has along half- life because it concentrated in RBC via carbonic anhydrase and protein binding. The elimination plasma half-life is occurring in range between 63 and 69 hrs. which showed a key advantage which enables a once daily dose, even when given zonisamide with an enzyme inducer AEDs, half-life of it remains above 24 hrs. (Abou-Khalil BW, 2022).

Zonisamide metabolized by cytochrome P450 via isoenzyme 3A4, zonisamide does not have metabolism products and also not enzyme inducer in liver (Eisai Inc, 2004).

Zonisamide may be broken down via two primary pathways:

acetylation, which results in the formation of N-acetyl zonisamide, and reduction, which results in the formation of open-ring metabolite 2-sufamoylacetyl phenol (SMAP). Unlike some AEDs, it doesn't have any active metabolites, zonisamide excreted through kidneys.

### 1.6.2.4. Drug – Drug Interactions

Serum concentration of zonisamide altered by drugs that either inhibit or induce CYP3A4. The given carbamazepine and phenytoin with zonisamide was been demonstrated to elevate plasma level of zonisamide clearance (Hakami, 2021).

## 1.7. *Gastrodia Elata*

*Gastrodia elata*, Rhizoma Gastradine, and Gastradin ,*Gastrodia elata* is a traditional chines famous herb from orchidaceae family which used for centuries from the ancient time. Dried rhizome of *Gastrodia elata (GE)*, considered the main part in medicinal uses of *Gastrodia elata* plant. The bioactive main component of Rhizome Gastrodiae is Gastrodin .

**Pic.1.1. *Gastrodia Elata* (Whole plant)**

*Gastrodia elata*, also called Tian ma, is a species of orchid that is a member of the family orchidaceae and the genus Gastrodia. Its natural habitat is mostly in eastern Asia, and more specifically in mountainous regions of China, Korea, India, and Japan (Chinese Pharmacopoeia Commission, 2015). The names Guiduyou, chijian, Mingtianma, and Duyaozhi were used to refer to *Gastrodia elata* in ancient times; however, these names are no longer often used today. *Gastrodia elata* can only be found in moist and damp environments, particularly in moist alpine regions, and it is reliant on the Armillaria mellea fungus for its nutritional requirements (Yuan Liu *et al*,. 2018).

The whole species of *Gastrodia elata* lacks the chlorophyll that gives

plants their distinctive botanical quality. It may reach heights of 30–150 centimeters, and its stems are upright, reddish red, and cylindrical in shape. Its inflorescence is a fringy raceme that may be up to 30 centimeters long and is golden in color. Its leaves have a scale-like appearance, are membranous and nervulose, and their length is around 1-2 centimeters. The rhizomes are pachyntic, oblong, and 10 centimeters long, and their diameter is approximately 3-4.5 centimeters (Chinese Academy of sciences 2004) as show in picture 1.1.

### 1.7.1. Rhizoma Gastrodiae and its Traditional and Medical Uses

It is also known as Tian ma G. elata, Gastrodiea Rhizome, Gastrodia root, and Gastrodiea tuber. Rhizoma Gastradiae is the dried rhizome component of Gastrodia elata. The dried rhizome has an oval form and measure between 6 and 10 centimeters in length and between 2 and 5 centimeters in diameter. Its surface is yellowish white, and their texture is strong and resistant to breaking (Chines pharmacopoeia - Commission, 2015; Liu *et al.,* 2018).

The use of rhizome from the Gastrodiae plant for therapeutic purposes was first documented more than 2,000 years ago. Rhizoma Gastrodiae was referred to as "a top grade medicine" in Shennong's classic of material medicine, which was the first monograph chinese herbal in history and was written during the Han Dynasty (GU and Yang, 2013).

Rhizome gastrodiae used extensively in ancient times in treatment of spasm, headache, epilepsy, dizziness, infantile convulsion, stoke, amnesia and many other disorder in China. Large number of studies and researches in recent decade have shown many pharmacological properties that result in used it in different therapeutic effects like anti–inflammatory, antioxidant, antiepileptic, anticonvulsive, neuroprotective, and antidepressant effects (Zhan *et al.,* 2015).

### 1.7.2. Gastrodin

Gastrodin is a phenolic glycoside of 4-hydroxybenzyl alcohol, it's one of the main bioactive components of GE (Zheng HF *et al.,* 2020). Gastrodin usually is obtained by either plant extraction or chemical synthesis. Traditional method to obtain gastrodin is the direct extraction from *Gastrodia elata* for the first time can isolated from ethanolic extract of rhizome gastrodiae. Many extracting methods have been developed in the following years like extraction with water methanol or ethanol with backflow, microwave – assisted (Yuan Liu *et al.*, 2018).

### 1.7.3. Gastrodin Pharmacokinetics

The gastrodin absorption is very rapidly in intestine and oral bioavailability is greatly different among various species. The intestinal absorption process may affect by Glucose transporters (GLTs) inhibitors (Cai *et al.*, 2013).

Gastrodin is rapidly and widely distributed after entering the systemic circulation mainly to lung, kidney, liver, spleen, brain and GIT in rats. However, it hardly distributed to the other part such as fat and muscle. Gastrodin elimination is quick, short half- life in human and rat plasma. It is excreted unchanged in the urine which may be due to low molecular weight less than 300 g/mol, small parts of gastrodin excreted by biliary elimination (Liu *et al.,* 2015). The pharmacokinetics of gastrodin in the brain have received a lot of attention, which is not surprising given that the majority of gastrodin's actions manifest in the central nervous system. Gastrodin is able to penetrate the BBB and enter the circulatory system, following which it is rapidly transported throughout the brain (Liu *et al.,* 2015).

### 1.7.4. Effects of Gastrodin on Epilepsy, Oxidation and Inflammation

Although there are many types of anti – epileptic drugs available, the rate of recovery from disease has not been certain improved so, there is a urgent need to discover a new medication that might be able to treat or protect against epilepsy.

Pre-treatment with gastrodin demonstrated that gastrodin has ability to increase or prolong the onset of fit (latency) of seizure and also decrease seizure severity, shorten duration of seizure, decrease rate of mortality and accelerate recovery in vivo.

The key mechanism that associated with neuronal abnormal activities is imbalance between the activities of excitatory and inhibitory neurotransmitters. GABA is the major inhibitory neurotransmitter in the brain (Liu *et al.,* 2018) and enhancement the activity of GABA was found to be very useful in epilepsy treatment.

Baek *et al.*, 1999, reported that gastrodin pre-incubation could inactivate the succinic semialdehyde dehydrogenase (SSADH) irreversibly, a GABA degradative enzyme in vitro. Later study in vivo showed that gastrodin has able to inhibit GABA transaminase (GABA-T) activities and also succinic semialdehyde reductase (SSAR) inhibition which were enzymes responsible for GABA degradation in the hippocampus of gerbil's seizure-sensitive (An *et al.,* 2003). From these results, it has been concluded that gastrodin could reverse the decreasing in level of GABA by inhibiting degradation of it in the synaptic cleft. Gastrodin was able to decrease the glutamate activity which is an important excitatory neurotransmitter in the brain (Chen and Tian, 2009). It has been shown that inflammation in the brain plays a significant role in the development of epilepsy (Vezzani *et al.,* 2011).

Regulation of the given associated etiological factors may help in treatment strategy for epilepsy. Hen *et al,* 2017 highlighted that gastrodin has the ability to inhibit the increasing in both (TNF-α) and (IL-1β) levels and reverse the

decreasing in IL-10 level in mice brain. Gastrodin also has an anti-oxidative effect which was involved in the antiepileptic and neuroprotective effects (Zhong *et al.*, 2016).

Gastrodia elata might inhibit an expression increasing of TLR4, IL-1β, IL18, downstream of NLRP3; so, the regulation of this pathway is critical for protection the microglia and inhibit the NLRP3 inflammasome (Zheng *et al.*, 2022).

*Gastrodia elata* plant in present study acts through maintaining the endogenous antioxidant GSH levels, cell survival reduced lactate dehydrogenase (LDH) leakage and increased endogenous antioxidant non-enzyme GSH level in the injury, model (Chen *et al.*, 2020).

Gastrodin pretreatment could inhibit the decrease of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and total antioxidant activity (TAOA), and also decrease the level of MDA that increasing in rat model that reported by (zhong *et al.*, 2016).

Additionally, gastrodin can also found to cause an inhibition in apoptosis of cells via increasing B-cell lymphoma 2 (Bcl-2) (gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death) decreasing and expression of the caspase -3 in the brain rat model (bian *et al.*, 2016).

# 2.1. Materials

## 2.1.1 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℃, a room humidity of 60-65 percent and the environment was maintained in 14-hour light – dark cycles provided with a certain type of commercial diet and water. The selected rats were randomly divided into 8 groups after 14 days of acclimatization. Five rats have been involved in each group of experiment. This study started from 1/10/2022 to 1/1/2023 and was conducted at the Animal House in the College of Medicine.

## 2.1.2. Instruments and Equipment:

Table 2.1. Instruments and equipment.

| No. | Instrument / Equipment | Company/ country |
|---|---|---|
| 1. | Autoclave | Memmert/ Germany |
| 2. | Centrifuge | Hettich, Germany |
| 3. | Deep freeze | GFL/Germany |
| 4. | Disposable 1cc syringe | Shanachuan/china |
| 5. | Disposable test tube (10 mL) | Meheco, China |
| 6. | Distilled water | Iraq |
| 7. | Electronic scale | SDT/ China |
| 8. | Elisa (reader, washer, printer) | Biotek/USA |
| 9. | Eppendorf plastic tubes | Gondong/ China |
| 10. | Eppendrof tubes | Sigma/ England |
| 11. | Filter paper | Citotest/ China |
| 12. | Gloves | Top glove/ Malaysia |
| 13. | High speed cold centrifuge | Hettich /Germany |

| No. | Instrument / Equipment | Company/ country |
|---|---|---|
| 14. | Homogenizer drill | Jiao Jie/ China |
| 15. | Incubator | Memmert/ Germany |
| 16. | Micro plate reader | BioTek/USA |
| 17. | Micropipettes (different volumes) | Eppendorf/ Germany |
| 18. | Normal saline | Pionner/Iraq |
| 19. | Refrigerator | Concord/ lebanon |
| 20. | Sensitive balance | Sartorius/ Germany |
| 21. | Spectrophotometer | BioTek/USA |
| 22. | Surgical set | China |
| 23. | Tube rack | China |
| 24. | Vortex | CYAN/ Belgium |
| 25. | Water bath | Memmert/ Germany |

**2.1.3. Kits:** Table 2.2. kits utilized in this investigation, along with their manufacturers and countries of origin.

| No. | Kit | Company | Country |
|---|---|---|---|
| 4 | GSH ELISA Kit | Elabscience biotechnology | United states |
| 7 | Lipid Peroxidation kit | Bilişim Destek Hizmetleri | Turkey |
| 6 | Nitric oxide kit | Bilişim Destek Hizmetleri | Turkey |
| 1 | Rat IL-1 ELISA Kit | Elabscience biotechnology | United states |
| 2 | Rat IL-6 ELISA Kit | Elabscience biotechnology | United states |
| 3 | Rat TNF-α ELISA Kit | Elabscience biotechnology | United states |

| | | | |
|---|---|---|---|
| 5 | T-AOC Colorimetric Assay Kit (ABTS, Enzyme Method) | Elabscience biotechnology | United states |

### 2.1.4. Chemicals and plant

The list of chemical and biological ingredients employed in this study are in table 2.3. below:

**Table 2.3. Chemical materials with their remarks**

| No. | Chemicals | Company | Country |
|---|---|---|---|
| 2 | Formaldehyde10% | AL-Jubail | KSA |
| 6 | Gastrodia elat blume | mayway | USA |
| 4 | Lacosamide 100mg | Union Chimique Belge ucb | Belgium |
| 1 | Pentylenetetrazol 5g | Santa Cruz | United state |
| 3 | Phosphate buffer saline 7.2 | HiMedia | India |
| 5 | Zonisamide100mg | Sun Pharmaceutical | India |

### 2.1.5. Preparation of plant and chemicals

The *Gastrodia elata* rhizome (Tian Ma) powder was dissolved in distilled water for intra gastric (by oral) administration. It's a single herbal extract, 5th March, 2015 was the date first available. Powder dosage form manufactured by Chinese medicine company**,** lacosamide100mg dissolved in 40ml N.S, zonsamide dissolved in 40ml N.S and PTZ40mg dissolved in 4 ml N.S and were administered by oral for lacosamide, zonisamide and i.p. for PTZ.


**Picture 2.1. *Gastrodia elata* rhizome (Tian Ma).**

### 2.1.6. Experimental Model of Seizure

The sub-convulsive dose of PTZ 40 mg/kg has been used dissolved in each other days for 15 days till the animals had shown complete kindling and rat had been reached to step 5 or 6 of the Racine's scale table 2.4., model had been used were rats model because the behavioral changes obviously to shown, no. of rats were 5 rats in each group it is acceptable number for study.

## 2.2. Method

### 2.2.1. Ethical approval

This study was approved by the committee of publication ethics at the College of Medicine, University of Babylon, Iraq. The study protocol and the subject information and consent form were reviewed and approved by a

local ethics committee according to the document number 4-3, at 06/07/2022 to get this approval.

### 2.2.2. Study Design and Experimental Protocols:

Animals: The selected rats were divided randomly into 8 groups with 5 rats in each group as following:

**Group 1:** Control group in this group the rats exposed to the same condition and each rats received i.p., p.o., normal saline only.

**Group 2:** Rats received i.p. PTZ 40 mg/kg each 48 hrs. for 15 days (Arooj Mohsin Alvi *et al.*, 2021).

**Group 3:** Rats received GE 883.56 mg/kg orally by a gavage for 14 days then induction of seizure by 40 mg/kg PTZ after that GR 883.56 mg/kg, for 5 days after kindling as pre-treatment (Ka Lai Yip *et al.*, 2020).

**Group 4:** Rats received GE 883 56 mg/kg and PTZ 40 mg / kg.

**Group 5:** Rats received lacosamide 50 mg/kg and PTZ 40 mg/kg (Wasterlain *et al.*, 2011).

**Group 6:** Rats received lacosamide 50 mg/kg orally.

**Group 7:** Rats received zonisamide 100 mg/kg orally (Singh G, 2021).

**Group 8:** Rats received zonisamide 100 mg/kg and PTZ 40 mg/kg.

### 2.2.3. Behavioral Evaluation by Racine's scale

In order to analyze the behavioral features, a modified version of Racine's magnitude has been utilized; seizure activity has been watched for a period of 30 minutes post-PTZ has been administered. Behavioral features including convulsion, length, and latency, severity or intensity phases have been recorded for a period of thirty minutes post-every dosage of PTZ, and the following measures from Racine's scale **(**Alvi AM, *et al*., 2021) have been observed and documented in table 2.4 below:

**Table 2.4: Modified Racine's Scale**

| 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 |

### 2.2.4. Preparation of Sample & decapitation of Rat Brain

#### 2.2.4.1. Isolation of the Brain

On the day15 after PTZ-kindling, the animals sacrificed by decapitation 24 hrs. after the last treatment. The brains were removed after dissection of a skull from foramen magnum posteriorly (Pic. 2.2.). Olfactory bulbs and cerebellum were cut and the brain was removed gently from the skull. The mid and forebrain were dissected out, washed with phosphate buffer saline solution, and weighted, kept in a sterilized Eppendorf tubes and was frozen on dry ice and then kept in deep freeze at -20 ℃.

#### 2.2.4.2. Preparation of Samples for Histopathology

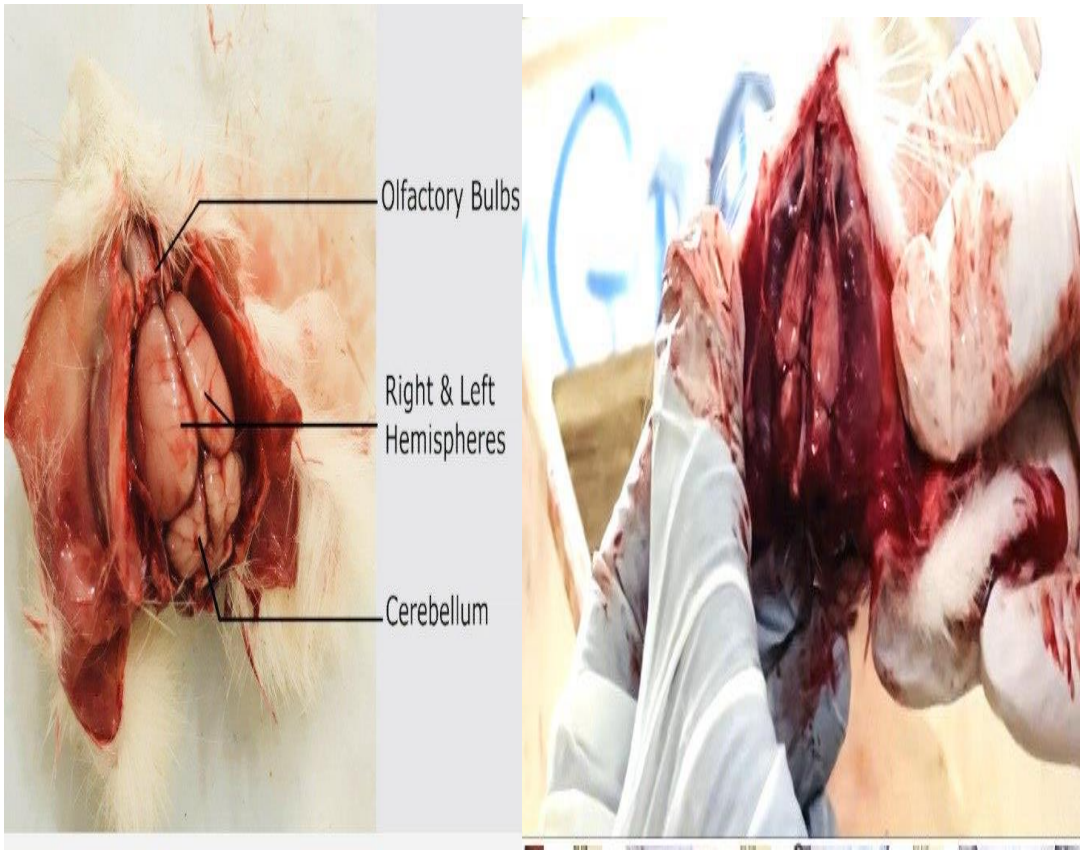
The brain coronal sections have been set in 10% formalin for at least 24-48 hrs. followed by tissue processing by automated tissue processor for 20 hrs., then dehydration with ethanol alcohol in different concentrations until absolute alcohol, after that clearing in xylene, then impregnation in wax, embedding, and formation of paraffin blocks. For histopathological analysis preparation, they have been cut down to 5 μm with the help of a microtome and stained with Hematoxylin and Eosin (H&E) stains (Liaquat *et al*, 2019).

### 2.2.4.3. Damage Scoring of brain in Histological Findings

Table 2.5. shown the damaging scores that used to determine the inflammation grade in brain (Pokela 2003).

**Table 2.5. damaging score by inflammation**

| Inflammation score | description |
|---|---|
| 0 (normal) | no morphological signs of damage |
| 1 (mild) | edema or dark neurons (pyknotic) |
| 2 (moderate) | small hemorrhages at least two |
| 3 (severe) | histological tissues sever changing ( infarcted foci clearly present) |




**Picture 2.2. Skull dissection of the rat.**

### 2.2.4.4. Preparation of Phosphate Buffer Saline (PBS) solution

According to the manufacturer directions; suspend 10.79 g in 1000 ml distilled water or purified water, sterilized by autoclaving at 115℃ for 10 minutes, keep it cool between 15-30℃ away from the direct sunlight.

### 2.2.4.5. Steps in the preparation of brain samples

1. Brain homogenization: any remaining blood had been washed away with a pre-cooling phosphate buffer saline solution (pH=7.2)
2. Piece of tissue cut by using razor blade.
3. A portion of the tissue should be weighed (0.2 gram), and then the specimen should be placed back on ice so that it does not get heated.
4. utilized a pair of scissors or a razor blade with a single edge, cut the specimen tissue into tiny pieces and place them on a sheet of ice while you work. It's indeed essential that the tissues be kept at a cool temperature.
5. Added (3 ml) of phosphate buffer (pH=7.2).
6. The mixture has been homogenized on ice by being ground with a mortar for a number of minutes, up to the point when there were no more visible pieces.
7. The material has been divided into aliquots and placed in Eppendorf tubes pre-being centrifuged in the refrigerator at a speed of 14,000 x g for 600 seconds. Collect the supernatant with extreme care.
8. Specimen kept in Freeze at -20℃ and then thaw at 2-8℃ for biochemical test.

## 2.3. Biochemical Assessments

### 2.3.1. Interleukin 1 (IL-1) ELISA Kit

**Test principle:** This kit has been based on the sandwich-ELISA design philosophy. The micro ELISA plate provided with the kit has been thus

pre-coated with an antibody which is particular to rat IL-1. This antibody has been included in the kit. In the wells of the microplate, either specimens or standards have been introduced, and then the mixture has been incubated with the particular antibody. Post-that, biotinylated detecting specific antibody for rat IL-1 and attached Avidin-Horseradish Peroxidase (HRP) has been added to every micro plate well progressively. Post-that, the mixture has been incubated, and any loose ingredients were removed by washing. every well had the substrate solution that has been put to it. The color blue has been observed in just those wells that had rat IL-1, Avidin-HRP conjugate, and biotinylated detecting antibody present in their contents. Post-inserting stop solution to bring the enzymatic reaction to an end, the color changes from green to yellow. Spectrophotometric analyzing of optical density (OD) is performed utilized a wavelength of (450±2) nm as the reference point. The magnitude of OD has been directly proportional to the amount of rat IL-1 that is present. We were able to determine the amount of rat IL-1 that has been present in the specimens by making a comparison between the standard curve and the OD of the specimens.

**Reagent preparation**

1. Pre-utilized any reagents, bring them to room temperature (between 18 and 25 degrees centigrade), and remove just the chemicals and strips that are required for the current experiment.
2. Wash Buffer: To make a volume of 750 mL of washing buffer, dilute 30 mL of concentrated bash buffer with 720 mL of D.W. or deionized water. This will bring the volume up to 750 mL. If crystals are developed in the amount, you should reheat it in a water bath at 40 degrees centigrade and blend it slowly in order to thoroughly dissolve the crystals.
3. The typical work solution had been centrifuged at a speed of 10,000 g for one minute. Benchmark standards and specimen diluent totaling 1.0

mL were both added, and then the tube has been allowed to stay for 600 seconds pre-being firmly inverted several times. When it had wholly dissolved, it has been carefully blended utilized a pipette. Effective solution of 1000 pg/mL obtained either by this reconstitute or by inserting 1 mL of specimen diluent and controlling standards, letting it stand for 1-2 minutes, and then mixing it with a vortex meter operating at a low speed. Post-that, necessary serial dilutions were prepared. The following is the suggested dilution gradient for the specimen volume: 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL. The procedure for diluting has been as follows: 7 EP tubes were used, and 500 µL of controlling standards and specimen diluent have been introduced to every tube. To create an operational solution with an amount of 500 pg/mL, transfer 500 µL of the 1000 pg/mL standard solution into the first tube, and blend it well. In accordance with this procedure, 500 µL of the solution has been transferred from the previous tube into the subsequent one utilized a pipette.

4. The operational solutions for the biotinylated detecting antibody: the needed quantity has been determined pre-the experiment (100 L per well), and the operational solutions has been prepared. During preparation, it is important to make a little bit extra than what has been estimated. biotin - conjugated antibody at 800 g for one minute, and then they diluted the 100x concentrate biotinylated detecting antibody to a 1x reaction mixture utilized dilution biotinylated detecting Ab (condensed biotinylated: diluent = 1: 99).

5. The operational solutions for HRP conjugates: Pre-beginning the experiment, I calculated the necessary quantity (100 L per well). During preparation, you should also make somewhat extra food than has been originally predicted. The 100x HRP conjugate has been diluted to 1x standard solution utilized HRP conjugate diluent, and then the

concentrate HRP conjugate has been centrifuged at 800g for one minute. The ratio of condensed HRP conjugate to HRP conjugate diluent has been 99 to 1.

**Assay procedure**

1. The liquid detected from every well don't wash 100 μL added, of biotinylated detecting antibody operational solutions immediately to every well, the plate covered with a new sealer and incubated at 37℃ for 90 minute.
2. Pour the liquid out of every well, but do not wash your hands. Quickly add one hundred microliters (L) of the operational solutions for the biotinylated detecting antibody to every well. Post-covering the plates with a fresh sealer, it has been placed in an incubator for one hour at 37 degrees centigrade.
3. When the solution has been drained from every well, 350 μL of washing buffer will be applied to every well. Socket for one minute and then decanted the liquid from every well pre-patting it dry on clean paper absorbent. This washing phase has been performed three times, and for this stage a micro plate washer can be employed. Do not wait for the wells to dry out pre-utilized the tested strip; instead, utilize them immediately post-the wash phase.
4. One hundred microliters of an HRP-conjugated operational solutions were poured through every well of the plate, and then the plate has been sealed with a fresh layer of sealer and placed in an incubator at 37 degrees centigrade for half an hour.
5. When the solution has been drained from every well, proceed with the washing procedure five more times in the same manner as described in stage 3.
6. Added 90 L of substrate and the agent to every well, encased the plates with a new sealant, followed by incubation at 37 degrees centigrade for

approximately 15 minutes, and shielded the plate from the light. Depending on the actual change in color, the response time might be extended or shortened, but it could not be longer than 30 minutes. The optical density (OD) reading has been taken post-the micro plate reader had been preheated for approximately 15 minutes.

7. Inserting 50 L of the stop solution to every well; the addition of the stop solution must follow the same protocol as the addition of the substrate solution.

8. utilized an optical density (OD) reader with a wavelength setting of 450 nm, measure the OD magnitude all at every reading well.

### 2.3.2. Interleukin 6 (IL-6) ELISA Kit

**Test principle**: This kit operated according to the Sandwich-ELISA concept. The micro ELISA plate that has been included in the kit has been already pre-coated with a specific antibody against rat IL-6. This antibody has been included in the kit. In the wells of the microplate, either specimens or standards have been introduced, and then the mixture has been incubated with the particular antibody. Post-that, biotinylated detecting specific antibody for rat IL-6 and conjugated Avidin-Horseradish Peroxidase (HRP) has been applied to every micro plate well progressively. Post-that, the mixture has been incubated, and any loose components were removed by washing. every well had the substrate solution that has been put to it. The color blue has been observed in just those wells that include rat interleukin-6, Avidin-HRP conjugate, and biotinylated detecting antibody present in their contents. Post-inserting stop solution to bring the enzyme-substrate reaction to an end, the color changes from green to yellow. Spectrophotometric analyzing of optical density (OD) is performed utilized a wavelength of (450±2) nm as the reference point. The magnitude of OD is directly proportional to the amount of rat IL-6 that is present. We were

able to determine the amount of rat IL-6 present in the specimens by analyzing the relationship between the standard curve and the optical density of the specimens.

**Reagent preparation**

1. Pre-to their usage, all of the reagents were allowed to warm up to room temp (between 18 and 25 degrees centigrade). I set up the micro plate reader by following the instructions in the booklet, then I preheated it for 15 minutes pre-measuring the OD.

2. Wash Buffer: To make a volume of 750 mL of wash buffer, dilute 30 mL of concentrated bash buffer with 720 mL of D.W. or deionized water. This will bring the volume up to 750 mL. If crystals have developed in the concentrate, you should reheat it in a water bath at 40 degrees centigrade and mix it slowly in order to thoroughly dissolve the crystals.

3. The standard operational solutions has been centrifuged at a speed of 10,000 g for one minute. Controlling standards and specimen diluent totaling 1.0 mL were both added, and then the tube has been allowed to stay for 600 seconds pre-being slowly inverted several times. When it had wholly dissolved, it has been carefully blended utilized a pipette. Operational solutions of 800 pg/mL obtained either by this reconstitution or by inserting 1 mL of specimen diluent and controlling standards, letting it rest for 1-2 minutes, and then mixing it with a vortex meter operating at a low speed. Post-that, necessary serial dilutions have been prepared. The following is the suggested dilution gradient for the specimen volume: 800, 400, 200, 100, 50, 25,12.5, and 0 pg/mL. The procedure for diluting has been as follows: 7 EP tubes were used, and 500 µL of controlling standards and specimen diluent have been introduced to every tube. To make an operational solution with an amount of 400 pg/mL, transfer 500 µL of the 800 pg/mL operational solutions into the first tube, and mix it well. In

accordance with this procedure, 500 µL of the solution has been transferred from the previous tube into the subsequent one utilized a pipette.

4. The operational solutions for the biotinylated detecting antibody: the needed quantity has been determined pre-the study (100 µL per well), and the operational solutions had been prepared. During preparation, it is important to make a little bit extra than what has been estimated. biotinylated detecting antibody at 100 g for one minute, and then diluted the 100 x concentrated biotinylated detecting Antibody to a 1 x operational solutions utilized diluent biotinylated detecting antibody (concentrated biotinylated: diluent = 1: 99).

5. HRP The working answer for conjugate: Pre-beginning the experiment, I estimated the necessary quantity (100 µL per well). During preparation, you must also make somewhat extra food than has been originally predicted. HRP Operational solutions conjugated to one utilized conjugate diluent for conjugation HRP.

**Assay procedure**:

1. The standard operational solutions has been added to the first two columns. Every amount of the solution has been put in duplicate, to one well every, side by side (100uL for every well). The specimens that were also introduced to the wells in question (100uL for every well). The plate has been then sealed utilized the sealer that has been included in the package. Incubate at 37 degrees centigrade for one hour and 600 seconds, inserting solutions to the wells bottom of the micro ELISA plate. Try to prevent contacting the inner wall of the well and creating foaming as much as you can.

2. You should not wash the liquid that has been withdrawn from every well. Immediately post-that, pour 100 µL of the operational solutions for the biotinylated detecting antibody into every well. Use the Plate sealer to

cover the plate. Incubate at 37 degrees centigrade for one hour post-giving it a gentle stir.

3. Remove the solution from every well utilized a vacuum aspirator, and then add 350 µL of wash buffer to every well. Post-soaking for one to 120 seconds, aspirate or decant the solutions from every well, and then dry the wells by patting them on clean absorbent paper. This stage of the washing process done three times

4. Poured one hundred microliters of the operational solutions for the HRP conjugate into every well. Incubate at 37 degrees centigrade for half an hour while wrapped with the plate sealer.

5. Decanted the solutions from every well, and then repeated the washing procedure for a total of five times as described in step 3.

6. Put 90 microliters of the substrate reactant into every well. The reaction time may be decreased or prolonged depending on the actual color change, but it should not exceed half an hour. The plate should be covered with a fresh plate sealer, protected from light, and incubated at 37 degrees centigrade for about 900 second.

7. Fifty microliters of Stop Solution were poured into every well. The addition of the stop solution must follow the same protocol as the addition of the substrate solution.

8. utilized a micro-plate reader with the 450 nm setting, I measured the optical density, also known as the OD magnitude, of every well all at once.

### 2.3.3. Tumor Necrosis Factor Alpha (TNF-α) ELISA Kit.

**Test principle:** This kit operated according to the Sandwich-ELISA concept. The micro ELISA plate that has been included in the kit has been already pre-coated with a specific antibody against rat TNF-α. This antibody has been included in the kit. In the wells of the microplate, either standards or specimens have been introduced, and then the mixture has

been incubated with the particular antibody. Post-that, biotinylated detecting monoclonal antibodies for rat TNF-α and coupled Avidin-Horseradish Peroxidase (HRP) has been introduced to every micro plate well progressively. Post-that, the mixture has been incubated, and any loose elements were removed by washing. every well had the substrate solution that has been put to it. The color blue has been observed in those wells that include rat interleukin-6, Avidin-HRP conjugate, and biotinylated detecting antibodies present in their contents. Post-inserting stop solution to bring the enzyme-substrate reaction to an end, the color changes from green to yellow. Spectrophotometric analyzing of optical density (OD) has been performed utilized a wavelength of (450±2) nm as the reference point. The magnitude of OD is directly proportional to the amount of rat TNF-α that is present. We were able to determine the amount of rat TNF-α present in the specimens by analyzing the relationship between the standard curve and the optical density of the specimens.

**Reagent preparation**

1. Pre-utilized any reagents, bring them to room temp (between 18 and 25 degrees centigrade), and remove just the chemicals and strips that are required for the current experiment.
2. Wash Buffer: In order to make a wash buffer with a capacity of 750 mL, 30 milliliters of concentrated bashing buffer are first diluted with 720 mL of D. W. or deionized water. If crystals have developed in the amount, you should reheat it in a water bath at 40 degrees centigrade and combine it slowly in order to thoroughly dissolve the crystals.
3. The industry-standard operating solution: The standards have been centrifuged for one minute at an acceleration of 10,000 g. Controlling standards and specimen diluent totaling 1.0 mL were both added, and then the tube has been allowed to stay for 600 seconds pre-being slowly inverted

several times. When it had completely dissolved, it has been carefully blended utilized a pipette. Operational solutions of 1000 pg/mL obtained either by this reconstitution or by inserting 1 mL of specimen diluent and controlling standards, letting it stand for (60-120) minutes, and then mixing it with a vortex meter operating at a low speed. Post-that, necessary serial dilutions had been prepared. The following is the suggested dilution gradient for the specimen volume: 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL. The procedure for diluting has been as follows: 7 EP tubes were used, and 500 µL of standard reference and specimen diluent have been introduced to every of them. To create an operational solution with an amount of 500 pg/mL, transfer 500 µL of the 1000 pg/mL standard solution into the first tube, and mix it well. In accordance with this procedure, 500 µL of the solution has been moved from the previous tube into the subsequent one utilized a pipette. reference

4. The operational solutions for the biotinylated detecting antibody: Pre-beginning the experiment, the needed volume has been determined to be 100 µL per well. During preparation, it is important to make a little bit extra than what has been estimated. Biotinylated detecting antibody at 800g for one minute, and then diluted the 100x concentrated biotinylated detecting Antibody to 1x operational solutions utilized Dilution biotinylated detecting Ab (concentrated biotinylated: diluent = 1: 99). 5. HRP conjugate operational solutions: Pre-beginning the experiment, I calculated the necessary quantity (100 µL per well). During preparation, you should also make somewhat extra food than has been originally predicted. post-the concentrated HRP conjugate has been centrifuged at 800g for one minute, the 100x HRP solution has been diluted. Conjugate to a 1 operational solutions utilized the HRP conjugate diluent (the ratio of concentrated HRP conjugate to HRP conjugate diluent should be 99 to 1).

## Assay procedure

1. The Standard operational solutions has been added to the first two columns. Every solution amount has been put in duplicate, to one well every, side by side (100µL for every well). The specimens that were also introduced to the wells in question (100µL for every well). The plate has been then sealed utilized the sealer that has been included in the package. Incubate at 37 degrees centigrade for one hour and 600 seconds, inserting solutions to the wells' bottom of the micro ELISA plate. Try to prevent the solutions from contacting the inner wall of the well and creating foaming as much as you can.

2. Pour the liquid out of every well, but do not wash your hands. Quickly add one hundred microliters (L) of the operational solutions for the biotinylated detecting antibody to every well. A fresh layer of sealer has been applied over the plate. Incubate for one hour at 37 degrees centigrade.

3. Remove the solution from every well, and then add 350 L of wash buffer to every one of the wells. Post-soaking for one minute, aspirate or decant the solution from every well, and then dry the wells by patting them on clean absorbent paper. Doing this stage of the washing process three times. Note that a microplate washer might also be utilized in other washing procedures in addition to this one. The tested strips, utilized directly post-the phase in which the wells were washed, kept the wells from becoming dry.

4. Poured one hundred microliters of the operational solutions for the HRP conjugate into every well. Incubate at 37 degrees centigrade for half an hour while covered with the plate sealer.

5. When the solution has been drained from every well, proceed with the washing procedure five more times in the same manner as described in step 3.

6. Added 90 L of substrate and the agent to every well, encased the plate with a new sealer, incubated at 37 degrees centigrade for approximately 15 minutes, and shielded the plate from the light. Depending on the actual change in color, the reaction time might be extended or shortened, but it has been unable to be longer than 30 minutes. The optical density (OD) reading has been taken post-the micro plate reader had been preheated for approximately 15 minutes.
7. Add 50 μL of the stop solution to every well; the addition of the stop solution should follow the same protocol as the addition of the substrate solution.
8. utilized an optical density (OD) reader with a wavelength setting of 450 nm, determine the OD magnitude all at once for every well.

### 2.3.4. GSH (Glutathione) ELISA Kit

**Test principle:** The Competitive-ELISA approach is used here with the ELISA kit. This kit comes with a micro ELISA plate that has already been pre-coated with glutathione (GSH). During the process, GSH that is found in the specimens or in the Standard competes with a constant quantity of GSH that is present on the solid phase support for locations on the biotinylated detecting antibody that is particular to GSH. The plate is then washed to remove any excess conjugate as well as any unbound specimen or standard. Then, Avidin that has been coupled to Horseradish Peroxidase (HRP) is added to every microplate well, and the plate is then incubated. The following stage is to add a TMB substrate solution to every individual well. The enzyme-substrate interaction has been brought to a halt by the injection of the stop solution, and the spectrophotometric analyzing of the color change is performed at (450±2) nm wavelength. Then, the optical

density (OD) of the specimens is comparison with the standard curve in order to get the value for the amount of GSH in the specimens.

**Reagent preparation**

1. Pre-utilized any reagents, bring them all to room temp (between 18 and 25 degrees centigrade), remove the strip and reagent that will be used in in the current experiment, and then store the remainder strip and reagent in the appropriate conditions.

2. Wash Buffer: Diluted 30 milliliters of concentrated wash buffer with 720 mL of distilled water deionized; or, to produce 750 mL of wash buffer, that unless crystallites had also created in the concentrate, heated up it with a water bath having a temperature of 40 degrees centigrade and mixed it slowly until the crystals have wholly dissolved.

3. The industry-standard operating solution: The standard has been centrifuged for one minute at a speed of 10,000 g. Post-inserting 1.0 mL of controlling standards and specimen diluent, the container has been allowed to stay for 600 seconds pre-being inverted slowly a few times. When it had finished dissolving wholly, it has been thoroughly combined utilized a pipette. This procedure results in an operational solution that has a amount of 100 g/mL (alternatively, add 1 mL of controlling standards and specimen diluent, let the mixture rest for 1-2 minutes, and then vigorously blend it utilized a vortex meter set to a low speed). By specimens were centrifuged the mixture at a slow enough speed, one may eliminate the bubbles that were produced by the vortex. Post-that, do any necessary serial dilutions. The following dilution gradient has been suggested by the manufacturer: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 g/mL. Technique of diluting: utilized seven EP tubes, everyone should have 500 µL of the controlling standards and specimen diluent added to it. To make an operational solution with an amount of 50 g/mL, transfer 500 µL of the 100 g/mL operational solutions into the first tube, and mix it well. According

to the instructions for this step, transfer 500 µL of the solution from the first tube to the second tube utilized a pipette.

4. The operational solutions for the biotinylated detecting antibody: the needed quantity has been determined pre-to the experiment (50 L/well), and it has been calculated. During preparation, it is important to make a little bit extra than what has been estimated. Biotinylated detecting antibody at 800 g for one minute, and then diluted the 100x concentrated biotinylated detecting Antibody to a 1x operational solutions utilized Diluent biotinylated detecting Antibody (concentrated biotinylated: diluent = 1: 99).

5. The operational solutions for HRP conjugates: Pre-beginning the experiment, I calculated the necessary quantity (100 L per well). During preparation, you should also make somewhat extra food than has been originally predicted. The 100x HRP conjugate has been diluted to 1x operational solutions utilized HRP conjugate diluent, and then the concentrated HRP conjugate has been centrifuged at 800g for one minute. The ratio of concentrated HRP conjugate to HRP conjugate diluent has been 99:1.

**Assay procedure**

1. the number of wells used for the diluted standard, the blank, and the specimens were estimated. Place fifty microliters of every dilution of the standard, the blank, and the specimen in the respective wells. Soon post-that, fifty microliters (L) of biotinylated detecting Ab operational solution has been added to every well. The sealer that has been included in the package should be used to cover the plate. Incubate for 45 min at 37 degrees centigrade. The solutions must be put to the well's bottom in the micro ELISA plate. It is essential that they prevent contacting the inner

wall of the well, and foaming should also be prevented to the greatest extent feasible.

2. Remove the solution from every well, and then add 350 L of wash buffer to every one of the wells. Post-soaking for one minute, aspirate or decant the solution from every well, and then dry the wells by patting them on clean absorbent paper. This stage of the washing process was doing three times.

3. Put one hundred microliters of HRP conjugate operational solutions through every well, resealed the plate, and placed it in an incubator at 37 degrees centigrade for half an hour.

4. Post-removing the solution from every well, continue the washing procedure five times in the same manner as described in step 2.

5. Added 90 L of the substrate reagent to every well, sealed the plate with a fresh sealer, and then placed it in an incubator at 37 °C for about 15 minutes. As long as the plate is shielded from light, the response time may be decreased or prolonged in accordance with the real color change, but it cannot exceed thirty minutes. Pre-measuring the OD, preheating the microplate reader for around 15 minutes is required.

6. Fifty microliters of the Stop Solution were poured into every well. The addition of the stop solution is to be performed in the same sequence as the addition of the substrate solution.

7. utilized a micro-plate reader with the 450 nm wavelength setting, measure the optical density (OD magnitude) of every well all at once.

### 2.3.5. Lipid Peroxidation kit

**Principle** Thiobarbituric acid reactive compounds were used to conduct an analyzing of lipid peroxidation (TBARS). The TBARS test provides a simple technique for assessing lipid peroxidation in serum that is standardized, repeatable, and easy to use. The malondialdehyde-

thiobarbituric acid (MDA-TBA) adduct can be evaluated either calorimetrically at 530-540 nm or fluorometrically with a wavelength range of 515 nm and an emission wavelength of 555 nm. This adduct has been designed by the reaction of MDA and 1,3-Diethyl-2-thiobarbituric acids (DETBA) under high temperature (90-100°C) When evaluated utilized fluorometry, the sensitivity of this response is significantly increased.

**Reagents preparation**

The working solution was prepared by dissolved 0.514 g of thiobarbituric acid (TBA) in D.W then added 25g of trichloroacetic acid (TCA), thereafter added 0.5 mL of HCL, subsequently complete the volume to 190 ml by using D.W then added 0.5 g of sodium dodecyl sulfate (SDS) then completed the volume to 200 ml and mixed well. Procedure shown in the table 2.6.

**Table 2.6. Show the details of the present method.**

| **Reagents** | **Test** | **Blank** |
|---|---|---|
| Serum | 100 µl | ---- |
| Distilled water | ---- | 100 µl |
| Working solution | 2 ml | 2ml |
| The sample was vortexed and heated in a 90º C water bath for 50 min, and then allowed to cool. | | |
| The sample has been centrifuged for 5 mins at a speed of 5000 rpm and the absorbance of the supernatant has been measured utilized spectrophotometry at a wavelength of 532 nanometers vs a reagent blank. The process for preparing the reagent blank has been the same as the one described previously, with the exception that the specimen has been replaced with DW. | | |

$$serumMDA = \frac{Absorbance}{d\ X\ \zeta}\ X\ D.F$$

d = 1cm, ε =extinction coefficient = 1.56 x $10^5$ $M^{-1}cm^{-1}$

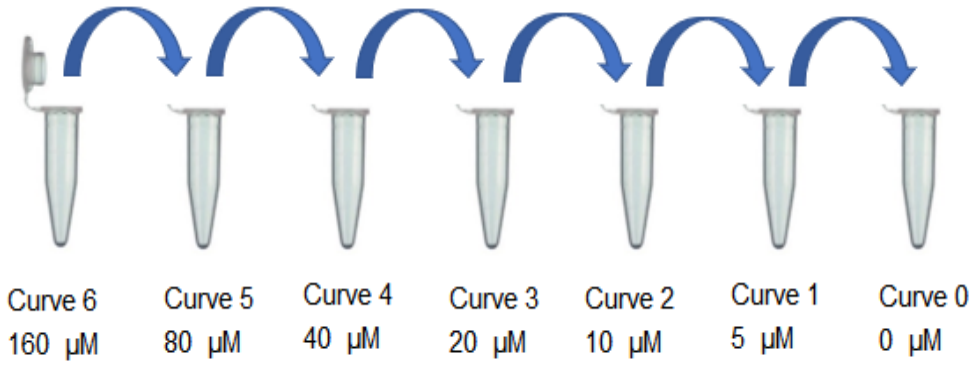
D.F = dilution factor

### 2.3.6. Nitric oxide kit

Griess reagent (Preparation in the dark) 0,1g N-(1-Naphthyl) Ethyl-Enediamine,1g sulfanilamide, 90ml, deionized water, 2,5ml orthophosphoric acid, store at 2ºC and 8ºC.

**Prepare standard sodium nitrite solution for standard curve:**

Dissolved 0,06899g NaNO2 (10mM Sodium Nitrite / 10,000µM) in 100 mL distilled water and place in dark flask at room temperature. Perform the 96-well plate assay or spectrophotometer. Put the reagents at room temperature. Prepare the standard curve by the serial dilution form. Use 7 micro tubes identified with the different concentrations prepared from the initial nitrite concentration,acidic one no primer microtubule, 8 µL nitrite (10mM/10.000µM) + 492 µL PBS.



Transferred 250 µL from the initial tube to the second and last tube, which must contain 250 µL for serial dilution, since the final volume will be 250 (50 µL for each quadruplicate well). Used the last well to place the blank (supplemented culture medium only). Added 50 µL of sample then added 50µL of Griess reagent to all wells containing samples and waiting for 10 minute (Yucel *et al.*, 2012).

### 2.3.7. Total Antioxidant Capacity (TAOC) Colorimetric Assay Kit (ABTS, Enzyme Method)

**Principle**: This is an explanation of the basic idea behind the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) technique, which is used to determine the TAOC. If a suitable oxidant has been used, ABTS will be converted to green ABTS. This transformation may be stopped if antioxidants are present. If the absorbance of ABTS is measured at either 414 or 734 nanometers, the TAOC of the specimen may be identified and computed from those readings. Trolox is a derivative of VE and has many of the same properties, including its ability to act as an antioxidant. Trolox is frequently utilized as a standard for comparing the effectiveness of other antioxidants.

**Reagent preparation:** Preparation of reagent 3 application solution:

Diluted reagent 3 with double distilled water at a proportion of 1:39.

Prepared the new solutions pre-used.

Production of the ABTS Substrate Solution First, determine how much ABTS operational solutions you will require and subsequently produce it in accordance with the proportion (reagent 1: reagent 2: reagent 3 application solution = 152:10:8). The prepared solution has been kept at room temperature with some mild shade, and it has been used up post-thirty minutes. The following steps are required to prepare the reagent 4

application solution: Pre-using, the amount of reagent 4 should be diluted with reagent 1 in the ratio of 1:9. Prior to its application, the fresh solution had been manufactured.

**The measurement of samples**

Standard well: To every of the wells, 10 µL of test solution with a various amount has been added. Specimen well: Put ten microliters of the specimen into every of the wells. Stage 1 has been completed by inserting 20 µL of the reagent 4 applications solution to every well. Stage 2 required the addition of 170 µL of the ABTS reaction mixture to every well. Wholly combine the ingredients and let them stand at room temp for six minutes. utilized a microplate reader, determine the optical density of every well at a wavelength of 414 nm.

## 2.3.8. Statistical analysis

In every trials experiment, the number of technical replicates used has been three and used five biological replicate (that is, n=5 for each group). The results were presented utilized the mean together with the standard deviation (SD). The statistical analyses were carried performed utilizing SPSS version 1.0.0.1406 utilizing either a one way or a two - way ANOVA (with a Dennett's adjustment for multiple comparison test). Differences were considered significant where the $p \leq 0.05$.

# Chapter Three

# Results

## 3.1. Behavioral tests result within the group

**3.1.1. Latency:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE, oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on latency (min) in rat's brain. Results (n=5) are expressed as the mean (± SD). Latency (mins) has been obviously significantly increased in 3,4,5, and 8 groups in comparison to PTZ group.

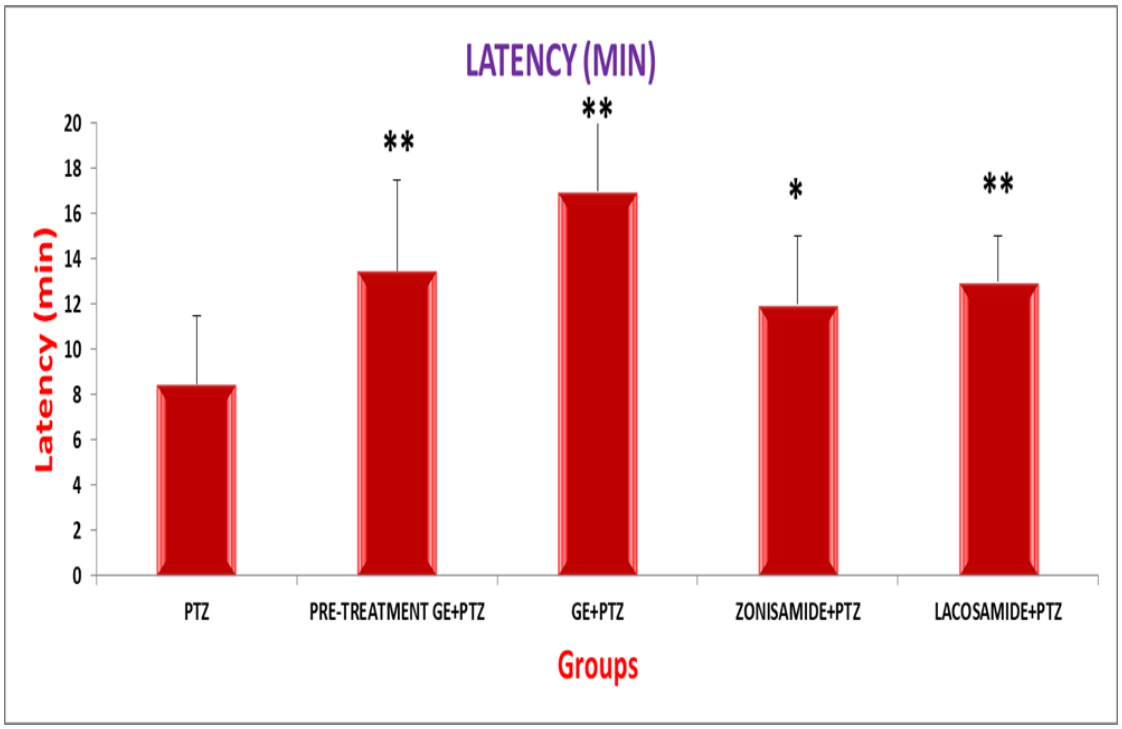



**Figure 3.1.** Effect of PTZ, pre-treatment GE + PTZ, GE+ PTZ, lacosamide + PTZ, and zonisamide + PTZ on latency (min) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.1.2. Duration:** Effects of PTZ, i.p., every other day pre-treatment with GE, p.o., for 14 days prior to kindling with PTZ, after that 5 days GE, oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on duration (min) in rat's brain. Results (n=5) are expressed as the mean (± SD). Duration of fits was highly significantly declined in groups 3,4,5, and 8 as compared to PTZ group.

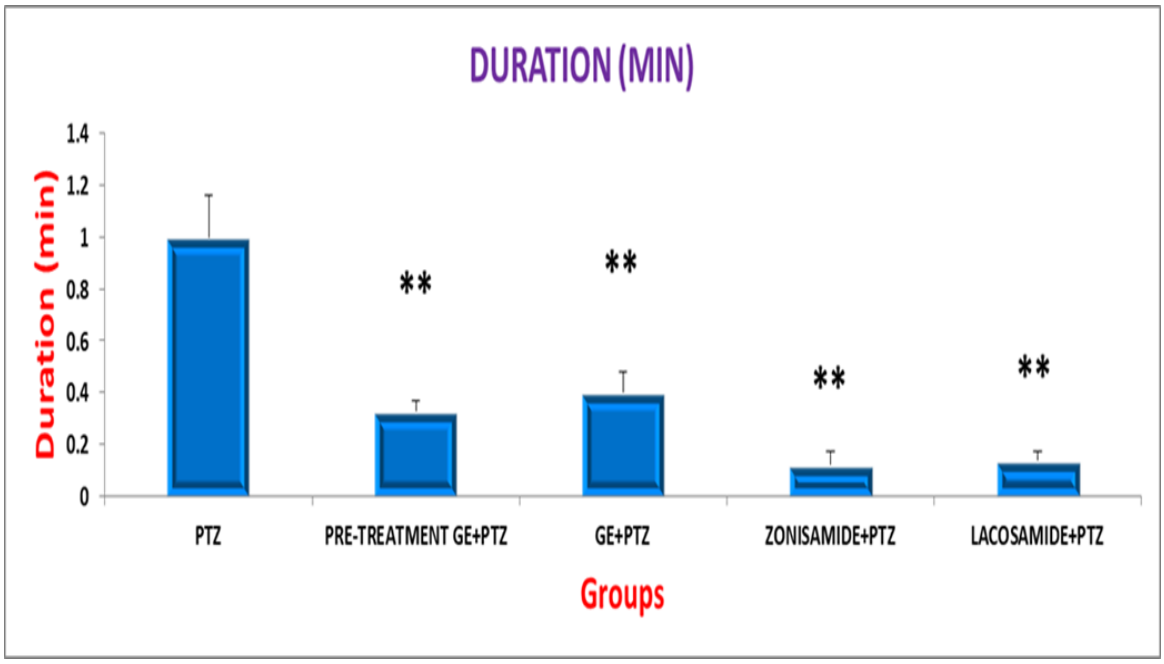



**Figure 3.2.** Effect of PTZ, pre-treatment GE + PTZ, GE + PTZ, lacosamide + PTZ, and zonisamide + PTZ on duration (min) in rat's brain after every other day for PTZ group, and 15 days' exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.1.3. Severity:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days prior to PTZ-kindling, then GE administered for 5 days, oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on severity (fits / min) in rat's brain. Results (n=5) are expressed as the mean (± SD). Number of fits / min. was found to be highly significantly lowered in 3,4,5, and 8 groups compared to PTZ group.

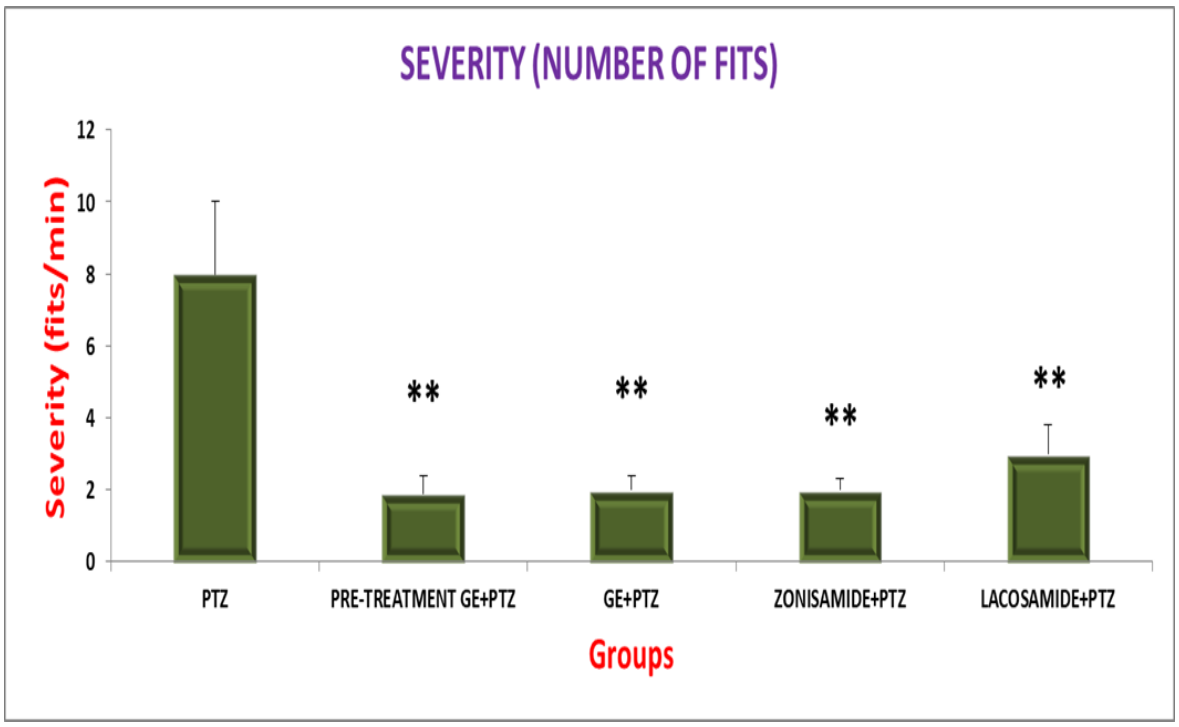



**Figure 3.3.** Effect of PTZ, pre-treatment GE + PTZ, GE + PTZ, lacosamide + PTZ, and zonisamide + PTZ on severity (fits / min) in rat's brain after every other day for PTZ group, and 15 days' exposure for other groups. *(*p<0.05), (**p<0.001).*

## 3.2. Biochemical tests results

### 3.2.1. Oxidative stress biomarker:

**3.2.1.1. GSH biomarker:** Effects of PTZ, i.p, every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE. Oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on GSH level (µg/ml) in rat's brain. Results (n=5) are expressed as the mean (± SD). This figure shows a significant decline in GSH level in PTZ group in comparison to control. However, in groups 3 to 8, GSH level surged significantly compared to PTZ group.

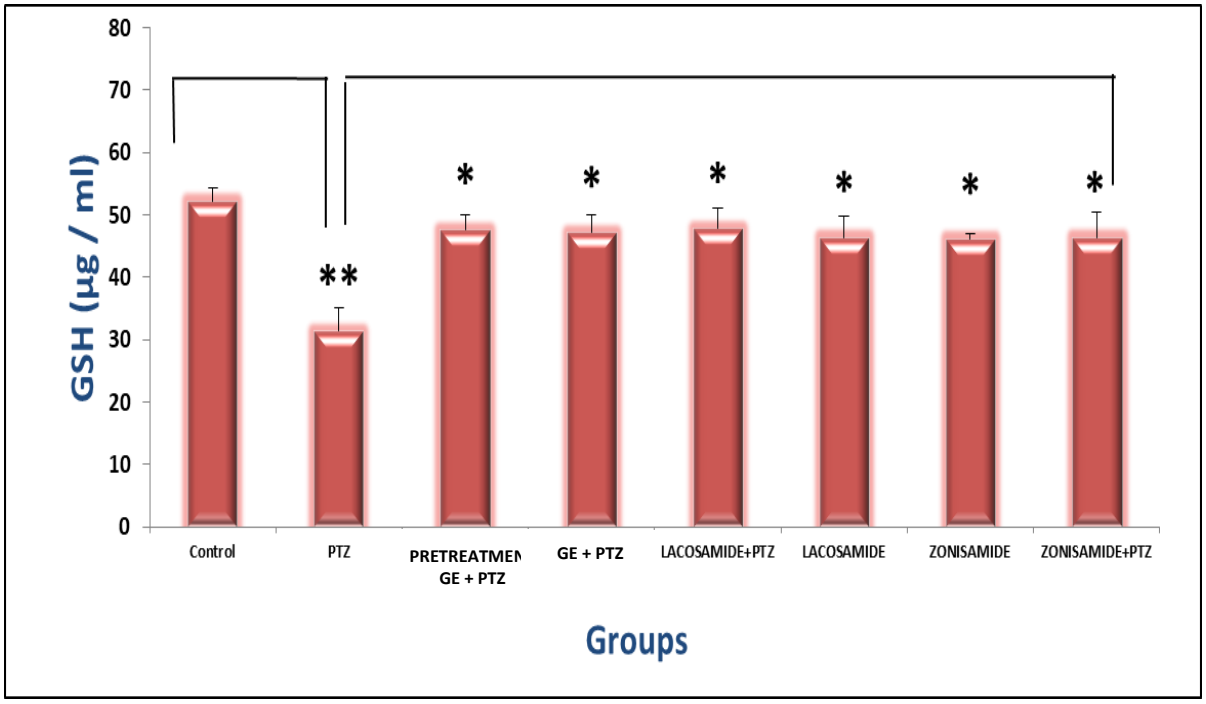



**Figure 3.4.** Effect of PTZ, pre-treatment GE + PTZ, GE + PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on GSH level (µg/ml) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.2.1.2. MDA biomarker:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE. Oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on MDA level (µmol/L) in rat's brain. Results (n=5) are expressed as the mean (± SD). This figure shows a significant increase in MDA level in PTZ group in comparison to control. However, in groups 3 to 8, MDA level decreased highly significantly compared to PTZ group.

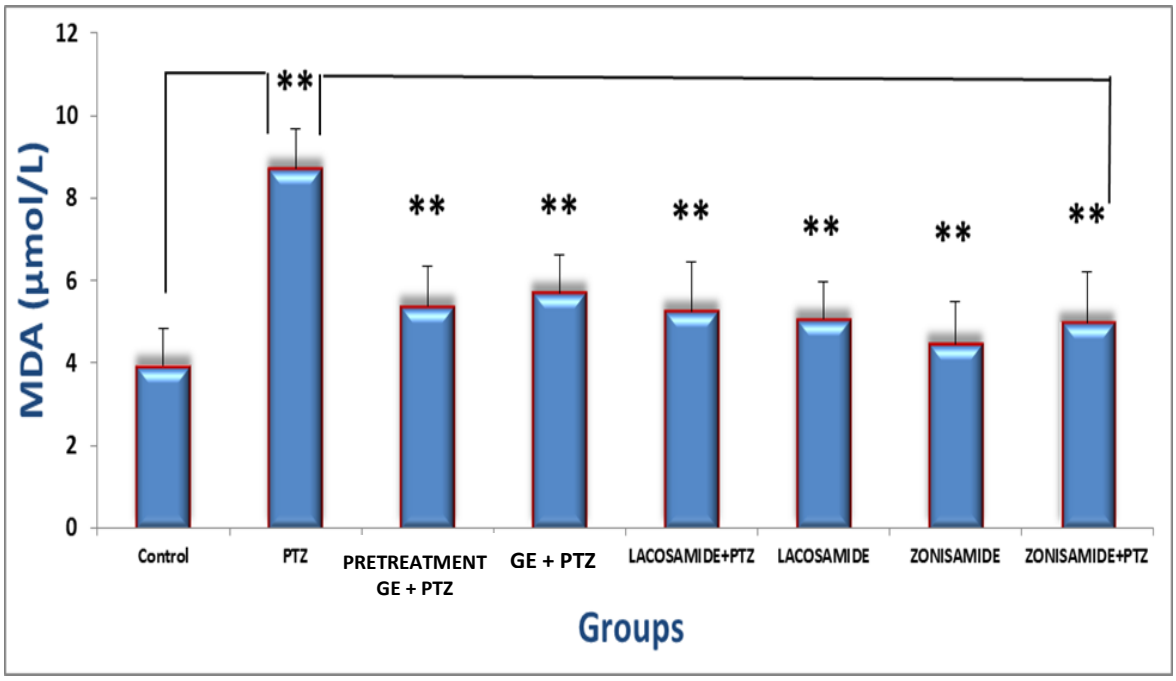



**Figure 3.5.** Effect of PTZ, pre-treatment GE + PTZ, GE + PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on MDA level (µmol/L) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.2.1.3. NO biomarker:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE. Oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on NO level (µmol/L) in rat's brain. Results (n=5) are expressed as the mean (± SD). It has been shown that PTZ increased NO level significantly compared to control. Moreover, in groups 3 to 8, a significant alleviation of NO was detected compared to PTZ group.

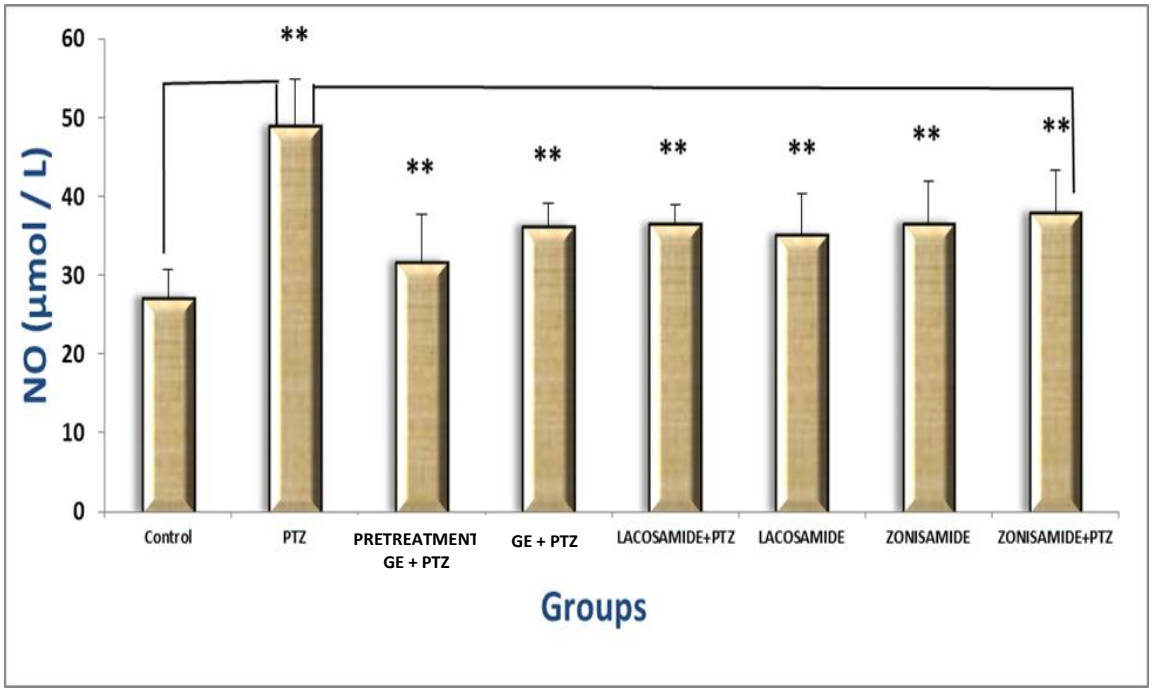



**Figure 3.6.** Effect of PTZ, pre-treatment GE + PTZ, GE+ PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on NO level (µmol/L) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.2.1.4. TAOC biomarker:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE. Oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on TAOC level (µmol/g) in rat's brain. Results (n=5) are expressed as the mean (± SD). There was a significant decrease in TAOC in PTZ group compared to control, however, a high significant increase in groups 3 to 8 in comparison to PTZ group.

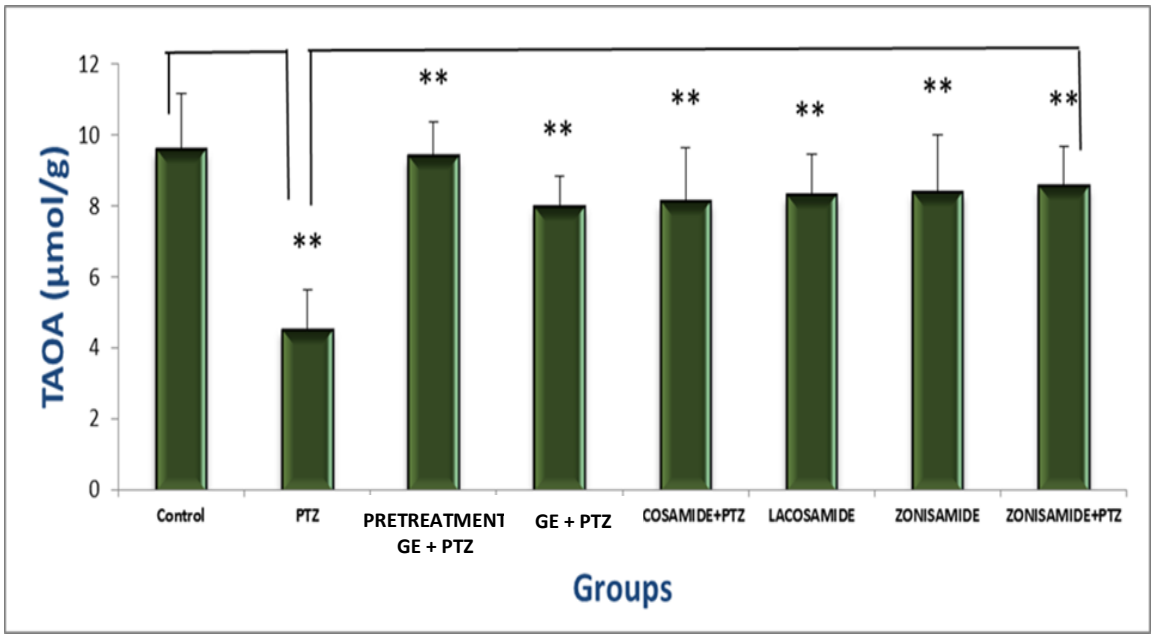



**Figure 3.7.** Effect of PTZ, pre-treatment GE + PTZ, GE + PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on TAOA level (µmol/g) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

## 3.2.2. Inflammation biomarkers:

**3.2.2.1.TNF-α biomarker:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE, Oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on TNF-α level (µg/L) in rat's brain. Results (n=5) are expressed as the mean (± SD). This figure shows a significant increase in TNF-α level in PTZ group in comparison to control. However, in groups 3 to 8, TNF-α level decreased highly significantly as compared with PTZ group.

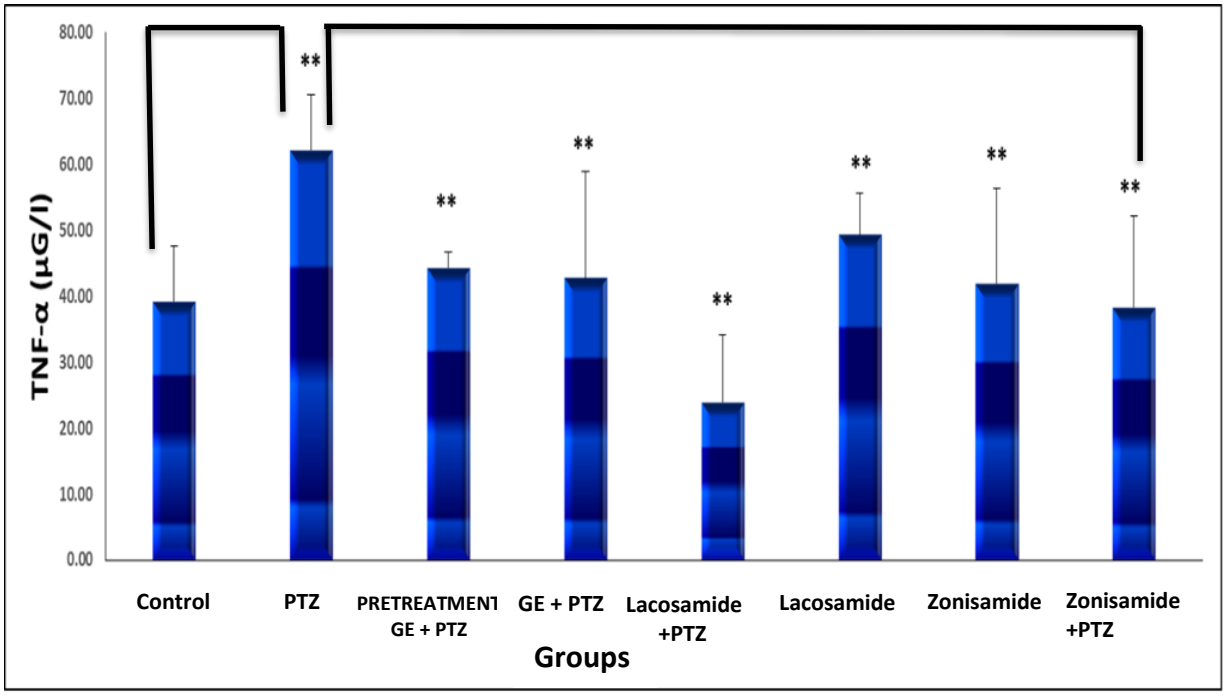



**Figure 3.8.** Effect of PTZ, pre-treatment GE + PTZ, GE+ PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on TNF-α level (µg/L) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.2.2.2. IL-6 biomarker:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days then, induction with PTZ, after that 5 days GE. Oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on IL-6 level (µg/L) in rat's brain. Results (n=5) are expressed as the mean (± SD). IL6 was significantly increased in PTZ group compared to control. However, it was highly significantly decreased in groups 3 to 8 as compared to PTZ group.

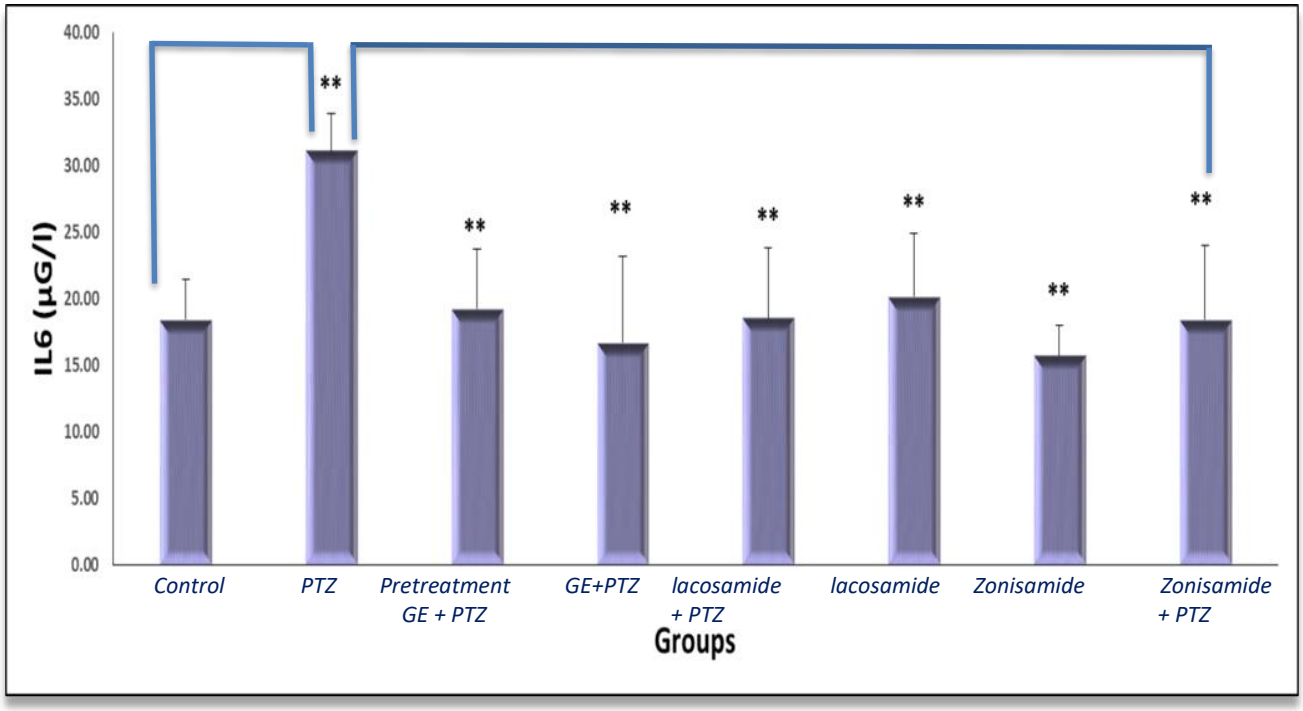



**Figure 3.9.** Effect of PTZ, pre-treatment GE + PTZ, GE+ PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on IL-6 level (µg/L) in rat's brain after every other day for PTZ group, and 15 days' exposure for other groups. *(*p<0.05), (**p<0.001).*

**3.2.2.3. IL-1 biomarker:** Effects of PTZ, i.p., every other day, pre-treatment with GE, p.o., for 14 days prior to kindling with PTZ, after that 5 days GE, oral administration of lacosamide, zonisamide, and GE for 15 days with kindling every other day on IL-1 level (µg/L) in rat's brain. Results (n=5) are expressed as the mean (± SD). This figure shows a significant increase in IL-1 level in PTZ group in comparison to control. However, in groups 3 to 8, IL-1 level decreased highly significantly compared to PTZ group.

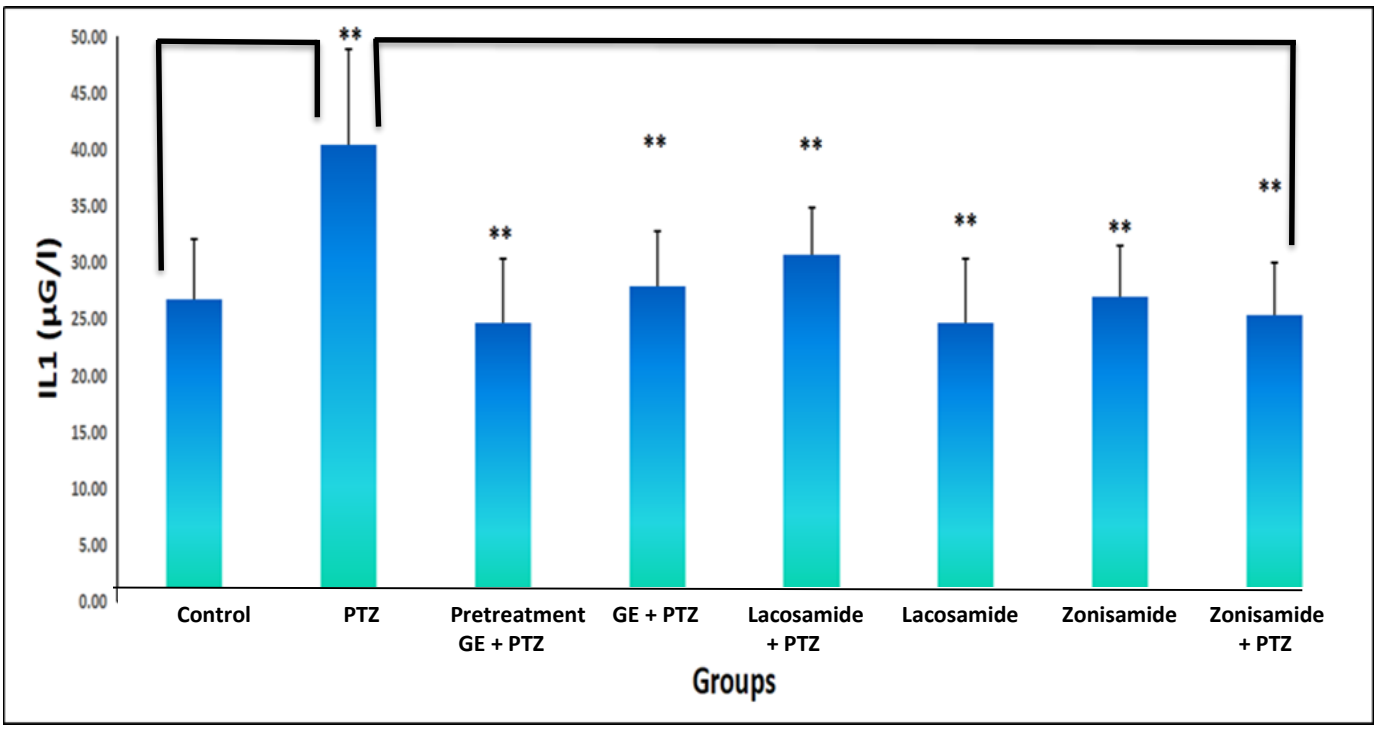
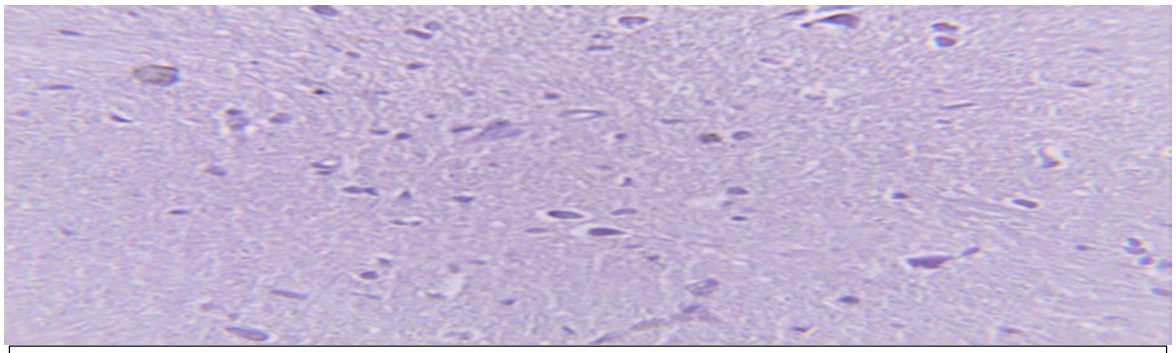


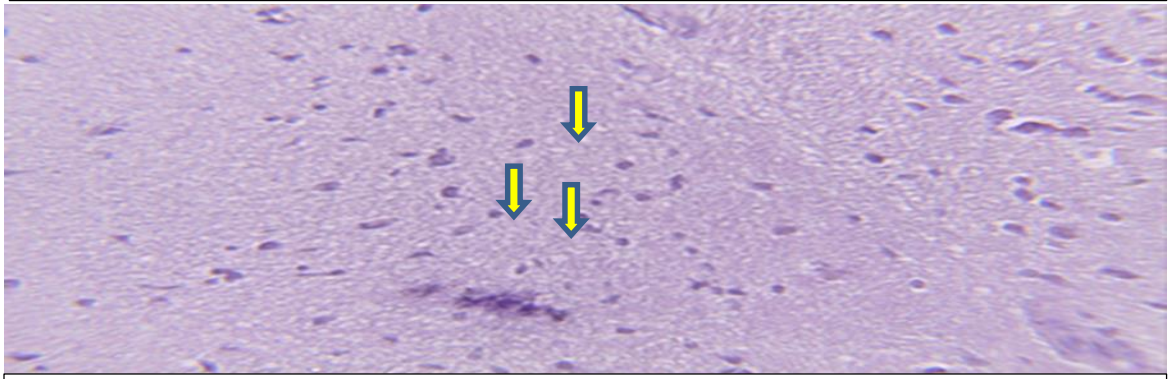

**Figure 3.10.** Effect of PTZ, pre-treatment GE + PTZ, GE + PTZ, lacosamide + PTZ, lacosamide, zonisamide, and zonisamide + PTZ on IL-1 level (µg/L) in rat's brain after every other day for PTZ group, and 15 days exposure for other groups. *(*p<0.05), (**p<0.001).*

## 3.3. Histological results

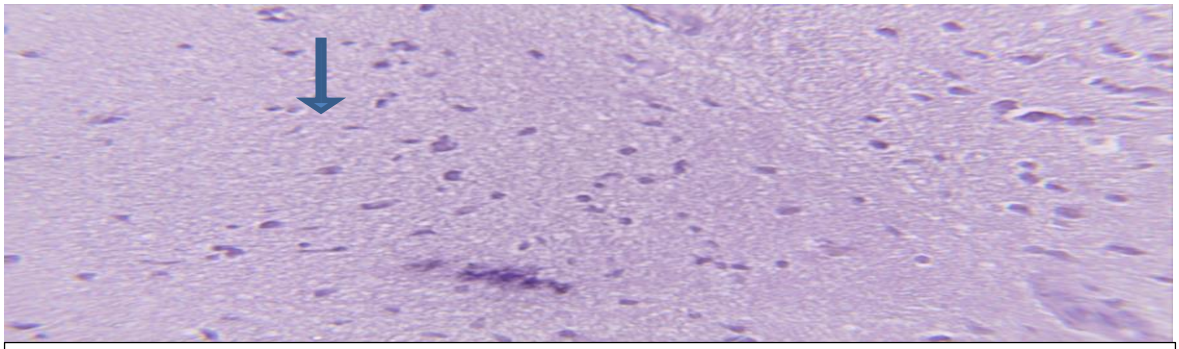
A cross section of rats' brains in control group showed a normal brain appearance. So, it scored 0 scores. Group 2 (PTZ-kindling group) shown frequent acute inflammatory cells infiltrate and histological tissue changes, PTZ group scored 2 scores. However, the other groups (3,4,5,8) presented with mild changes in brain tissues, eosinophil, edema, and pyknotic (dark neurons) so, this groups scored 1scores as explain in table 2.5. Following photomicrograph describe tissue changes in each group.



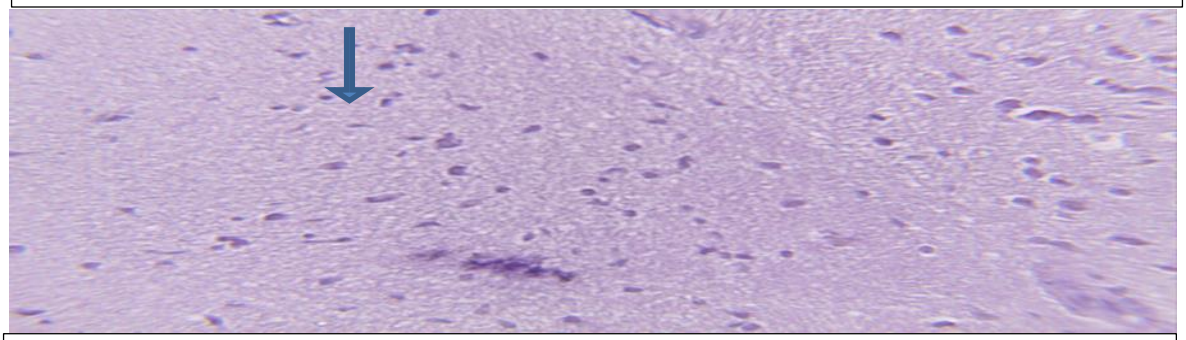
**Pic. 3.1.** Rat brain, normal histology shows astrocytes with capillary blood vessels (H&E ×400) (control group 1). Score (0).



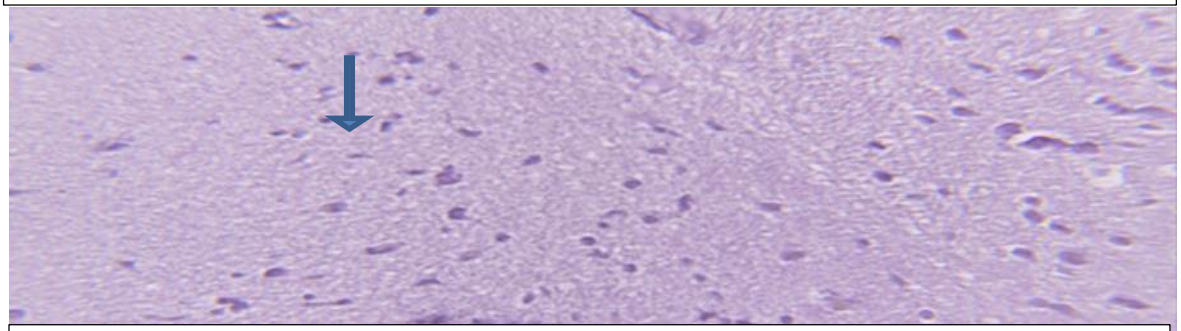
**Pic. 3.2.** Rat brain, show moderate inflammation with frequent acute inflammatory cells infiltrate arrow (H&E× 400) (PTZ group 2). Score (2)



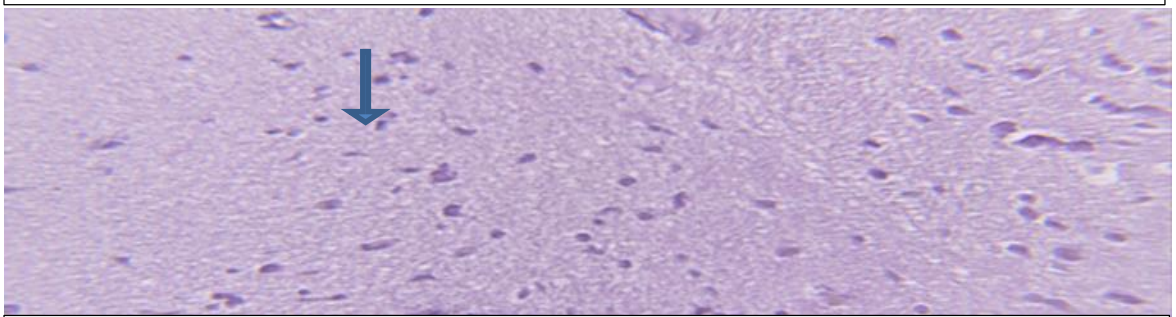
**Pic. 3.3.** Rat brain, mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E ×400) (pretreatment GE+PTZ group3). Score 1



**Pic 3.4.** Rat brain, mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E ×400) (GE+PTZ group 4).Score 1



**Pic. 3.5.** Rat brain, mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E ×400) (LCM+PTZ group 5). Score 1



**Pic. 3.6.** Rat brain, normal to mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E ×400) (ZNS+PTZ group 8). Score 1

## 4.1. Effect of Gastrodia elata, Zonisamide, and Lacosamide on Behavioral evaluation.

When compared to the rats in the control group (group 1) as seen in figure 3.8, the rats that were given PTZ-Kindling exhibited significant generalized tonic-clonic seizures. These seizures have been represented obviously by a greater average seizure intensity score that corresponded to stage 5 or 6 in the modifying Racine scale (Table 2.4) compared to control (group1) fig (3.8).

Group of PTZ-treated, resulted in a highly significantly little time in the latency period as opposed to control group; this result was in agreement with previous studies which indicated an immediate onset of seizures after PTZ administration successfully. (Alvi *et al.*, 2021).

Duration and severity of seizures with PTZ- kindling (group2) were increased significantly comparing to control (group1) fig. (3.9), (3.10). It has been highlighted that the PTZ- kindling model of seizure was by antagonizing the gamma aminobutyric acid (GABA) – A receptor (Shimada & Yamagata, 2018), PTZ suppressed the of inhibitory synapses function and subsequently caused an increased in neuronal activity, this antagonism leads to generalized seizures in animals (Shimada & Yamagata, 2018).

Upon treatment with GE in both pretreatment and treatment (group 3 and 4) respectively, it has been found that the rats exhibited significant prolongation in latency period of seizures initiation compared to PTZ (group2) and also improvement in mean seizures severity and shorter duration of seizures.

Current results were in agreement with other studies revealed that GE treatment prevented a large proportion of rats from developing seizures, delayed the onset time, reversed the decrease of GABA level in the synaptic cleft via inhibiting its

degradation (Yuan Liu *et al.*, 2018), as well as, decreased the glutamate (Glu) activity (chen and Tian 2009) who found that GE could reduce the number Glu immune- histo-chemically positive cells in PTZ-induced rat model.

So, in current study, the findings implied a potential role of GE in preventing the possible subsequent seizure frequency. Furthermore, previous studies that demonstrated the significant effect of GE as antiepileptic which was by down regulation of overexpression of para-mammalian target of rapamycin (P-mTOR) level. This down regulation was found to reduce the severity of epilepsy. GE may up regulate the under expression of SEMA3F (semaphorin-3F) levels, So the regulation of P-mTOR and SEMA3F may accompanied by GE antiepileptic effect (Yip *et al.*, 2020).

In group 5, LCM+ PTZ, the result of present study found that the latent onset of PTZ induced seizures increase and significant decrease in duration of seizures and severity compared to PTZ (group2). Lacosamide is an AED approved to use as anticonvulsant agent that prevents the seizures recurrence. This result is in agreement with many previous results that approved the effect of LCM on suppression of epileptiform activity, limiting the fits numbers of and decreasing their long -term consequences when administered early (Wasterlain *et al.*, 2011).

Lacosamid selectively inhanced the delayed inactivation of voltage- gated sodium channel slow inactivation without changing any of the other features of sodium channel particularly their recovery rate (Errington *et al.*, 2008).

Zonisamide + PTZ (group8) in present study founded that ZNS caused prolongation in latency period and a decrease in duration and severity comparing to (group2) PTZ-kindling rats.

This result went with many previous studies that presented the role of ZNS drug

in treatment of epilepsy as a first line for refractory epilepsy because it has many mechanism of action to act as AED like, binding to GABA-benzodiazepine receptor complex and lead to facilitate the neurotransmission of both serotonergic and dopaminergic, blocking voltage dependent Na+ channels, lowering voltage dependent T-type inward Ca+2 currents, reducing Ca+ dependent K+ triggered the release of glutamate intracellularly (Sandeep Kumar & Govind Singh, 2021).

## 4.2. Effect of Gastrodia Elata, Zonisamide, and Lacosamide on Oxidative Stress

### 4.2.1. Effects on GSH biomarker

There seems to be an ever-growing amount of data to support the hypothesis that oxidative stress may have neurotoxic effects (Sarasvati *et al*., 2009). This study has shown that 40 mg / kg, PTZ, i.p., every other day for 15 days produced oxidative stress by significantly decreasing the GSH level in comparison to control group.

However, pre-treatment with *Gastrodia elata*, 883.56 mg / kg, p.o, for 14 days prior to kindling, oral administration with zonisamide (100 mg / kg), lacosamide (50 mg / kg) and *Gastrodia elata* for 15 days with kindling were found to increase GSH level significantly in comparison to PTZ group (group 2).

Kindling is employed in research as a model for epilepsy, and PTZ has been shown to be effective as a convulsing drug in a variety of different experiments. Mason and Cooper were the first researchers to report the kindling that was caused by PTZ in rats (1972). After receiving many doses of PTZ, those who experience this phenomenon have a higher risk of developing epileptic seizures. The drug PTZ acts as an antagonist for the gamma aminobutyric acids (GABA-A) receptor, PTZ has ability to suppresses the normal functioning of inhibitory synapses leading to increased neuronal activity.

Glutathion is a tripeptide, γ-l-glutamyl-l-cysteinylglycine it considers a key

antioxidant in all mammalian tissues at 1-10mM concentrations (Shelly C. Lu., 2013). GSH has a unique structure in that the peptide bond linking cysteine and glutamate of GSH through glutamate γ-carboxyl group rather than the conventional α-carboxyl group.

Gamma – glutamyl transpeptidase GGT is the only enzyme that able to hydrolyze this unusual bond and it presented only on the external surface of certain types of cells (Shelly C. Lu., 2013). The findings of current study demonstrated that the level of GSH decrease with PTZ- Kindling (group2) comparing with control group (group1) as in fig. (3.1). PTZ-kindling was find to cause neuronal cell death due to increased activity of glutamenergic transmitters that generate the free radicals so this mechanism might have played a crucial role (Batool Rahmati *et al.*, 2013).

So, PTZ-kindling increase glutamate release, NMDA receptor activation lead to increased oxidative stress. *Gastrodia elata* was found to up regulate the innate antioxidants GSH level and inhibit the decreased in GSH level (Zhong *et al.*, 2016). *Gastrodia elata* plant in present study acts through maintaining the endogenous antioxidant GSH levels, cell survival reduced lactate dehydrogenase (LDH) leakage and increased endogenous antioxidant non-enzyme GSH level in the injury, model (Chen *et al.*, 2020) as showed in fig. (3.1).

Lacosamide (LCM), a new AED used for the treatment of seizures and it was found to have antioxidant effect when comparing the net results of PTZ + LCM (group 5) with kindling(group2) fig (3.1). With previous results from other studies, there was a great agreement because it was presented that LCM decreased the free radical's formation after seizure induced by pilocarpine and increased GSH levels (Nirwan, *et al.*, 2018).

High dose of LCM, 30mg /kg, managed to increase the activity of antioxidant

enzymes and could protect proteins from oxidation and also reduce the undesired functional and structural changes in some key enzymes (Nirwan *et al.*,2018). The antioxidant effect of LCM in rat's model was implicated to be an important mechanism underlying the AED ability to mitigate seizure. Additionally, GSH is a non-enzymatic tripeptide antioxidant which plays a crucial role in the CNS defense from overproduction of ROS in both intra-and extracellular medium (Valdovinos-Flores & Gonsebatt, 2012).

Zonisamide (ZNS) drug was found to have antioxidant effect and might be able to increase the level of antioxidant GSH in astroglial, C6 cell (Glial cell line in rat brain) but not in dopaminergic neurons (Kyoko Mashima *et al*., 2018). ZNS increased GSH markedly 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 neuroprotection against oxidative stress (Asanuma *et al.,* 2010). In the current study, ZNS+PTZ (group 8) was found to increase GSH level comparing with PTZ (group 2) as shown in fig. (3.1).

### 4.2.2. Effects on MDA biomarker

Free radicals were found to generate the process of lipid peroxidation in an organism. MDA is one of the final productions polyunsaturated fatty acids peroxidation. An increase in free radicals caused overproduction of lipid peroxidation byproducts (Stefan Gawel *et al.*, 2004).

PTZ- Kindling model in rats was found to increase the MDA level and raise lipid peroxidation combined with a decrease in antioxidants activity compared with control group this results also had been shown in many others previous recerches implying the oxidative stress impact in the pathophysiology of epilepsy (Kilinc, 2021).

Because of high lipid content and brain's limited antioxidant capacity, it is found to be more susceptible to oxidative damage (Arooj Mohsin Alvi *et al.,* 2021). In this current study, PTZ induce fit (group2) was found to elevate level of MDA as compared with control group(group1), fig. (3.2) that was in line with previous studies (Abdelaziz M. Hussein *et al.,* 2019).

Current study, highlighted that PTZ-kindling model lead to a significant surge in oxidative insult state in the tissues of brain, as evidenced by significant increase in concentration of MDA which reflected the increase in ROS in the pathophysiology of PTZ - induced epilepsy. PTZ-binding has been noticed to enhance free radical's production include hydroxyl radicals, PTZ increased the production of ROS by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which causes increase in MDA level (Shao *et al.,* 2017), which might be the major cause of oxidative stress and subsequently neuronal damage.

*Gastrodia elata* was found to suppress the PTZ- inducing seizures progression via modulating oxidative stress in (group 3) pretreatment, *Gastrodia elata* was significantly decreased the level of MDA compared to PTZ-kindling (group 2) to a manner more than that found in (group 4) as presented in fig. (3.2). *Gastrodia elata* might be able to protect from oxidative stress by inhibiting glutamate and also prevents glutamate induced ($Ca^{2+}$) influx, blocks the activation of the calmodulin - dependent Kinase II (Jiang *et al.,* 2014).

On this basis, these results suggest that GE might act as free radical scavengers and to some extent has neuroprotective effects (Jin Yu, *et al.*,2005). In the present study, lacosamide + PTZ (group 5) was found that LCM alleviate lipid peroxidation MDA compared to (group 2) PTZ –kindling, as explain in fig. (3.2), and managed to restore the MDA level near to normal as in control (group 1). These results were in consistent with other previous study which has showed that LCM decreased

formation of free radicals 48 hrs. after induction of seizure by epileptic inducer, since, the authors reported reduce in level of MDA (Nirwan *et al.,* 2018).

The decrease in the levels of free radicals occurred by LCM could be responsible sequence for attenuation the seizure of the rats treated with it through epileptogenesis. (Shishmanova Doseva, *et al.,* 2021).

Zonisamide + PTZ (group 8), presented the effect of zonisamide in reducing oxidative stress via significantly decrease the level of MDA compared with (group 2) PTZ kindling as shown in fig. (3.2). These findings went well with several studies that had been explained the mechanism of antiepileptic effect of ZNS which may involve in the protection of neurons from damaging by free radicals via scavenging hydroxyl toxic ROS generated from the decomposition of $H_2O_2$ and nitric oxide radicals (Borowicz-Reutt, *et al.,* 2020).

Moreover, scavenging the activity and an inhibitory effect on lipid peroxide formation in rat's model with iron-induced epileptogenic foci.

### 4.2.3. Effects on NO biomarker

According to previous studies, statistically significant increase in NO levels detected in PTZ- kindling rat's models (Hale Maral Kir *et al.,* 2013). In this current study, PTZ-kindling model (group2), was in agreement with the previous studies as NO level was increased significantly compared to control (group1) as in fig. (3.3). PTZ induced seizure might lead to produce NO from the rising action of the excitatory neurotransmitter glutamate is supposed to be included in pathophysiology in models of epilepsy (Hale Maral Kir *et al.*, 2013). So, high levels of NO lead to oxidative stress. GE in this study was found to have antioxidant role and significantly decreased the NO level in both pretreatment (group 3) and also in treatment (group 4) comparing with (group 2) of PTZ-kindling as shown in fig (3.3).

Gastrodia elata inhibited NO production and expression of inducible nitric oxide synthase (iNOS) (Eun-Kyoung Ahn *et al.,* 2007).

Lacosamide + PTZ (group 5), NO level decreased which reflected the antioxidant effect of LCM that might have scavenging role on free radicals comparing to PTZ-kindling (group 2) through the same effect explained in this study above with MDA.

Zonisamide + PTZ (group 8) ZNS decreased the level of NO compared to PTZ-kindling (group2) because it decreases free radicals that goes with another results that approved it as it had a role in elimination of free radicals, ZNS was found to reduce NO synthase activity accelerated by NMDA (Singh G, 2021).

### 4.2.4. Effect on TAOC 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 and NO level that means PTZ decreased TAOC because, TAOA is a measure of the whole amount of antioxidant .TAOC increased with pretreatment and treatment group 3, and 4 respectively and also increased with AED, LCM and ZNS, group 5, and 8 respectively as the same clarification discussed before for GSH, MDA, and NO levels as explained briefly in 4.1.1, 4.1.2, and 4.1.3.

## 4.3. Effect on Anti- inflammatory biomarkers

Rats who have had their seizures artificially produced in experiments have been shown to have a strong inflammatory response in regions of the brain that have been involved in the propagation of the initiation of epileptic activity (De Simoni *et al.,* 2000).

According to previous studies, seizures induced either electrically or chemically

resulted in cytokines increase in brain of rat's model (Vezzani, *et al.,* 2011). PTZ (group 2) in present study demonstrated a significant increase in pro -inflammatory cytokines IL-1β, IL-6 and TNF-α compared to the rats in control (group1) as explained in fig. (3.5), (3.6), (3.7).

This results agreed with another previous studies that approved IL-1β, IL-6 and TNF-α which were expressed at very lows levels in the brain of normal animals, their pro-inflammatory cytokines were rapidly rising after the seizures induction during (≤ 30min) decreasing to basal levels during 48 - 72 hour from the onset of seizures (De Simoni *et al.,* 2000). PTZ- kindling resulted in increase of the IL-1β, IL-6 and TNF-α via increase brain synthesis of cytokine was determined in microglia and astrocytes (Dinarello, 2000), up regulation of IL-1 receptor antagonist (RA) a naturally occurring antagonist, as well as, IL-1β, has been detected after acute kindling seizures (Plata-Salaman *et al.,* 2000).

The brain is less effective than the periphery by which a crucial mechanism induced the termination of the elevated sustained endogenously IL-1β, IL-6 and TNF-α rapidly (Plata-Salman *et al.,* 2000).

Additionally, rats kindling could might exacerbate α-amino-3-hydroxy -5-methylisoxazole - 4 - propionic acid (AMPA) - receptor that mediated the excitotoxicity, depending on their length time of tissue exposure and extracellular concentration of these cytokines during the injury (Bernardino *et al.,* 2005), IL-6 receptor and signaling transducer protein of it gp/30 (albumin binding protein) increased in forebrain of the rats after seizures. However, the expressing of specific types of cells that this receptor has not been diagnosing (Lehtimaki *et al.,* 2003).

*Gastrodia elata* in both (group3) pretreatment and (group 4) treatment had produced a reduction in the IL-1β, IL-6 and TNF-α levels in different degrees

compared with PTZ-kindling (group 2), these results have agreed with previous studies that highlighted the administrated of GE could reversed the increase of inflammatory cytokines levels in brain tissues of rats with PTZ -induced seizures,

so, GE may have potential value in the alleviation of inflammation accompanied by seizure (He *et al.*, 2022). Gastrodia elata might inhibit an expression increasing of TLR4, IL-1β, IL18, downstream of NLRP3; so, the regulation of this pathway is critical for protection the microglia and inhibit the NLRP3 inflammasome (Zheng *et al.*, 2022)

Lacosmide decreased microglial cells activation of brain and TNF-α expression (Kumar *et al.*, 2018). In present study, it has been found a significant decrease in cytokines levels of TNF-α and IL- 1 β, IL-6 in (group 5) LCM+PTZ, compared to (group2) PTZ- kindling, which was in agreement with other recent studies that demonstrated decrease levels of these pro-inflammatory cytokines and produced a mild amelioration in inflammatory effects in the hippocampus (Savran *et al.*, 2019; Al-Massri *et al.*, 2018).

Zonisamide + PTZ (group 8), ZNS approved to have anti-inflammatory effect in present study as it decreased the cytokines levels significantly comparing to the (group2) of PTZ - kindling rats, these results were in consistent with other previous studies that presented the effect of ZNS in attenuating the inflammatory cascade associated with progression of seizures. ZNS can be further behaved as neuroprotective agent in epileptic seizures (Kumar *et al.*, 2018).

## 4.4. Histological Alteration:

Kindling with PTZ has been found to display significant morphological alterations and sever inflammation. PTZ-kindling (group2) in present study demonstrated histological alteration in the brain of rat model and caused moderate inflammation compared to control group1, (pic.3.2.).

This results were consistent with the previous studies which concluded that the PTZ administration induced histological aberration in the brain hippocampus with significantly elevated inflammation infiltration, pyknosis, and necrosis as compared to normal rats which did not show any pyknosis (Tao *et al.,* 2020). PTZ-kindling induced abnormal migration of newborn granule neurons (Lee *et al.,* 2013).

Treatment with GE has been found to ameliorate the histopathological damage by greater number of surviving neurons and mild inflammation area of brain compared to PTZ group2. In current study GE in both pre-treatment and treatment groups 3,4 respectively, have decreased the effect of PTZ - kindling that produced inflammation by decreasing the inflammatory cells infiltration, congestion, and necrosis pyknosis as compared to PTZ-Kindling group 2 as explain in pic. (3.3., 3.4.).

These results were in agreement with other studies data that further suggested that GE prevented the activation of microglia in rat's neuro inflamed hippocampus (Zheng *et al.,* 2022).

Lacosamide in group 5, (LCM+PTZ) was found to decrease the inflammation as mentioned before so it reduced the effect of PTZ from moderate to mild as compared with group 2, PTZ- Kindling, in present study pic. (3.5.).

Previous results by other studies were furtherly demonstrated the effect of LCM on inflammation by decreasing the activated microglia with increasing of ramified

microglia under inflammatory conditions caused by pathological or induction with chemical agents, this role of LCM supported its beneficial effect in epilepsy associated with neuro-inflammation. (Corvace *et al.*, 2022).

Zonisamide + PTZ, group 8, in current study has shown the effect of ZNS as anti- inflammatory through reduction of the inflammation to normal/mild one as shown in pic. (3.6). These results were in agreement with other experimental studies that demonstrated the ZNS role in amelioration of inflammation by decreasing the microglia expression along with that of pro-inflammatory cytokines and also NADPH oxidase (Hossain *et al.*, 2020).

## Conclusions

1. Behavioral changes (decreasing latency period, increasing severity and duration of fits) which induced by pentylenetetrazole were found to be opposed by *Gastrodia elata*, zonisamide, and lacosamide via attenuating the severity and duration of fits and by increasing the latency period of fits in male rats.
2. *Gastrodia elata* possess antioxidant properties can minimize the pro-oxidant induction by PTZ decreasing malondialdehyde, nitric oxide levels, and increasing reduced glutathione and total antioxidant capacity in male rats' brain.
3. *Gastrodia elata* shown anti-inflammatory effect via alleviation of Interleukin-1, Interleukin -6, and tumor necrosis factor-α level in male rats' brain.
4. Zonisamide and lacosamide were found to decrease inflammatory mechanisms by decreasing Interleukin -1, Interleukin-6, and tumor necrosis factor-α level in male rats' brain.
5. Oxidative stress induced by pentylenetetrazole was found to be minimized by administration of zonisamide and lacosamide through decreasing malondialdehyde, nitric oxide levels, and increasing reduced glutathione and total antioxidant capacity in male rats' brain.

## Recommendations

1. Future studies that intends to comprehend the fundamental processes of epileptogenesis and to find new therapeutic targets should focus on the investigation of the link between Gastrodia Elata, epilepsy, inflammation, oxidative stress, and approved AEDs.
2. Utilizing larger sample size of seizure models in vivo, future studies ought to seek to confirm their results. It would also enable concurrent exploration of drug pharmacokinetics and their pharmacodynamics impacts, as well as a better knowledge of how oxidative insult and inflammation could impact seizures and whether this seems to have any bearing on the changes in behavior. Chronic exposed to GE and certain AEDs could also be made possible, that is more clinically relevant.

3. Likewise, more effort is required to explore the full range of oxidative stress and inflammatory markers, with the addition of measures of superoxide dismutase activity, glutathione peroxidase activity

4. *Gastrodia elata* might be used as a putative, adjunctive therapy with commonly used antiepileptic drugs as it has antioxidant, anti-inflammatory, and a putative anti-seizure activity.

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**الخلاصة**

الصرع هو اضطراب عصبي وأحد أكثر أمراض الدماغ شيوعًا والذي يرتبط بنوبة أو نوبات غير مستثارة تحدث في أقل من 24 ساعة على حدة. يحدث الصرع في جميع الأعمار. تم تصنيف ستة محاور للصرع المسبب للمرض من قبل الرابطة الدولية لمكافحة الصرع وهي: مسببات هيكلية، وراثية، معدية، استقلابية، مناعية وغير معروفة. الصرع ليس حالة عصبية ثابتة، ولكنه اضطراب مستمر، تقدمي، يثار خلال فترة الحياة. لا تزال الآليات الفيزيولوجية المرضية لعملية تكوين الصرع غير مفهومة بشكل جيد. في الآونة الأخيرة، هناك حاجة ماسة للحصول على أدوية مضادة للصرع يمكن تحملها بشكل أفضل وتكون فعالة كمواد واقية للأعصاب و / أو مضادات الاختلاج. في الوقت الحاضر، هناك أدلة جديدة على العلاقة بين الإجهاد التأكسدي و / أو الالتهاب والصرع. ومع ذلك، فإنه لا يزال غير معروف فيما إذا كانت سببًا أو نتيجة لذلك.

*Gastrodia Elata* هو عشب صيني تقليدي مشهور من عائلة زهرة الاوركيد والذي تم استخدامه لعدة قرون. أظهرت العديد من الدراسات في العقد الأخير أنه يستخدم على نطاق واسع كمضاد للالتهابات، مضاد للأكسدة، مضاد للصرع، حماية للأعصاب، ومضاد للاكتئاب.

تم تقسيم أربعين من ذكور الجرذان إلى ثماني مجموعات المجموعة الأولى (مجموعة التحكم) لم تتعرض لـ PTZ ولم تتلق أي علاج بينما تعرضت المجموعة الثانية لـPTZ40mg/kg عن طريق حقنه في البطن كل يومين لمدة 15 يومًا، تلقت المجموعة الثالثة نبتة *Gastrodia Elata* 883.56mg/kg، لمدة 14 يومًا ثم استحثاث النوبة بمقدار PTZ40mg/kg، ثم إعطاء النبتة بعد الاستحثاث لمدة 5 ايام، المجموعة الرابعة تم تعريضهال *Gastrodia Elata* / kg 883.56 mg وPTZ 40mg/kg، تلقت المجموعة الخامسةLCS 50mg/ kg عن طريق الفم و PTZ 40mg/kg. تلقت المجموعة السادسة LCS 50mg/kg. تلقت المجموعة السابعة ZNS 100 mg/kg عن طريق الفم، وتلقت المجموعة الثامنة ZNS 100mg/kg PTZ40mg/kg.

في هذه الراسة كان الهدف هو قياس فعالية النبتة المستخدمة كمضاد للصرع، مضاد للأكسدة ومضاد للالتهاب. تم تقييم التأثير المضاد لنوبات الصرع بعد استخدام الـGE ، والأدوية المضادة للصرع، عن طريق قياس الزمن اللازم لحدوث النوبات وشدة ومدة النوبات باستخدام مقياس Racine's scaleفي المجموعة الثانية، انخفض كل من GSH و TAOAبشكل ملحوظ (p <0.05)في المقارنة مع مجموعة التحكم المجموعة الأولى، ومع ذلك، زادت MDA و NO و IL-1و IL-6و TNF-αبشكل كبير .(p <0.001) في المجموعة 3 إلى المجموعة 8، زاد كل من GSH و TAOAبشكل كبير(p <0.001) ، ولكن انخفض MDA و NOو IL-1 و IL-6و TNF-αبشكل كبير (p <0.001) مقارنة بالمجموعة الثانية، انخفضت مدة وشدة النوبات بشكل كبير(p <0.001) ، ومع ذلك، زاد زمن الانتقال بشكل ملحوظ (p <0.05) مقارنةً بالمجموعة الثانية في المجموعات 3 , 4 , 5 , 8، كانت المدة والشدة انخفض بشكل كبير (P <0.001)، ولكن، كان فترة حدوث النوبات مرتفعًا بشكل كبير (P <0.001) مقارنة بالمجموعة الثانية.

في الاستنتاج، قد يكون لـ *Gastrodia Elata* تأثير مضاد لنوبات الصرع ومضاد للأكسدة ومضاد للالتهابات كما هو موضح في نتائج الاختبارات السلوكية والكيميائية الحيوية التي أظهرت أن GE زاد من المؤشرات الحيوية المضادة للأكسدة ويقلل من العوامل المضادة للالتهابات. علاوة على ذلك، خفضت من شدة النوبات ومدتها ولكنها زادت من زمن الوصول الى استثارة النوبات مثل العمل الذي قامت به الادوية المضادة للصرع.

**جمهورية العراق**

**وزارة التعليم العالي والبحث العلمي**

**جامعة بابل/ كلية الطب**

# النشاط المضاد للصرع لكاستروديا ايلاتا (*Gastrodia Elata*): مفهوم الخصائص المضادة للأكسدة والمضادة للالتهاب في نموذج الجرذان

رسالة

مقدمـــة إلـــى مجلس كليـــة الطـــب / جامعـــة بابـــل

كجزء من متطلبـات نيـل درجـة الماجستيـر في الأدوية / الأدوية والسموم

من قبل

**نور الهدى محمد خمير العبادي**

(بكالوريوس صيدلة 2013-2014)

الجامعة المستنصرية

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**م. د**

**رياض هادي الموسوي**

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دكتوراه في الكيمياء الحياتية السريرية

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