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THE REPUBLIC OF TURKEY  
VAN YUZUNCU YIL UNIVERSITY  
INSTITUTE OF HEALTH SCIENCES

**INVESTIGATION THE EFFECT OF  
PENTHYLENTETRAZOLE INDUCING EPILEPSY  
MODEL USING EXTRACTION OF *Epilobium hirsutum***

Sara Sami DZHAFAR  
THE DEPARTMENT OF PROFESSIONAL PHARMACEUTICAL SCIENCES  
(PHARMACOLOGY)  
MASTER THESIS

SUPERVISOR  
Asst. Prof. Dr. Oruc ALLAHVERDİYEV

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## ABBREVIATIONS

|                  |   |                                                                     |
|------------------|---|---------------------------------------------------------------------|
| AED              | : | Anti-Epileptic Drugs                                                |
| K <sup>+</sup>   | : | Potassium Ion                                                       |
| Na <sup>+</sup>  | : | Sodium Ion                                                          |
| Ca <sup>+2</sup> | : | Calcium Ion                                                         |
| GABA             | : | $\gamma$ -aminobutyric acid                                         |
| NMDA             | : | N-methyl-D-aspartate                                                |
| AMAP             | : | Alpha-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic<br>Acid |
| Mg <sup>+2</sup> | : | Magnesium Ion                                                       |
| EEG              | : | Electroencephalographi                                              |
| MRI              | : | Magnetic Resonance Imaging                                          |
| SV2A             | : | Synaptic vesicle                                                    |
| CNS              | : | Central Nervous System                                              |
| MIC              | : | Minimum Inhibitory Concentration                                    |
| PTZ              | : | Pentylentetrazol                                                    |
| i.v              | : | Intravenous Injection                                               |
| i.p              | : | Intraperitoneal Injection                                           |
| s.c              | : | Subcutaneous Injection                                              |
| DNA              | : | Deoxyribonucleic acid                                               |
| ROS              | : | Reactive Radical Oxygen                                             |
| RON              | : | Reactive Radical Nitrogen                                           |
| OH               | : | Hydroxide                                                           |
| H2O2             | : | Hydrogen peroxide                                                   |
| O2               | : | Oxygen                                                              |
| ONOO             | : | Peroxynitrite                                                       |
| HNO              | : | Dünya Sağlık Örgütü                                                 |
| NO               | : | Nitric Oxide                                                        |
| (cGMP)           | : | Guanosine 3',5''-cyclic monophosphate                               |
| VG               | : | Vagus nerve                                                         |

|          |                                               |
|----------|-----------------------------------------------|
| MDA      | : Malondialdehyde                             |
| XO       | : Xanthine oxidase                            |
| Zn       | : Zinc                                        |
| NS       | : Normal saline                               |
| Cu       | : Copper                                      |
| NaCl     | : Normal saline                               |
| WHO      | : World Health Organization                   |
| IQ       | : Intelligence quotient                       |
| VNS      | : Stimulation of the vagus nerve              |
| RNS      | : Responsive neurostimulation                 |
| DBS      | : Deep brain stimulation                      |
| OTC      | : Over the counter                            |
| NADPH    | : Nicotinamide adenine dinucleotide phosphate |
| USA      | : United States of America                    |
| ROS– RNS | : Oxygen / nitrogen species                   |
| GSH      | : Glutathione                                 |
| CAT      | : Catalase                                    |
| GSH-Px   | : Glutathione peroxidase GSH-Px               |
| SOD      | : Superoxide dismutase                        |
| NS       | : Normal saline                               |

## 1. INTRODUCTION

Epilepsy is one of the most complicated hyperactivity neurological disease that affects different parts of the normal brain performance like movement, awareness, sensation, consciousness, and behavior. Seizure duration can take a few seconds to a few minutes; however, backing up to the regular stage was gradually with consciousness, and awareness return, which considered the end-stage of seizure episodes (Fisher *et al.*, 2005). Epilepsy believed to be the second most prevalent neurological disorder (1% of the world population) after stroke, with no full treatment, nor save prevention of drug side effects or drug interactions (Sucher and Carles, 2015).

It cannot be classified as a disease of its self; rather it is a medical condition, in which chemical interactions affect the normal pathways of the neurons to generate a complex electrical response that transmits to neighboring neurons. Thus, when a neuron gets excited it sends signals through the axon to communicate with other neurons, which are either excited or inhibited. And when two neurons get excited all at once, seizures can attain that further spontaneous recurrent seizures, end with the phenomenon known as epilepsy (Chang and Lowenstein, 2003; Loscher, 2011). Epilepsy classified as a chronic condition, which can develop in one region of the brain hemispheres specifically the medial temporal lobe or both hemispheres (Gregory, 2004; Loscher, 2011). Evaluation of (MDA) level by biochemical assays was a clue for lipid peroxidation generation, which results from the formation of free radicals (ROS) that attacks the brain membranes tissue with every seizure provoke or with a chronic seizures. On the other hand, evaluation of antioxidant enzymes like (glutathione peroxidase, superoxide dismutase, and catalase) was a clue for successful treatment against oxidants formation, which showed an increase in their level in the present of seizure inducer or the chemical PTZ kindling in experimental animals (Ilhan *et al.*, 2005; 2006).

According to (Martinc and Grabnar, 2012), epilepsy has divided into 3 phases, the (early phase), in which the seizure onset occurs within a week, the (late phase), in which seizure develops into chronic recurrent onset that could take years from the initialed onset, and the (latent period), which is the period between the injury or the

acute phase and the late chronic phase, in this period epileptic factors develop or undergo to neuroprotection by pharmacotherapy to prevent any later progressing in neuroinjury.

Medical drugs showed various side effects, tolerances, drug interactions and economic demands, which made many researchers looking for alternative therapies with positive reflection on the human health. An agent for seizure control needs to have a potent antioxidant effect and has to prevent the generation of (ROS) free radicals, which in turn controls the hyperactivation of the neuron by the organizing of neuron the neurotransmitters. Thus, different discoveries studied the extracted plant antioxidant compounds and their role in controlling seizures provoke using experimental animals.

*Epilobium hirsutum* that belongs to (Onagraceae) family that has been used since the ancient in treating different health problems such as inflammation, prostate hypertrophy, gastrointestinal, menstrual disorders (Kiss *et al.*, 2011; Stolarczyk *et al.*, 2013; Vitali *et al.*, 2006). The effect was suggested to be, due to their bioactivity and pharmacological antioxidant compounds of ellagic acid, flavonoids, phenolic acids, steroids, and tannins that found in the extracted plant of *Epilobium hirsutum* (Kiss *et al.*, 2006; Stolarczyk *et al.*, 2013). *Epilobium hirsutum* used by the folk of Van in Turkey, for controlling symptoms of convulsion by using the aerial parts of the plant after drying it and drinking it as an herbal tea. However, the Ethnopharmacologic science that is based on studying the traditional usage of the plant by the folk and introduce these data to pharmacology labs provided us basic information of the history of *Epilobium hirsutum* plant.

Our aim in this study was proving the interest of Ethnopharmacology in pharmacology lab by exploring the anticonvulsant effect of *Epilobium hirsutum* using experimental male mice and PTZ seizure inducer. Moreover, open field, as well as Rota rod tests, supported our research by providing additional information of animal locomotors behavior and muscular relaxation. We have enhanced our experiment using biochemical assay to evaluate the brain defend system in the presence of seizure inducer of PTZ.

## **2. GENERAL INFORMATION**

Usually, the epileptic patient faces unsocial life of a hardship and high risk of psychiatric comorbidities, memory loss, confusion, anxiety, and depression symptoms (Esmailpour *et al.*, 2017). Besides, 30% of autistic individuals by their adulthood have shown the development of complex partial seizures (Meidenbauer *et al.*, 2011). However, 70% of the patient got benefit effect of anti-seizure drugs, while the remaining 30% said to be drug-resistant and preferred to be treated by alternatives such as surgical intervention, the ketogenic diet, physical exercise or using different medicinal plants (Bromfield *et al.*, 2006; Devinsky *et al.*, 2014).

### **2.1. Epilepsy**

In the ancient epilepsy was called the brain disease and named as the (Sacred disease), which derived from the Greek word “epilepsy” that referred to the condition of seizures, or possessed that resulted from God curse. In “Farfadiya” book of Hausa who has changed the old reckon of God curse and come up with the modern scientific concept, treatment of using magic, herbs, rituals and, incantation (Muazu and Kaita, 2008). While John Hughlings who considered the father of epilepsy was the first who established seizures origination from different parts of the brain and according to that, had classification seizures to partial and general ones (Hippocrates, 1965).

### **2.2. Epidemiology**

Globally epilepsy considers as a chronic burden brain electrical disorder, that approached to reach, up to 50 million of the world population (Allahverdiyev *et al.*, 2017; Shin *et al.*, 2011), while (WHO, 2016) reported that 2.4 million people are diagnosed with epilepsy each year. According to WHO report, the prevalence of epilepsy is about 80% in low and middle-income countries, while annual cases of high-income countries were between 30 and 50 per 100 000 (WHO, 2016).

In Turkey, despite the few studies done around the country, the prevalence of epilepsy showed to reach (5.3/1,000), separated between 8.8 in 1,000 in rural areas and 4.5 in 1,000 in urban areas, which considered for developed countries. While the lowest prevalence, 1.5 per 1,000 reported being in Japan. (Aziz *et al.*, 1997; Velioglu *et al.*, 2010).

Although epilepsy can appear at any time of the age life, the onset is often high in the children at the age of 6-9 years old and more common in girls than in boys; however, 71% of patients believed to be due to supernatural causes. (Aziz *et al.*, 1997; Çalışır *et al.*, 2006).

### **2.3. The pathologic of epilepsy**

Epilepsy can arise either from idiopathic (genetic) or symptomatic (acquired) factors or even the both. Although the controversial cause of seizures is still unknown; suggested causes like stroke, brain tumors, infections of meningitis, or injury of the both brain or spinal cord, (lead to spell out the glutamate continents into the extracellular space that stimulation the presynapse to release extra glutamate), or due to neuronal death that results from impairment oxygen and glucose supply (lead to high accumulation of glutamate in the extracellular), or due to birth defection, which all were stranded as acquire causes for epilepsy provoke (Martinc and Grabnar, 2012).

On the other hand, the idiopathic is the term, which associates with genetic malformation of central nervous system (CNS) that seen in mitochondrial respiratory chain complex by increasing its permeability influx or release its continent of oxygen that acts as a precursor for initiation of (ROS). Malformation of voltage channel, legend channels function, synapses, and receptors (Martinc and Grabnar, 2012). Moreover, biochemical and physiological changes condition were also seen, such as changes in cerebral blood flow, distribution in blood-brain barrier, increased intracranial pressure, ischemic hemorrhages resulting from the breaking of the red blood cells and therefore, increase in hemoglobin level as well as irons releasing level, which mediate (ROS) as catalysts and induce hyperexcitability of the neuron and cause its death.

Other studies have investigated the causes of seizures by testing the blood sugar level, which showed excessive decrease in the blood sugar (Hypoglycemia) and dehydration are two serious phenomena of seizures provoke due to large gather of glutamates around the synapse causing excessive increasing in  $\text{Ca}^{2+}$  concentration intracellular that end with excitotoxicity or initiation of apoptosis. Hyponatremia (low blood sodium) is another cause of the seizure provoke, low blood oxygen (hypoxia), genetic disorders, and medication was evaluated as causes for epilepsy initiation (Schachter *et al.*, 2013). However, assessing a patient with epilepsy or determining the types of seizures were standing on physical diagnosis of seizures, and using clinical known devices like an electroencephalogram (EEG) (a device placed near the brain surface to recording the electrical impulse) or using MRI image (Ridley *et al.*, 2017).

#### **2.4. The mechanism of epileptic action**

In general explaining, the mechanism of seizure is based on the concept of the imbalance between excitation and inhibition neurotransmitters. The hyperexcitable state of the repeated action potential, increases the synaptic excitatory neurotransmissions function like an opening channel for  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  ions, and glutamate, or decrease in inhibitory neurotransmission like  $\text{K}^+$ ,  $\text{Cl}^-$  and GABA to understand the mechanism of seizures provoke, explaining the normal action potential of a healthy neuron would be helpful in understanding the mechanism of epilepsy (Bromfield *et al.*, 2006).

An initiation of an action potential makes a rapid change in the permeability of the neuron membrane toward  $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$  ions, which inter throughout the voltage-gated channels by opening and closing mechanism. An opening of the voltage-gated channel in the presynapse cause the high concentration of extracellular  $\text{Ca}^{2+}$  ions to penetrate into the cell body of the neuron, which in turn influence the vesicles by opening and releasing their continents to the cleftic space to get locked with the postsynapse receptor by ligand-gated mechanism, and give way for  $\text{Na}^+$  ions to enter in and to change the voltage rate from 70  $\text{vm}$  to - 60 - 55  $\text{vm}$  (thresholds) by causing membrane depolarization of postsynapse (more positive inside and negative outside) (Bromfield *et al.*, 2006; Katzung *et al.*, 2012).

The most affected receptors in epilepsy are NMDA, AMPA, GABA, Na<sup>+</sup> and T-type Ca<sup>+2</sup> channels while the most neurotransmitters affect these receptors are, glycerin, which binds to NMDA site, glutamate bind to NMDA and AMPA sites, barbiturate, GABA and benzodiazepine that binds to GABA receptor site; however serotonin, acetylcholine, endocannabinoid have also reported in epilepsy provoke (Büget *et al.*, 2016; Werner and Coveñas, 2011). Thus, the key and the locking phenomenon played a significant role in controlling the normal brain function or generate brain malformation by losing the electrical balance and propagate epilepsy disorder (Katzung *et al.*, 2012).

Glutamates that belong to ionotropic receptors of the alpha-amino-2, 3-dihydro-5-methyl-3-oxo-4-isoxazole-propanoic acid (AMPA), as well as kainate receptors, and N-methyl-D-aspartate (NMDA), play a significant role in excitation of the postsynaptic membrane. The number and the type of the receptor determine the membrane transmutation potential. In the normal state AMPA receptors are sensitive to a weak stimulation of glutamates by an opening of the channel and entering of Na<sup>+</sup> in and K<sup>+</sup> ions out but not for Ca<sup>+2</sup> ion; this happened in the resting stage or studying and understanding or (long-term potential LTP). A small number of glutamates are released and cause depolarization of the membrane (Bromfield *et al.*, 2006).

Despite in weak stimulate or in resting stage a few ions enter and exit NMDA receptor channel, which is blocked by Mg<sup>+2</sup> ion extracellular; however, it is getting removed by higher action potential or rather, the higher number of glutamates and give free space for Na<sup>+</sup> and Ca<sup>+2</sup> ions to enter and initiate of an action potential (Katzung *et al.*, 2012).

In epilepsy the displacement of Mg<sup>+2</sup> becomes permanent, and the flow of Ca<sup>+2</sup> influx becomes continually, which lead to further depolarization and phosphorylation of more NMDA reproductions on the postsynapse that usually end with neuronal injury or death, under the condition of excessive neuronal activation. Figure 1 elucidates the general mechanism of GABA in epilepsy (Bromfield *et al.*, 2006; Katzung *et al.*, 2012).

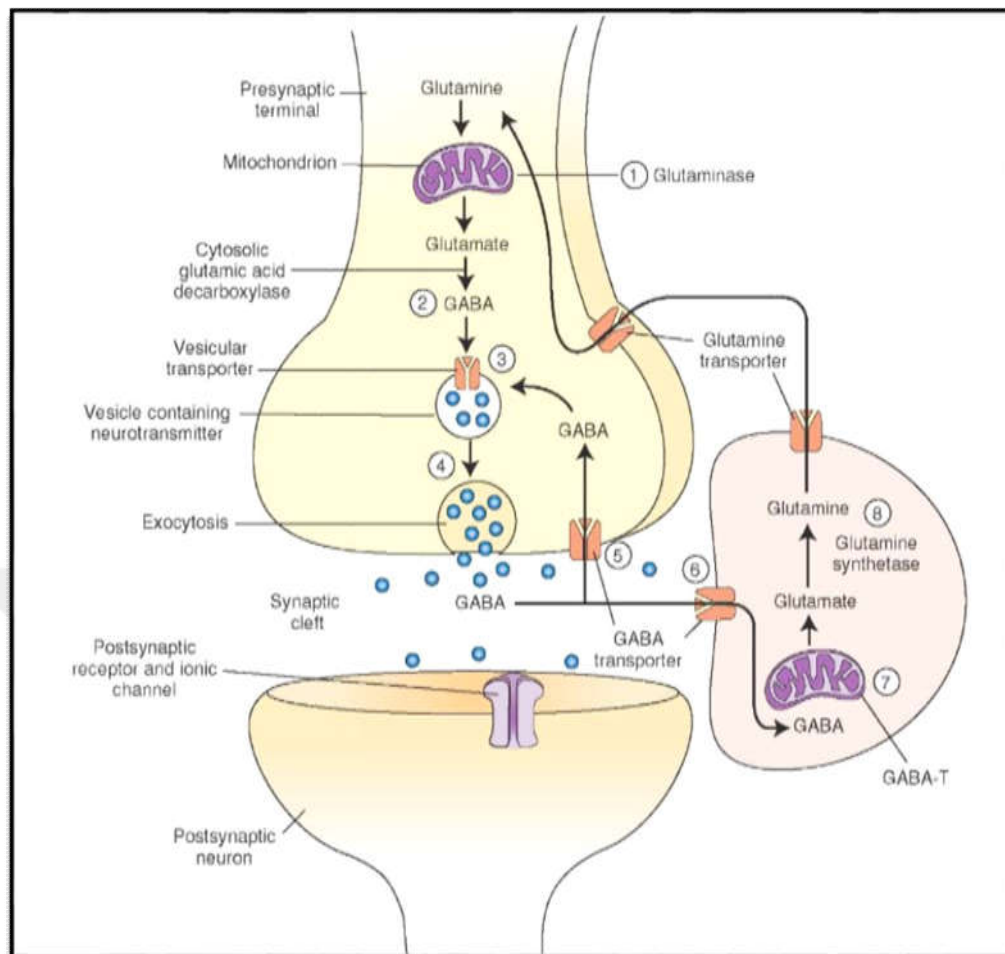


**Figure 1.** The general mechanism of GABA in epilepsy. The neurotransmitter of GABA synthesis in the brain from glutamate neurotransmitter, using the enzymes glutamic acid decarboxylase (GAD) the active form of vitamin B6 (pyridoxal), which are cofactors and from glucose. After the GABA, release to the cleftic space its reuptaked by the presynaptic nerve terminals and surrounding glial cells for utilization. In Gallia, GABA synthesis of glutamine, which has been converted by glutamine to glutamate taking the pathway of, enters the GABA shunt.

## 2.5. The brain structure

The brain is composed of gray matter (neurons and interconnections) and white matter (axons surrounded by a myelin sheath). However, it consists of two hemispheres

and four lobes, which are the frontal lobe (sensation), temporal lobe (feeling), parietal lobe (numbness and tingling) and occipital lobe (visual distribution and hallucination). The variety of epilepsy symptoms depends on its initiation of seizure in one of these lobes or more than one part and classification according to these as Figure 2 shows (Dabbs *et al.*, 2012).

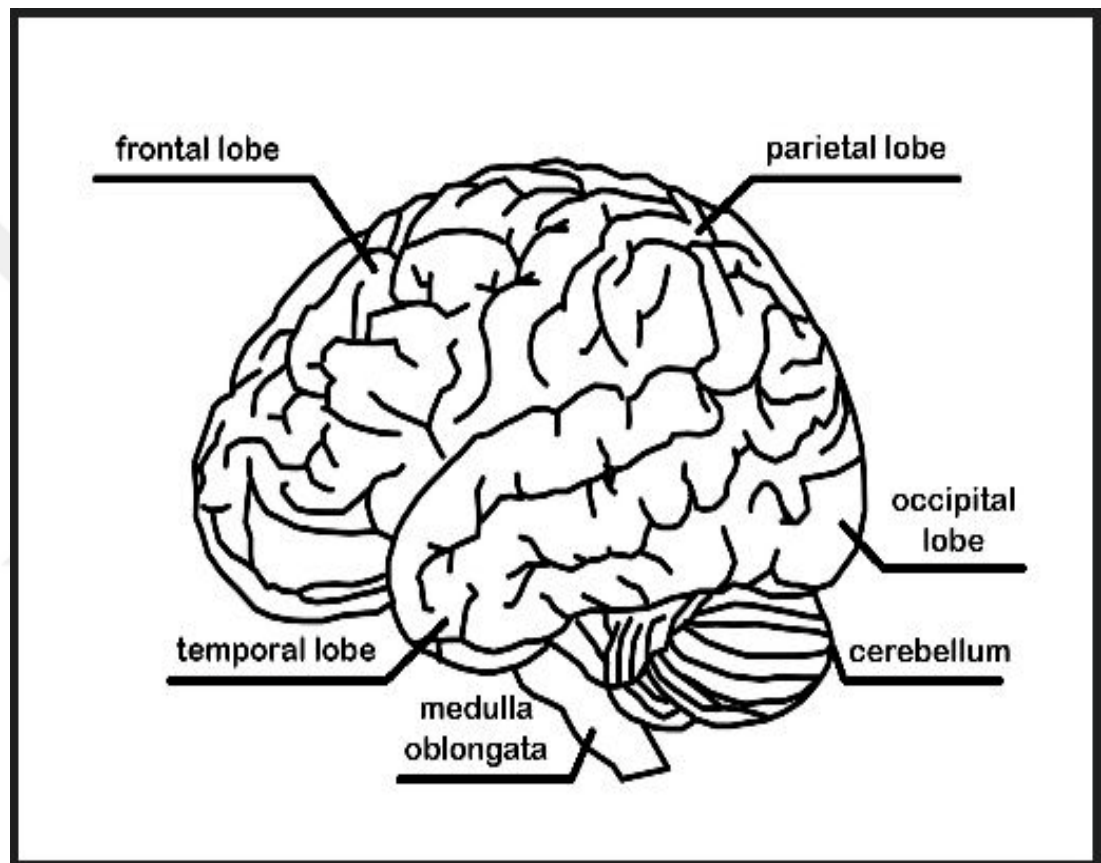


**Figure 2.** Brain structure

## **2.6. The classification of epilepsy**

### **2.6.1. Partial epilepsy**

Partial seizure or focal seizure or even localized seizures, is located in one part of the brain hemispheres or parts of hemisphere lobes. However, partial seizures can be determined by the consciousness, which starts suddenly and very brief and last typically from 60 to 120 seconds.

The term partial seizure, itself divided into a simple partial seizure, which usually affects the temporal lobe and affects about 60% of adult persons; however, when it is developed to complex partial seizure it is known as (aura). While when it is spread to the adhesive parts of the hemisphere, it is known as secondarily generalized seizure or (tonic-clonic seizure) (Engel, 2001; Wiebe *et al.*, 2001).

#### **2.6.1.1. Simple partial seizures**

Partial seizures can be rise from a small area of the temporal lobe, such as the amygdala and hippocampus. Its symptoms divided into somatosensory symptoms of sudden inexplicable feelings of anger, fear, happiness, sadness, or nausea, sensations symptoms like alternation in smelling, hearing, seeing, tasting, sensory illusions or hallucinations symptomes like dissociation from the environment and remembering events in detail. Autonomic seizure (alternation in heart beating rate or sweating) and partial psychotic seizure (influence the way we think, feel and talk, labored speech or inability to speak at all). Sudden discomfort in gastrointestinal tract, paresthesia (tingling sensation of the fifth finger rising to the forearm) (Bromfield *et al.*, 2006).

#### **2.6.1.2. Complex partial seizures (with impairment of consciousness)**

These seizures tend to have a warning or aura before they occur and usually affect the motoric seizure symptoms of muscles activates (tonic-colonic) that result in abnormal movement of one part of the body or spread to adjacent areas of the same side of the body or even both sides with a loss of the consciousness that alter the person's ability to interact normally with the environment around. Paralysis can be seen in one part of the body or more for minutes or hours or even more (Roffman and Stern, 2006).

### **2.6.1.3. Partial seizures evolving to secondarily generalized Seizures**

The partial seizures spread to become secondarily generalized seizure or (tonic-clonic) seizure of trunk, arms, and legs stiffen (the tonic phase) or flexed extended position, and then jerk (the clonic phase). Because of the difficulties to differentiate between secondarily partial seizure and the primarily generalized seizure electroencephalogram (EEG) and neuroimaging test (TC or MRI) were useful in identifying the type of the seizures (Bromfield *et al.*, 2006).

## **2.6.2. General epilepsy**

### **2.6.2.1 Typical and atypical absence seizure**

Absence seizure is the most common aspect of generalized seizure as it shows brief episode of sudden awareness impairment and responsiveness that usually last for (30-20 seconds). Head or face injury was suggested causes of absence seizure; however, the patient often discernible of his surroundings and may follow with hand movement or eye blinking or slight twitching movements of the lips, hyperventilation during their seizure. They usually resolve themselves without complication.

Despite the similarity of absent and partial complex seizures, they have distinguished by studying the period of the unconsciousness; however, the differences between typical and atypical absence became clearer by using EEG device. Seizure in typical absence child, which usually start at the age of 4 - 14 and resolve by the age of 18 or might persist during the adulthood with normal development and intelligence.

The opposite is true with a child of atypical seizure the patient shows normal activity, but with more slowly or with mistakes, loss of the muscle tone of the head, trunk or limbs have also been reported. Impairment of the conscious last longer, up to few minutes with little confusion after regaining of awareness. The causes were unclear, but some suggestion was due to brain abnormalities that were present at the time of birth (congenital) or from a head injury or trauma, or from complications of kidney or liver; however, this type of seizures may continue into adulthood (Goldenberg, 2010).

#### **2.6.2.2. Myoclonic seizures**

The epileptic myoclonic seizure usually bilateral that affect the neck, shoulder, upper arms, body and upper leg and may see a brief shock-like jerk of a muscle or a group of it. However, it can see in epileptic people as well as non-epileptic ones. Consciousness difficulties cannot be seen because of its brief duration of < 1 second while repeating of rhythm can be termed as a clonic seizure. It considers as progressive, degenerative epilepsy (Goldenberg, 2010).

#### **2.6.2.3. Tonic seizures**

It characterized by a sudden drop of the postural tone usually of the head nods or jaw drop. However, consciousness often impaired for several seconds or more than 1 minute. This seizure can come with myoclonic symptoms (Goldenberg, 2010).

#### **2.6.2.4. Clonic seizures**

Clonic seizures can occur during the sleep and characterized by flexion of the neck and the waist with flexion and extension of the upper or lower extremities; however, it can be bilateral nearly symmetric manner duration of 5 - 20 seconds and more common in people with abnormalities (Goldenberg, 2010).

#### **2.6.2.5. Tonic - Clonic seizure**

Known convulsive seizures as well that start with the tonic phase of stiffening, legs and arms extended and continue with consciousness loss. Drooling or foaming resulting from lack of excessive salivation and impair of swallowing, biting the cheek or lip and the tongue, causing bleeding and bladder or bowel incontinence that last for 30 - 20 seconds (Goldenberg, 2010).

Another sign of seizure is the nonconvulsive status epilepsy while the EEG report abnormalities of the consciousness that last for at least 30 minutes without convulsion. Most of these signs are confusion, agitation, hallucinations, facial

automatisms, nausea, papillary abnormalities and cardiorespiratory syndrome (Treiman and Walker, 2006).

### **2.6.3. Neonatal seizures**

Encephalopathy seizure or what is known West syndrome epilepsy of the first year. It occurs more frequently in neonatal and early childhood. However, the IQ of this age of seizure is lower and usually shows problems with learning and behavior actions than the normal children. Moreover, it is considered a significant sign for the high rate of mortality and morbidity than those of adult individuals because of less myelin development level. A neonatal seizure can be developed into clones, myoclonic, and tonic.

Despite the real cause unknown, they have suggested children with high fevers, abnormal of blood levels sugar (glucose), magnesium, calcium, vitamin B6, or sodium can be causes of seizures initiation; they are usually resolved as soon as the cause relieves. On the other hand, if the seizures repeat without such specific cause, the cause is likely to be due to the congenital disability or injury during birth, or brain disorder or genetic metabolic abnormality.

However, it starts at the age of 2 to 14 years and the most common drugs used in controlling it are steroids like Prednisolone 10 mg that was the first line treatment with gradual increase in its dose, besides to phenobarbital, phenytoin, and benzodiazepines; however, surgical epilepsy was the end alternatives option after medical intake (Hart *et al.*, 2015).

### **2.7. AEDs according to seizure type**

Antiepileptic drugs are the first choice treatment for people with epilepsy. However, choosing the appropriate drug is stand on patient's health, wellness, lifestyle, pregnancy, the type of seizure, and how frequently it sparks or which area of the brain it affects partial or general seizure. According to these factors narrow-spectrum, such as carbamazepine, benzodiazepines, eslicarbazepine acetate, ethosuximide, gabapentin,

lacosamide, methsuximide, oxcarbazepine, perampanel, phenobarbital, phenytoin, pregabalin, rufinamide, tiagabine hydrochloride, vigabatrin, or broad-spectrum such as clonazepam, clorazepate, ezogabine, felbamate, lamotrigine, levetiracetam, lorazepam, primidone, topiramate, valproic acid, and zonisamide, are described. Table 1 summarizes AEDs according to seizure type (Bromfield *et al.*, 2006).

**Table 1.** AEDs according to seizure types

| <b>According to their generation types</b> |                                                                                                                                                   |
|--------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>The first generation</b>                | Ethosuximide, phenytoin, phenobarbital, valproic acid, carbamazepine                                                                              |
| <b>The second generation</b>               | Zonisamide, oxcarbazepine, gabapentin, lamotrigine, levetiracetam, felbamate, rufinamide, tiagabine, pregabalin, topiramate, vigabatrin, clobazam |
| <b>The third generation</b>                | Ezogabine, lacosamide, perampanel                                                                                                                 |

## **2.8. The general treatment of epilepsy**

### **2.8.1. Medical treatment of epilepsy**

In general, the AEDs work on opposing the excitatory neuron either by affecting particular ion channel like inhibition the  $\text{Na}^+$  /  $\text{Ca}^+$  pumping or agonist of GABA A (g-aminobutyric acid) receptor or indirect influence the synthesis of GABA

neurotransmitters and increasing the potassium ion concentration intracellular as described in (Kwan *et al.*, 2001).

Many AEDs have used for controlling seizure and providing more comfortable relatable life. However, only 70% showed their effectiveness in controlling seizure while 30% said to be drug resistance. To understand how AEDs play a useful role in controlling seizures explaining the mechanism of some AEDs can give a clear conception.

Sodium channels consist four dominate subunits that any mutation in these channels, was an expression of epileptic genetic deformation; however, drugs like phenytoin, carbamazepine, oxcarbazepine (as well as its active metabolite licarbazepine), Lamotrigine and lacosamide showed to blocking the sodium channels (Meldrum and Rogawski, 2007; Stafstrom, 2007).

Felbamate showed to do the function of glycine by binding to NMDA site rather than the glycine and stop  $\text{Na}^+$  influx. While topiramate, which binds to the glutamate site of AMPA, prevent both channel opening for  $\text{Na}^+$  ion flow, and prevent glutamate binding to its sites and cause the action potential (Sohal *et al.*, 2003).

Barbiturates unlike benzodiazepines at a higher concentration can act directly on GABA A, which is not used in treating absence epilepsy as it lacks the  $\alpha 3$ -continent, rather than that, it can give opposite effect (Greenfield, 2013).

Most of the AEDs acting on GABA A receptor (ligand-gated chloride channel), while GABA B receptor, which is heterodimeric G protein-coupled receptors, activate for  $\text{K}^+$  ion and inhibit  $\text{Ca}^{+2}$  ions; however, it's didn't target for the most AEDs (Lothman, 1992).

Tiagabine bind to GABA receptor to induce  $\text{Cl}^-$  ion influx and prevent the reuptake of GABA neurotransmitter, by prolonging their existence extracellular. While vigabatrin showed to inhibit the converting enzyme of 4-aminobutyrate

aminotransferase (GABA transaminase) that inactivate succinic semialdehyde and glutamate and by this increase the whole level of brain GABA content in the external synapse (Rogawski and Cavazos, 2014).

Studying the action of vigabatrin on experimental mice showed during the first period of drug administration reduction in the seizure threshold while the anticonvulsant activity influenced later (Stuchlík *et al.*, 2001; Löscher *et al.*, 1989). Its use for complex partial seizure or secondary general seizure and can administrate as a monotherapy in treating West syndrome infinite spasms. (Haga *et al.*, 1995).

Blocking voltage-gated calcium channels were more used in the general absence seizure (Avoli *et al.*, 2001; Lambert *et al.*, 2014). The best first drug used to treat the absence epilepsy was the ethosuximide, which showed to bind to the T-type  $\text{Ca}^{+2}$  channel and stop  $\text{Ca}^{+2}$  from entering and initiation of seizure recurrent (Coulter *et al.*, 1989). Zonisamide an inhibitor of  $\text{Na}^{+}$  channels has also shown inhibition action on  $\text{Ca}^{+2}$  channel as well as valproate, which is widely used in absence epilepsy (Emilio, 2005).

Ezogabine is useful in treating the partial seizure by inhibiting potassium channels from Kv7.2 to Kv7.5 but not for Kv7.1 that is predominant in the heart tissue (Martin *et al.*, 2012; Rogawski, 2006).

Levetiracetam is the key lock of glycoprotein SV2A that found in the membrane of a vesicles of the presynaptic neuron and act as seizure protection beside to phenytoin (Kaminski *et al.*, 2012). On the other hand, the  $\alpha 2\delta$ -1 subunit protein of voltage-gated calcium channels presynaptic of the excitatory glutamatergic neurons,  $\alpha 2\delta$ -1 showed to be the key lock of gabapentin, and pregabalin (Stahl *et al.*, 2013). However, any malformation or mutation of these subunits result of absence seizure or rather enhancing seizure susceptibility (Brown and Andrew, 2005; Ivanov *et al.*, 2004; Rogawski and Bazil, 2008; Rogawski and Cavazos, 2015).

An extended period of AEDs administration can produce sustained free seizure due to the accumulation of the inhibitory neurotransmitter GABA and inhibition of its degradation, like phenobarbitals, valproic acid, and vigabatrin.

Disadvantage in resistance to drug treatment like benzodiazepines, which proved to decrease its efficacy with the prolonged period of drug intake. While phenobarbital, carbamazepine or phenytoin have shown the development of drug tolerance due to increasing drug elimination metabolism (Loscher, 2011).

Before starting with epilepsy drugs, each patient should be diagnosed with the right seizure type, whether it is a partial or general seizure. Although AEDs cannot treat the seizure, it can rather diminish the separation of abnormal firing into the neighbor parts of the brain and attenuate seizure development from partial to general seizure type.

Successful treatment starts with single/monotherapy with gradually increasing in its dose; however, most of the causes force the patient taking a combination of AEDs to get full seizure control.

When should AEDs be taken? Was the contravention decision by many physicians who said after the initiation of the first seizure; however, within 5 years another seizure could provoke besides of the initiated ones, which is enough to tell us that despite the treatment can reduce the risk 50 %; it cannot prevent the patient from second seizure attack. Figure 3 elucidate AEDs and their mechanism of action (Lasoń *et al.*, 2013; Rogawski and Cavazos, 2015; Singh and Brashier, 2014).

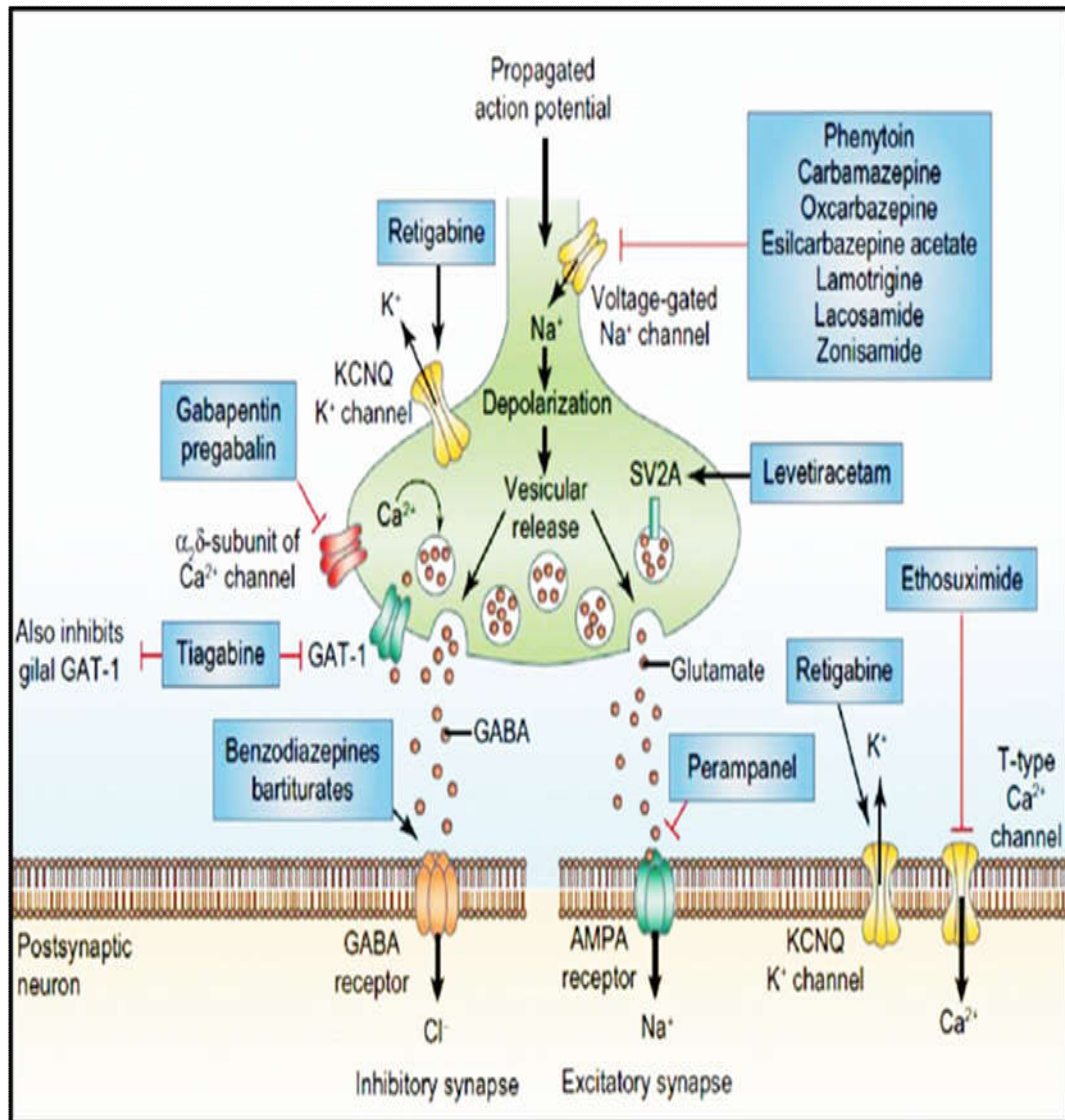


**Figure 3.** Mechanisms of action of antiepileptic drugs

### 2.8.2. Combination treatment

Studies have shown that combination of AEDs in treating seizure could lead to addiction. (Goldenberg, 2010). However, others have shown that AEDs of the same

mechanism of action, has aggravated side effects, while the ones of different mechanism of action may improve drug the effectiveness (Margolis *et al.*, 2014).

Long-term use of ADEs showed to cause several side effects like neurological, cognitive disorders, bone marrow hypoplasia, hormonal disorders and aplastic anemia, vitamin K and folate deficiencies, which disrupt the efficiency of the treatment (Sigrid *et al.*, 2015; Toledano and Gil, 2008).

In the pregnant women, the fetal can develop abnormalities malformation such, cleft lip, cleft palate, congenital heart disease, mental deficiency and slowed growth (Wlodarczyk *et al.*, 2012). Moreover, since antiepileptic drugs used for a long period, they can intervene with other drugs in taking such as cardiovascular drugs, anticoagulants, antibiotics, oral contraceptives, proton pump inhibitors, hemostasis group, tuberculosis medicines and antidepressants (Ortinski and Meador, 2004). Moreover, some AEDs can interact with herbal and dietary supplement via cytochrome P450 enzymes like garlic, grapefruit juice and echinacea or by increase the occurrence of seizures like ginseng, ginkgo, black cohosh, evening, ephedra, primrose dehydroepiandrosterone, and caffeine. That is why it is important to understand the active plant components and their interaction with AEDs.

### **2.8.3. Alternative treatments**

As AEDs showed many adverse effects and tolerance, sciences started to look for the substituted treatment with less opposing effect like supplement of pyridoxine, ascorbic acid, selenium, physiology treatment of relaxation or even surgical intervenes (Allahverdiyev *et al.*, 2017; Ramaekers *et al.*, 1994; Kwon *et al.*, 2004). Therefore, the development of new molecule with better therapeutic effect and fewer side effects than the AEDs was continually researched by science. The compounds derived from the natural medicinal plants had to have a great field of interest in their new therapeutic efficacy for the treatment of epilepsy.

One of the ends chose of unsuccessful alternative treatments was surgical intervention. Stimulation of the vagus nerve (VNS) was done surgically by inserting

small device into the affected area of the brain to send electrical impulse toward the vagus nerve and stimulate it every 10 minutes for 7 - 30 second, which has shown < 5% seizure free in the epileptic patient and was useful for complex partial seizure (Allahverdiyev *et al.*, 2017; Basim, 2000). While removing the affected part of the anterior temporal lobe along with the hippocampus and amygdala showed 70% - 80% successfulness seizure free (Gregory, 2004; Martinc and Grabnar, 2012). However, the side effects were like memory, language impairment, and brain infection. (Dalkilic, 2017; Kelly and Chung, 2011; Wiebe *et al.*, 2001).

#### **2.8.4. Medicinal plants**

Many plants all over the world have shown to have an effective role in relieving convulsion, and successful experimental methods have estimated their effectiveness toward seizure with less adverse effects. However, and as (Loscher, 2011) reported in his research the reason of failure to develop new AEDs from most of the plant extract agents with higher efficiency and less side effect, was due to fewer dare experiments have not been done with different methods or experimental animals. Most of the anticonvulsive therapeutic plant has made under the same conventional animal models, of maximal electroshock seizure, PTZ test, and rodents, which worked as a gatekeeper for their successful experiment.

Moreover, most of the extracted plant showed to contain more than one active components and to study each single compound in the extract solution needed massive time and enough financial support. On the other hand, some physicians were interested in homeopathy and megavitamin therapy, but less likely to be interested in folk remedies.

However, some of these plants, which investigated their effectiveness toward seizures were brassica nigra (Kiasalari *et al.*, 2012), cyperus rotundus rhizome (Khalili *et al.*, 2011), ferula assa foetida gum (Kiasalari *et al.*, 2013), that found in the areas of Iran, ginkgo biloba in Cine (Ilhan *et al.*, 2006), melissa that originally comes from southern Europe and found in coastal areas of Turkey, as well as in Iran (Mrlanova *et al.*, 2001; Kennedy *et al.*, 2004) and nigella sativa (Ilhan *et al.*, 2005) these and many other

else have shown positive effects on controlling brain electrical disorders and worked as valid anticonvulsant effect. The major constituent of these plants were their antioxidant compounds like  $\alpha$ -tocopherol, polyphenolics, and flavonoid which were the major constituent in curcumin and most *Epilobium* species, which worked as useful anticonvulsant agents against seizure.

In curcumin polyphenolics showed to prevent lipid peroxidation and maintain a balanced glutathione level (Shin *et al.*, 2011), while in ginsenosides the terpenoid saponins from *Panax ginseng* showed to have an anti-convulsive effect (Shin *et al.*, 2009a, 2009b; Lian *et al.*, 2006). Honeybee propolis, which consists caffeic acid, phenethyl ester, and various flavonoids, were maintaining antioxidant constituent of glutathione homeostasis as well as adenosine (Kwon *et al.*, 2004). Ginkgo biloba leaves that is rich in flavonoids, and terpene lactones had reported in suppressing seizure effect as other did (Ilhan *et al.*, 2006). However, some medicine plants can cause serious adverse effect especially when the density and the quality of the active ingredients are not usually known by the patient, which may interrupt the regime taken of AEDs from increasing their sedative effects such as (kava, chamomile, valerian, passionflower) or stimulative effect like (anecdotal, caffeine, ginkgo, ginseng and St. John's wort) or even containing epileptogenic compounds like (essential oil of hyssop, fennel, wormwood, rosemary, eucalyptus pennyroyal, thuja, turpentine, tansy, sage) or influence their mechanism of action like (grapefruit and pyrrolizidine) (Dzhafar and Allahverdiyev, 2017).

## **2.9. The concept of Ethnopharmacology**

When we introduced to know the science of Ethnopharmacology it comes in our minds the definition of the old science of ethnobotany, which describes the uses of the plant by the people for food, medical or shelter purpose; these use based on humankind culture and their understanding of the plant and as Stein defined in his book. Hence, ethnology studies the culture while botany study of plants (Schooley, 1997).

Ethnopharmacology considers the new scientific discovery that bases on collecting data from the folk and describe their uses of the plant as a traditional medicine cure. Then producing these data for pharmacology experiment to investigate of indigenous components and their rule in the biological activities. (Gertsch, 2009; Xia *et al.*, 1997).

Traditional medicine was the acquisition of 80% of world population, especially in developing countries as a source of primary health care (Farnsworth *et al.*, 1985). The first recorded report of medicine use was in 1597 by Gerard's Herabl, which obtained 16 prescribed drugs (Janick, 2003). In other studies, showed 94 species of plants used to produce 122 single national agents, 72% of them used clinically as ethnomedical (Fabricant and Farnsworth, 2001). However, in ethnomedical many therapeutic agents stranded as an incomplete date in the identification of the isolated compounds or study them biologically as a single agent (Cordell and Colvard, 2005).

The first fact in health care performance with using traditional plants is to be safe with no harm. The second fact was to provide the needed effect for the health purposed. Although this notion was familiar all over the world, consuming the phytotherapeutic products as over the counter supplements is no longer compatible after understanding the critical clinical interaction between these phytotherapeutic agents and traditional medicines (Ioannides, 2002). WHO has provided a guideline reference in improving the use of the traditional plant by improving the studying and the research unit of most of them.

Thus, Ethnopharmacology works on testing the hypothesis of observation, description, and experimental investigation of phytochemical compounds that stand on ethnomedicine plans and development of technology laboratories to transfer all phytotherapeutic active components as a single agent for a new drug investigation (Rogawski *et al.*, 2008).

However, as the investigation of every single compound would be an insufficient process for all components of the single plant and will need enough

financial support, coming up with the idea of studying the traditional use of the natural plant by the folk known and investigating the suggested compounds, as a single agent was more worthy. Hence, encouraging the science of Ethnopharmacology will help each country economically to develop their sustainable medicine agents with less financial demand.

### **2.10. *Epilobium hirsutum***

Before pharmacy science developed, people used natural plants to treat different health problems, including the CNS disorders like relieve depress, improve the mood, relaxation and memorizing, and many are still in the progress of investigation like *Epilobium hirsutum*, which reported from the ancient to treat inflammation, prostate, gastrointestinal, menstrual disorders (Ducrey *et al.*, 1997; Granica *et al.*, 2014; Smith, 1921, 1933; Turner and Bell, 1973) and in our studies control of epilepsy.

*Epilobium hirsutum* existed in the most areas of Van in Turkey and used by folk in relieving symptoms of seizures by boiling the plant after drying it and drink it as a herbal tea, which our experiment was based on and depending on folk used of the aerial parts of the plant at room temperature.

*Epilobium* covers more than 200 species the word wild; however, one of our interest species is *Epilobium hirsutum* that belong to (Onagraceae) family. The name derived from the Greek words ‘epi’ (upon) and ‘Lobos’ (a pod) according to the morphology shape of the flower arrangement upon long, thin, pod-like seed cases (Hippocrates, 1965). They are large flowering plant belonging to the willow herb *Epilobium* genus. The Latin name is *hirsutum* comes from its woolly leaves and stem, which is mean hairy and named hairy Willow herb due to its robust stems, which is profusely hairy as Figure 4 shows (Clapham *et al.*, 1987; Grigson, 1996). The hairy leaves of are 2 -12 cm long and 0.5 - 3.5 cm wide. While the flower has four notched petals of pinkish - purple color seldom white or yellow of erect style with 10 - 16 mm long. Thus, the stigma is white with four lobes and they usually long up to 2 meters in height sharply toothed edges and no stalk (Grigson, 1996).



**Figure 4.** The flower and the hairy stem and leaves of *Epilobium hirsutum*

Natively, North Africa and exist in most European up to the south of Sweden and parts of Asia (Cao *et al.*, 2012; Clapham *et al.*, 1987). It typically grows 2,500 meters above the sea level unshaded in moist soils of a long stream (Shamsi and Whitehead, 1974). However, we have collected it near the coastline of Van Lake especially in July-August as its peak flowering period (Shamsi and Whitehead, 1974). Elephant hawk moth insects, hoverflies, and bees are pollination syndromes (Clapham *et al.*, 1987).

Despite the limit study use of *Epilobium hirsutum* among *Epilobium* species, it showed to have nonspecific remedy; however, the Egyptian folk people showed a benefit in using the plant for treating inflammation, adenoma and prostate tumors (Kiss *et al.*, 2011). While in North American, folk had shown many different usages. They used in fusion roots to treat gastrointestinal ailments and diarrhea as well as persistent coughs (Rousseau, 1947; Smith, 1921, 1933; Turner and Bell, 1973 ), several of skin and mucous infection, infected sore, hemorrhages, and swelling relive (Bocek, 1984; Barrett and Gifford, 1933; Smith, 1921). Treating eczema has also reported by (Ellingwood, 1919).

Despite the selective anti-proliferation effect of inhibiting DNA synthesis, no significant difference in IC 50 value reported. The mechanism of inhibition the proliferation seems to be independent of Andorra-gene receptors that suggested in vitro experiments (Ducrey *et al.*, 1997; Granica *et al.*, 2014).

On the other hand, aqueous extract of *Epilobium hirsutum* showed to have an anti-inflammatory effect due to oenothien B compound. Nalgesic and anti-nociceptive activities were also reported (Kiss *et al.*, 2011; Tita *et al.*, 2001).

While the ethanolic extracts showed to have antimicrobial and antifungal activity with the minimum inhibitory concentration (MIC) from 81 to 650 mg/ml (Battinelli *et al.*, 2001).

The antiviral activity was reported towards influenza viruses vitro and vivo (H3N2 and H1N1), and this was due to the oenothien B component that is significantly dominated in polar extracts of *Epilobium* species (Ivancheva *et al.*, 1992; Villa *et al.*, 1989). Moreover, it showed to have effective anti-prostate, antitumor, hyperplasia, anti-inflammatory, immunomodulatory, and anti-viral activities (Sakagami *et al.*, 2000; Wang *et al.*, 1999). Until now, no study has been done to demonstrate brain GABA level changes with anti-convulsion activity for *Epilobium hirsutum* as we approached in this study.

Although we have not studied the chemical composition of *Epilobium hirsutum*, we find the following compounds existing from other studies done, which helped us to predict the effect of anticonvulsive in *Epilobium hirsutum* that was our primary concern.

The secondary metabolism of *Epilobium* species showed to be rich in antioxidants compounds as Serdar and his team of researchers estimated in their plant extract.

Determination of the phenolic acid derivatives such as ellagic acid contents carried by using LC-MS/MS analyses, which were  $880 \pm 11$  mg. It marketed as ellagitannins that commonly found in vegetables, fruits, nuts and beverages such as grape juice, tea, red wine; however, ellagic acid showed to have antioxidant, anticancer, anti-allergic, antimicrobial, and anti-inflammatory activities.

Flavonoids particular hyperoxide, guaiaverin, myricitrin, quercetin-3-O-alpha-larabinofuranoside, quercetin-3-O-beta-D-glucuronide, tannins, steroids specific beta-sitosterol, triterpenoids and fatty acids were also estimated in *Epilobium hirsutum* (Barakat *et al.*, 1997; Cao *et al.*, 2010; Nawwar *et al.*, 1997).

Valoneic acid dilactone, quercetin glycosides, and myricetin glycosides, reported by (Ducrey *et al.*, 1997; Hiermann, 1995; Hevesi *et al.*, 2006; Slacanin *et al.*, 1991). However, the major content anti-oxidant of *Epilobium* species was oenotherin B according to (Ducrey *et al.*, 1997; Granica *et al.*, 2014).

As we mentioned before, there was not a lot of research done for *Epilobium hirsutum* in vivo; however, we have found studies done to inoculation tumor cells of leucosis P - 388 and ascetic tumor of Ehrlich (ATE) cells. And that was by aqueous extract of *Epilobium hirsutum*, which was administered i.p 100 mg/kg b.w for 5 days subsequently on the experimental mice and the result was a significant increase of lifespan by the percent of (156% and 158% respectively) (Voynova *et al.*, 1991).

Other vivo experimental has done on studying the antioxidant effect of *Epilobium hirsutum* using the experimental rats and administered 37.5 mg/ kg b.w i.p

for 9 days of the water extract. The result was a significant increase of protein level and activation of phase II as well as the antioxidant enzymes glutathione peroxidase (GPx) and NADPH quinineoxi reductase1 (NQO1) (Karakurt *et al.*, 2016).

Vitali has studied the ethanolic extract of *Epilobium hirsutum* plant vivo on the experimental mice by administration doses from 5 to 200 mg/kg b.w i.p. and he found a useful effect of controlling diarrhea by inhibition of muscle contractility and mobility. However, the anti-diarrhea effect was due to polyphenol compounds as well as tannins compounds (Vitali *et al.*, 2006).

*Epilobium hirsutum* consider being safe and nontoxic; however, extensive studies have done studying the cytotoxicity in vital organs of Wister rats by oral administration of 1.5 ml of hydroalcoholic extract for 10 days, and the result was no cytotoxicity in rats' brain. While investigating the cytotoxic effect of *Epilobium hirsutum* on rat liver by water extract of 37.5 mg/kg was done by testing the serum transaminase enzymes; AST, ALT and LDH activities. Most of the results showed no acute toxicity or hepatocellular injury on rat livers. (Pourmorad *et al.*, 2007; Roman *et al.*, 2010).

Yakı otu, Qarapil is the local name of *Epilobium hirsutum* in Turkey especially in Van as Figure 5 shows. However, as our research aimed to understand the ethnobotanical use of the plant and apply it to pharmacology as an Ethnopharmacological science, we have prepared the plant extract based on traditional folk use.

According to folk of Van and their method of preparation as tea infusion of the plant at the room temperature, we have followed their traditional use and prepared our extract based on water extraction to get the beneficial lyophilized powder.

Plant materials (approximately 5 kg fresh weight) harvested in Kurubaş Village, Gürpınar, Van City, in the Eastern Anatolia Region of Turkey, (GPS coordinates 38° 21' 52.78" N; 43°23' 24.54" E) in August. Their identity confirmed at the Van

Pharmaceutical herbarium Pharmacy Faculty, Van Yuzuncu Yil University, Turkey. The aerial parts of the fresh plants were cleaned and dried at room temperature in the dark, and finally, the samples were ground and stored at - 20°C until analyzing.

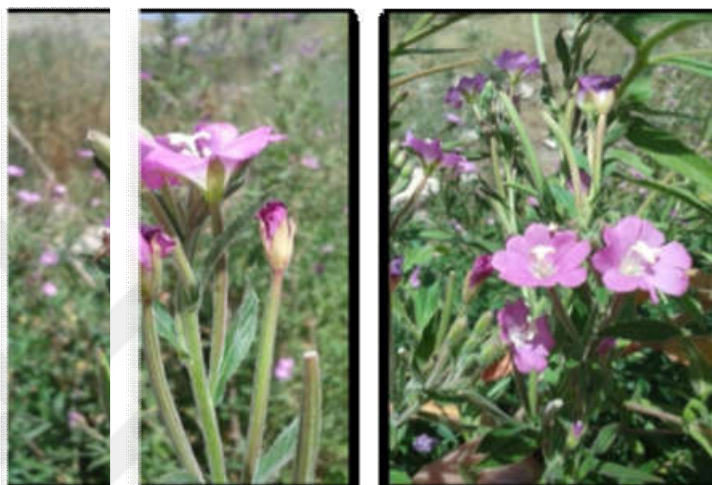


**Figure 5.** *Epilobium hirsutum* plant collected in Van

### **2.11. Chemoconvulsants used in animal models of epilepsy**

Kindling model was the most widely used to investigate any new AED based on using animal models with sub-convulsive repeated application doses to generate full seizure or epilepsy form. Kindling can be either electric stimulation of the amygdala, hippocampus, and frontal cortex or chemical inducers of repeated administration sub-convulsive doses or a single dose. Pentylenetetrazole PTZ is the most used kindling beside to bicuculline, picrotoxin, penicillin, and  $\beta$ -Carboline in inhibiting GABA neurotransmitters (Da *et al.*, 1998; Kumar *et al.*, 2016).

Neurotoxicants reported to alter neurotransmitter release and binding function and used in kindling models like bicuculline is an alkaloid, has an identical response to electrical kindling stimulation but with faster action in producing generalized seizures (Da *et al.*, 1998; Kumar *et al.*, 2016).

While methylation of the parent drug caused more active bicuculline methiodide forms that have rapid inhibition on the postsynapse without affecting the mechanism of hyperpolarization (Da *et al.*, 1998; Kumar *et al.*, 2016).

B-carboline is another kindling alkaloid that binds to benzodiazepine GABA receptors by repeated administration of sub-convulsive doses to produce full seizure (Da *et al.*, 1998; Kumar *et al.*, 2016).

Picrotoxin is a toxic crystalline compound that found in the fruit of a plant of *Anamira cocculus*, has a potent effect on reducing GABA inhibitory effect by very complex mechanisms (Da *et al.*, 1998; Kumar *et al.*, 2016).

Endosulfan is a pesticide belong to organochlorine, which said to block GABA receptors by repeated administration of sub-convulsive doses (Da *et al.*, 1998; Kumar *et al.*, 2016).

Landuce is another pesticide and known as a chlorinating hydrocarbon that used besides to the electrically stimulated paradigm to accelerate the consultative effect of blocking the GABA receptors. Trimethylolpropane phosphate use to facilitate the electrically kindling by repeated sub-consultative doses (Da *et al.*, 1998; Kumar *et al.*, 2016).

Local anesthetics have played invalid role in kindling model as well by repeated administration of a high dose, or by chronic abusers like cocaine, which is NMDA antagonist inhibition and enhance glutamate formation and release, while lidocaine showed to block GABA ionophore receptor complex (Erkeç and Arihan, 2015; Da *et al.*, 1998; Kumar, *et al* 2016).

Kainic acid is a natural marine acid present in some seaweed, and the agonist of kainite receptors induces  $\text{Na}^+$  channel opening (Zhu *et al.*, 2011).

A group of antibiotic used to evaluate the epilepsy state model in grand-mal epilepsy, such as penciling that caused neurodegenerative by intracortical injection of 1500 IU sub-convulsive doses. The effect of convulsions was between 4 - 5 min using EEG recording performance, which showed to block GABA A receptors (Akdogan *et al.*, 2008; Arık *et al.*, 2014).

### **2.12. Pentylenetetrazol**

Pentylenetetrazol (PTZ) kindling model has used for more than 60 years ago, and still the most used models for identification of any new AEDs and its development toward epilepsy (Loscher, 2011). Many different animals nominated for PTZ kindling models like rats, chickens, rabbits, monkeys, zebrafish, and mice; however, different routes of administration also applied like i.p, i.v, s.c, which used to identify compounds that involve in raising seizure threshold. However, testing an intravenous PTZ into the tail vein of the mice, gave a wider screen with its board spectrum mechanism (Mandhane *et al.*, 2007).

Initiation of seizure start within 5 min after administration of the latent dose of (65 mg/kg) and last for 30 seconds. However, the administration of sub-convoluted dose of (35 mg/kg) lead to up normality of the mouse behavior over the application of the 1 - 2 week while stronger phases of animal convulsion develop in the 3 - 4 week and full kindling achieve in the 4 - 6 weeks (Goddard, 1967; Kumar *et al.*, 2016).

PTZ is also known as metrazol, pentetrazol, which used for the circulatory and respiratory stimulant, but at higher doses, noticed to cause convulsion (Nestler, 2004). Although the real mechanism of PTZ is unknown, studies have suggested its influence on calcium and sodium influx and depolarization of the neuron membrane as well as it excitation of amino acids such as glutamate and induce of GABA inhibitory (Da *et al.*, 1998; Papp *et al.*, 1987).

### 2.12.1. The mitochondrial involve with PTZ induce oxidizing stress

Protein damages, mitochondrial destruction, distribution of lipid membrane, mutation of DNA – RNA, and alterations in antioxidant defense systems of the brain were all reasons for the oxidative stress of oxygen/nitrogen species (ROS – RNS) that induced by PTZ. However, other studies reported that long periods of AEDs intake could also induce ROS/RNS as well (Dhir, 2012).

The brain is the most organ susceptible to oxygen consumption by the mitochondria with maintaining an ionic equilibrium of (high intracellular  $K^+$ , low  $Na^+$ , and free  $Ca^{+}$ ) (Benard *et al.*, 2006; Bashkatova *et al.*, 2003; Emerit *et al.*, 2004). However, the mitochondrial product, it's self by defending the system of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, and other non-enzymatic like glutathione, vitamins E and C (Wilson, 1997).

The brain showed to have a low level of antioxidant enzymes, catalase (CAT), glutathione peroxidase (GPx), as well as of non-enzymatic antioxidants like vitamin E and glutathione (GSH). Moreover, it gets weaken with aging (Martinc and Grabnar, 2012).

The recurrent phenomenon of seizure, lead to increase oxygen consumption with every seizure provoke and as a consequence, the formation of (ROS) increase mitochondrial permeability toward  $Ca^{+2}$  which elevate during the latent phase and continue to the chronic phase, then end with necrosis and apoptosis of the nerve cells (Brown, 1999; Martinc and Grabnar, 2012).

As long as the brain is in rich with unsaturated fatty acids, oxidation of macromolecules like lipid, lead the formation of lipid peroxides, which indicate by measuring the endogenous enzyme level of malondialdehyde (MDA) that located in the pineal gland (Giusti *et al.*, 1996), or loss of glutathione homeostasis (Shin *et al.*, 2008) or decrease in (CAT) defending system or an increase of iron, copper ions, which are used by the brain for energy supply and act as a catalyze in inducing hydroxyl radicals formation (Oubidar *et al.*, 1996).

In the blood sample test, the antioxidant enzymes were studied (GPx, CAT, and Cu–Zn–SOD) and most of them were at low level with recurrent seizure (Ängelgren *et al.*, 2003).



### **3. MATERIAL AND METHODS**

#### **3.1. Material**

##### **3.1.1. Experimental animals model**

Swiss albino male mice were chosen according to the approval of the Institutional Animal Ethics Committee (IAEC) of Van Yuzuncu Yil University, Pharmacy faculty letter No TYL-2017-5873, which base on minimizing animal suffering and reduce the number of animal consumption. Mice were kept in Institute animal house and were exposed to appropriate food facility to gain significant weight of ( $35 \pm 50$ g) (mice of 8 - 10 weeks old), which was suitable for our present examination. At the first of the studies, 40 mice housed in hygienic of transparent plexiglas cages (polypropylene cage) of 40×30×20 cm maximum 8 mice/ cage, under standard laboratory conditions. All experiments were performed between 8.00 and 10.00 a.m. in

a silent room and constant temperature of 22 - 28 C and relative humidity of 50 - 70% with 12/12 h light/dark cycle and had free access to standard pellet food and tap water as Figure 6 shows (Bhosle, 2013; Pahuja *et al.*, 2013).



**Figure 6.** Animal model mouse

### **3.1.2. Preparation of lyophilized extracts**

Lyophilized extraction was more advantageous for our research, which provided us stable compounds, devoid from the microbial formation, active enzyme activation as well as oxidization reaction and able to save it for 5 years since it doesn't contain water or humidity.

The lyophilized extracts prepared as described previously (Dalar and Konczak, 2013), with minor modifications. Briefly, the ground, plant material was mixed with a 20 - fold volume of pure water (gr/mL) shaken for 2 h at room temperature (22°C) and centrifuged for 20 min (15320 g) at 4°C (Sorvall RC - 5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial No. 02U8152, USA) with the supernatant collected. The solvent evaporated under reduced pressure at 40°C. The derived fraction dissolved in purified water and freeze-dried under a vacuum at - 51°C to obtain a fine

lyophilized powder used for analysis as Figure 7 elucidate. However, our selected doses ( 100 mg/kg and 200 mg/kg) used base on previous studies done (Vitali *et al.*, 2006).



**Figure 7.** The extraction of *Epilobium hirsutum*

### 3.2. Methods

#### Experimental procedures

Animals were divided randomly into 5 groups, each cage 8 mice (Bhosle, 2013).

**Controlled group:** NS + NS (Physiological Normal saline 0.9% NaCl 0.5 mL; i.p).

**Kindling group PTZ:** NS +PTZ (65 mg/kg; i.p).

**Positive group valproate:** Valproate (100 mg/kg; i.p) 45 minutes later + PTZ (65 mg/kg; i.p).

**Extracted plant + PTZ group:** EH (100 mg /kg; i.p) of the plant extract 45 minutes later PTZ (65 mg/kg; i.p).

**Extracted plant + PTZ group:** EH (200 mg/kg; i.p) extracted plant 45 minutes later PTZ (65 mg/kg; i.p).

The first group or the control group was given normal the saline solution NaCl, 0.9% 0.5 mL; i.p for 7 days, which was prepared daily fresh (Bhosle, 2013; Pahuja *et al.*, 2013; Vitali *et al.*, 2006).

The second group is PTZ group, normal saline solution NaCl 0.9% of 0.5 mL; i.p gave daily for 7 days. On the test day or the 7 - day, PTZ (65 mg/kg; i.p) was dissolving it in 0.5% of the saline solution and administrated after 15 min from saline solution administration (Bhosle, 2013; Pahuja *et al.*, 2013; Vitali *et al.*, 2006).

The third group, saline solution NaCl 0.9% 0.5 mL; i.p was administrated for 7 days on the test day both PTZ (65 mg/kg), and valproate (100 mg/kg) were prepared freshly by dissolving them in 0.5 mL of normal saline solution. However, PTZ applied 20 minutes after valproate administration and used to compare the results of biochemical and mouse behavior of the controlled group and the tested group of the extracted plant (Bhosle, 2013; Pahuja *et al.*, 2013; Vitali *et al.*, 2006).

The fourth group, is the extracted plant of *Epilobium hirsutum*, which prepared daily fresh for 7 days by dissolving it in saline solution (100 mg/kg, 0.5 mL; i.p), on the test day PTZ (65 mg/kg) was applied 30 minutes after the extracted plant administration and the animal behavior was observed for 30 min. (Bhosle, 2013; Pahuja *et al.*, 2013; Vitali *et al.*, 2006).

The fifth group, was the other dose of the plant extract *Epilobium hirsutum* (200 mg/kg of 0.5 mL; i.p), which was prepared daily fresh for 7 days, on the test day PTZ (65 mg/kg) was applied 30 minutes after the extracted plant administration and the behavior animal was observed for 30 minutes. (Pahuja *et al.*, 2013).

### **3.2.1. Pentylene-tetrazole-induced convulsions**

To provide the potential for the anticonvulsant effect and the actual mechanism of *Epilobium hirsutum*, the animals observed for their response to PTZ seizure inducer and their attenuation of seizure standing on 5 phases of convulsion scale after the

administration of PTZ for about 30 min. Thus, immediately after PTZ administration (as previously explained) the mice were observed for, (1) duration of convulsion (number of mice showing convulsions), (2) onset of convulsions (elapsed time from PTZ injection until convulsion occurred) and (3) mortality for 30 min. (Erakovic *et al.*, 2001; Pahuja *et al.*, 2013; Swinyard *et al.*, 1952).

Stage 1: Ear and facial twitching.

Stage 2: Head nodding, head clonus, and myoclonic jerks.

Stage 3: Unilateral forelimb clonus.

Stage 4: Rearing with bilateral forelimb clonus.

Stage 5: Generalized Tonic–Clonic seizure, with a loss of righting reflex.

The animals considered kindled if they exhibited stage 4 or 5 of the consecutive trials; however, if no convulsion occurred within the limited time, the animals considered to be protected. After testing the mice with PTZ, they were immediately placed in the open field test and then Rota rod test to evaluate neurological deformities.

### **3.2.2. Evaluation of neurological deformities**

A neurological disorder is a result of dysfunction of one part or more of the brain areas or even affecting the general nervous system, as the consequent symptom of physiological and psychological was seen with most of the neurologic patients (WHO, 2016). In our research, we have evaluated our study by examining, brain electrical abnormalities, by observing paralysis, muscle weakness, poor coordination, and confusion behavior of the mice. After using PTZ test, animals immediately placed for open field test and Rota Rot test, which carried on the 7th day or the tested day.

#### **3.2.2.1. Open field test**

The open-field apparatus or locomotors activity device made of acrylic/plexiglas (transparent walls so the mice could be visible) with black floor, of the large size 72 x 72 cm divided into 8 squares of equal area. The lines divided the floor into sixteen 18 x 18 cm squares and the central square (18 cm x 18 cm) was drawn in the middle of cage

(The middle square is used to test the mouse locomotor activity and their behavior crossing the lines of the trailing chamber many times during a test session) (Brown *et al.*, 1999). It is used to measure mental activity, anxiety, exploration, depression, and locomotion as well as psychotic emotion. However, increase in the count of mice movement was regarded to central nervous stimulation while a decrease in the number of mice movement regard to central nervous depressant activity (Correia *et al.*, 2016) as Figure 8 shows.



**Figure 8.** Open field apparatus

### **The Procedure**

After PTZ administration of the 4 groups (PTZ group, the positive group valproate 100 mg/kg, and the plant extract groups 100 and 200 mg/kg; i.p), the mice of the all groups were immediately handled base of their tails in the center of the open field with the four paws and allowed to explore the apparatus for 5 minutes. After the open field test completed, they placed to be tested for Rota rod test while the open field was hygiene with 70 % ethyl alcohol and left to dry between with each mouse. The mice observed for their behavior of locomotor activity, and the scored were standing according to (Brown *et al.*, 1999; Machado *et al.*, 2015).

#### **3.2.2.2. Rota rod test**

Rota-rod test used to test skeleton muscles relaxation as it assesses motor coordination, motor learning, intoxication, sedation, stamina, motor memory (long-term skill or procedural memory) and balances of the mice. This by testing the ability of mice to remain on a rotating rod during the 300 seconds. However, the mouse failed off the rod rotating at different speeds or under continuous acceleration as Figure 9 shows (Asuntha et al., 2010).

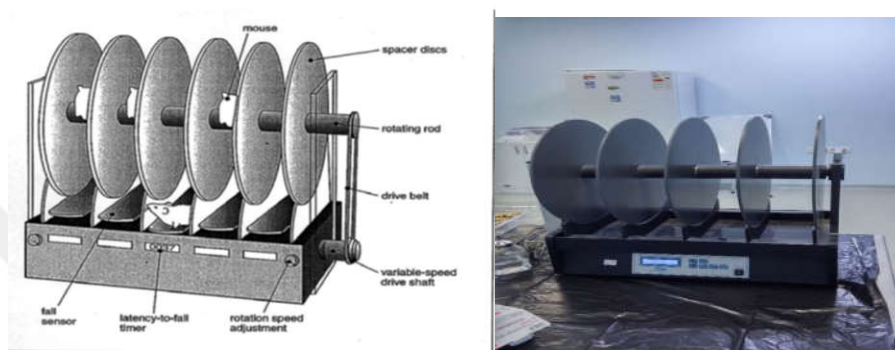


**Figure 9.** Rota rod apparatus

### **The Procedure**

In the 7 day or the test day, after PTZ (65 mg/kg) administration of the four groups, the mice of the all groups were handled from their tails at all times to be placed on the Rota rod stand of 25 mm diameter at the speed of 10 rpm for 3 minutes per run (300 seconds) with replacing the gray rubber foam prior with each new group (Machado *et al.*, 2015). The device initially rotated at 4 rpm with constant speed to allow the mice taking their positions. Once they get their position, we accelerated the rotating from 4 rpm to 10 rpm in 300 sec. The apparatus cleaned with water, and then with 50% ethanol with every group used for the test and the time assumed of the mouse who remained on the Rota-rode device along the 300 seconds.

### **3.2.3. Sample prepares for biochemical assay**

Animals were sacrificed by removing the brains quickly and washed them with cold saline solution two times, then placed them in depth glass labeled bottle of deep freeze of (- 30 C) until the biochemical assay processor.

The brains were cut into smaller pieces using scissors, and were homogenized using 5 ml ice-cold, Tris-HCL buffer of (1 mmol/L EDTA, 0.32 mol/L sucrose and 10 nmol/L Tris-HCl, pH 7.4) using (Ultra Turrax T25, IKA, Staufen, Germany), homogenizer and a glass of porcelain homogenizer (20 kHz frequency ultrasonic, Bandelin Sonupuls) that was homogenized for 8 min to break down the cell continents and gets solution A in this phase. (Ilhan *et al.*, 2004; Kiasalari *et al.*, 2012, 2013; Rahmati *et al.*, 2013).

However, the homogenate solution A was centrifuged at 9500 rpm for 30 min to separate the debris (Xia *et al.*, 1997). The Clear upper supernatant fluid was extracted to determine antioxidant enzyme superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activity providing that the temperature was constant +4 C at all preparation procedures. (Ilhan *et al.*, 2004; Rahmati *et al.*, 2013; Kiasalari *et al.*, 2012; 2013).

### **3.2.3.1. Total protein concentration determination**

Total protein concentration of homogenates was determined by the method of (Lowry *et al.*, 1951). Bovine A solution was used as a standard. Different concentrations (10 - 200 µg) of standard protein bovine A solution were processed for preparation of a standard curve. Measurements performed against blank at 695 nm wavelength. The values expressed as mg protein/g wet tissue (mg/g).

### **3.2.3.2. Malondialdehyde (MDA) evaluation**

MDA level was determined according to the method described by (Sushil *et al.*, 1989). As PTZ induced brain tissue to undergo to oxidative stress, lipids peroxide were evaluated by measuring MDA level. 2 mL of the 10% homogenate solution was added to 2 mL 30% Trichloroacetic acid than 2 mL of 0.8% Thiobarbituric acid was added to

the mixture. The tested tubes covered with aluminum foil and kept on shaking water bath for half an hour at 80 - 90 C and at PH 2 - 3 after half an hour; the tubes taken out and kept in ice-cold water for more than 30 minutes, where they placed in centrifuge at 3000 - x g for 15 min. However the supernatant solution absorbance was at 535 nm at room temperature against appropriate blanks (2 mL distilled water, 2 mL of 30% TCA and 2 mL of 0.8% TBA) (Fernandez *et al.*, 1997). The content of malonaldehyde (MDA), expressed as n moles formed per milligram of protein in the tissue, was calculated using the formula: Concentration = A x (V/E) x P. Where, A is the volume of solution, E is extinction Coefficient ( $1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$ ) and P is the protein content of tissue calculated as milligram of protein per gram of tissue (Bhosle, 2013; Ilhan *et al.*, 2004).

#### **3.2.3.3. Glutathione peroxidase (GSH-Px) evaluation**

GSH-Px level determined according to the method described by (Paglia and Valentine, 1967). The amount of glutathione in tissue expressed in  $\mu\text{mol/g}$  of tissue  $\mu\text{mol/ mg}$  wet tissue:  $[A/13600] \times \text{dilution factor} \times 1000$ .

#### **3.2.3.4. superoxide dismutases (SOD) evaluation**

Total SOD activity tested based on the Sun method (Sun and Oberley, 1988). The principle of the method based on the inhibition of nitroblue tetrazolium (NBT) reduction of the xanthine-xanthine oxidase system as a superoxide generator. It expressed in Units per milligram protein.

#### **3.2.3.5. Catalase (CAT) activity determination**

Catalase (CAT) measured according to the method of (Aebi, 1984). A solution of  $\text{KH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{H}_2\text{O}_2$  were mixed to be added to water to provoke an inhibition of 50% diluent rate (i.e., the uninhibited reaction). Spectrophotometrically absorption at 230 nm and results expressed as  $\mu\text{M/min}/\mu\text{g}$  protein and read against  $\text{H}_2\text{O}_2$  and water.

#### **3.2.3.6. Glutathione (GSH) evaluation**

Reduced brain glutathione (GSH) was measured according to the method of (Ellman, 1959). An equal quantity of homogenate was mixed with 10% trichloroacetic acid, which was taken to make solution A and centrifuged to separate the proteins. However, 0.01 mL of solution A was taken to 2 mL of phosphate buffer (pH 8.4), 0.5 mL of 5'5-dithiobis (2-nitrobenzoic acid) DTNB, and 0.4 mL of distilled water was added. The mixture was vortexed and the absorbance read at 412 nm against blank of distilled water, the amount of glutathione in tissue expressed as  $\mu\text{mol/g}$  of tissue (Bhosle, 2013).

#### **3.2.3.7. Statistical data analysis**

Results are expressed as  $X \pm \text{SEM}$  analysis of variance (ANOVA) was performed, and the statistical comparisons among the groups were carried out with *post-hoc* Tukey's test for normally distributed variables, or with nonparametric Benferroni test for non-normally distributed data using a statistical package program (SPSS 22.0 for Windows).

## 4. RESULT

### 4.1. Effect of *Epilobium hirsutum* on induction of kindling by PTZ

Testing the mice with the two different doses of EH (100 and 200 mg/kg; i.p) have caused severe conception during the 7 days, which caused a death of one mouse from EH (100 mg/kg) group while three mice from EH (200 mg/kg). However, we have noticed a decrease of normal animal activity from the first day after EH injection, at the both doses of EH (100 and 200 mg/kg; i.p). On the seventh day, all the mice were undergoing for PTZ kindling test-inducing convulsions except the controlled group.

Valproate is the most common AED used with the broad-spectrum activity in suppressing seizure provoke. However, we used the positive group (valproate 100 mg/kg + PTZ 65 mg/kg) to compare the results with PTZ (65 mg/kg) group and the other extracted plants (100 and 200 mg/kg; i.p).

In our present experiment, PTZ (65 mg/kg; i.p) group showed to develop a standard pattern of limbic 5 phases motor seizures of twitch, short-lasting clonic phase, then wild running, falling of animal and finally tonic phase.

Moreover, PTZ showed a significant decrease in the onset of convulsion by  $p < 0.05$  when it was compared with EH (200 mg/kg + PZ), and a significant increase in duration by  $p < 0.001$  comparing it with EH (200 mg/kg + PTZ) group. However, in PTZ group we have recorded one death of the mice (14%) comparing it with EH (200 mg/kg + PTZ) group. Table 2, as well as Figure 10 and 11, elucidate the explained results.

In EH (200 mg/kg + PTZ) group, showed the significant higher onset of convulsion of  $p<0.05$  compared with the positive group (PTZ + Valproate) and PTZ group, while the duration of convulsion was significantly lower  $p<0.05$  comparing with te positive group (PTZ + Valproate) and  $p<0.01$  comparing with PTZ group. Table, 2 as well as Figure 10 and 11, elucidate the explained results.

In EH (100 mg/kg + PTZ) group, showed lower onset of duration  $2.80 \pm 8.70$  comparing it with EH (200 mg/kg) group. However, EH (100 mg/kg + PTZ) group duration was significantly lower  $p<0.01$  comparing it with PTZ and  $p<0.05$  comparing it with the positive group (PTZ + Valproate). While the mortality rate was (14%), which is one death of the mice in EH (100 mg/kg + PTZ) group. Table 2, as well as Figure 10 and 11, elucidate the explained results.

In the positive group (PTZ + Valproate), the onset of convulsion showed to be significantly lower  $p<0.05$  compared with EH (200 mg/kg + PTZ) group. While the duration of convulsion was significant higher  $p<0.01$  comparing it with EH (200 mg/kg + PTZ) group and  $p<0.05$  comparing it with EH (100 mg/kg + PTZ) group. Table, 2 as well as Figure 10 and 11, elucidate the explained results.

**Table 2.** Effect of Epilobium hirsutum (EH) on the pentylenetetrazole-induced convulsion in mice

| Groups        | Onset of clonic convulsion (s)<br>$\bar{X} \pm \text{SEM}$ | Duration of convulsion (s)<br>$\bar{X} \pm \text{SEM}$ | The number of mortality |
|---------------|------------------------------------------------------------|--------------------------------------------------------|-------------------------|
| Control       | 0.00±0.00                                                  | 0.00±0.00                                              | 0.00±0.00               |
| PTZ           | 2.20± 5.28 <sup>c</sup>                                    | 4.33 ± 0.33 <sup>a,b</sup>                             | 1/7 (14%)               |
| 100 mg EH+PTZ | 2.80± 8.70                                                 | 2.00 ± 0.00 <sup>b,c</sup>                             | 1/7 (14%)               |
| 200 mg EH+PTZ | 4.50± 6.72 <sup>c,cl</sup>                                 | 1.80 ± 0.20 <sup>a,bI</sup>                            | 0/5 (0.0%)              |
| PTZ + VPA     | 2.04± 1.47 <sup>cl</sup>                                   | 3.60 ± 0.25 <sup>c,bI</sup>                            | 0/6 (0.0%)              |

a:  $p<0.001$ , b,b1,:  $p<0.01$ , c,cl:  $p<0.05$  (Similar letter fields show significance at that letter level).

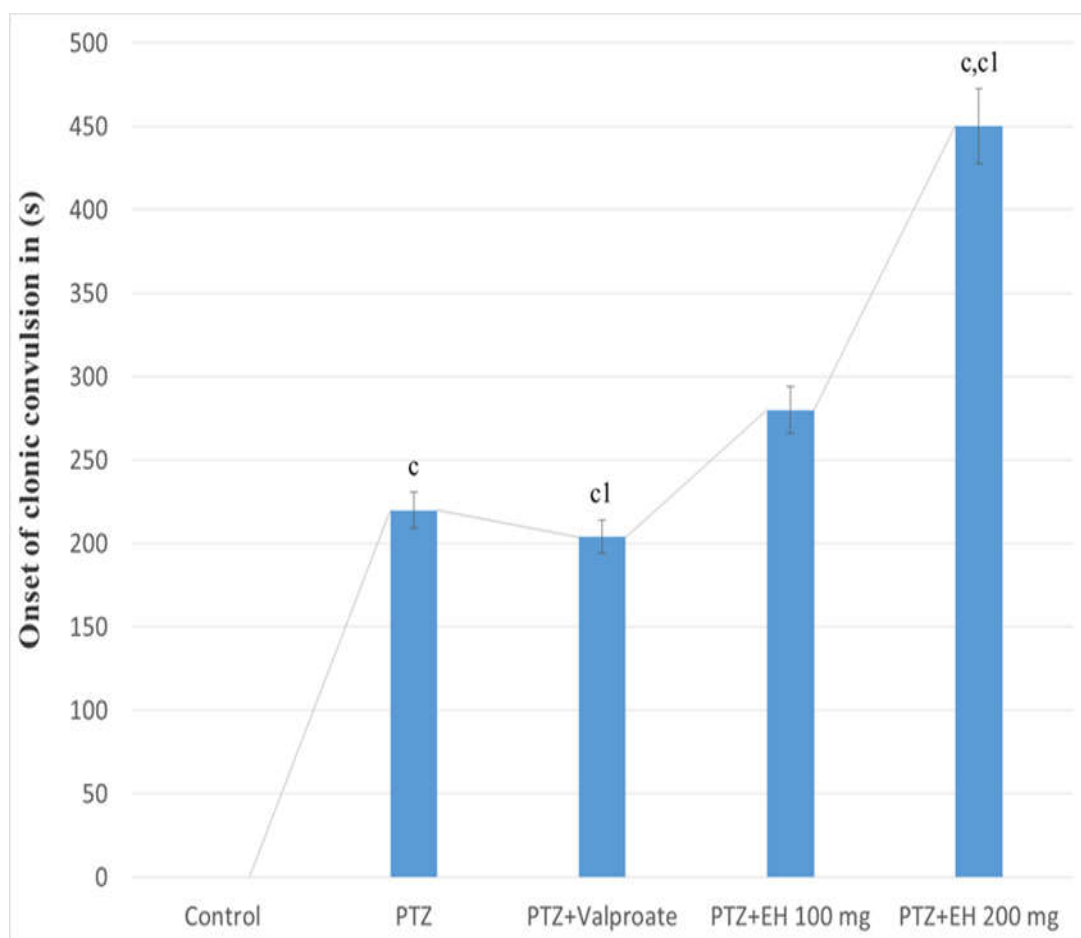


**Figure 10.** Effect of valproate (100 mg/kg) and two doses of *pilobium hirsutum* (100 and 200 mg/kg) on the latency of arriving to phase 5 of seizure. n = 8 in each group. VA and EH indicate valproate and *pilobium hirsutum* respectively.

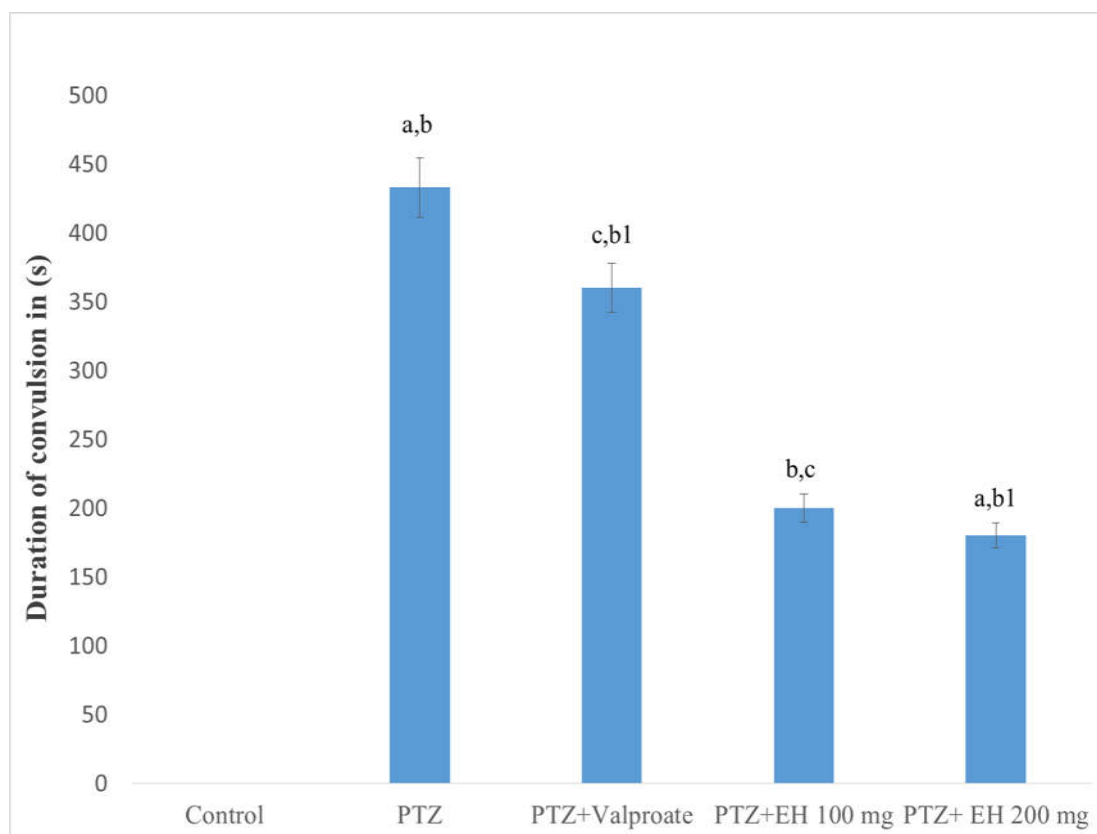


**Figure 11.** Effect of valproate (100 mg/kg) PTZ (65 mg/kg) and the two doses of *pilobium hirsutum* (100 and 200 mg/Kg) on the time in the phase 5 n = 8 in each group. a:  $p < 0.001$ , b, b1  $p < 0.01$ , c, c1  $p < 0.05$  shows significant difference as compared to PTZ-kindled group.

#### 4.2. Effect of *Epilobium hirsutum* on open field

The open field widely used to test gross animal behavior, the nerve excitability, anxiety, mental activity, exploration, and locomotion (Correia et al., 2016). However, the epileptic patient has shown to develop different psychotic impairments like anxiety and showed to be less mental activity comparing them with normal individuals.

In our current study, mice tested for their gross behavior and exploration ability by releasing them to the center of the open field. They have not shown any movement achievement after seizure provoked during the given time 5 minutes as Table 3 elucidated.

In the controlled group, mice showed almost normally movement by crossing some squares  $19.00 \pm 5.261$  during the given 5 minutes; however, exporting the center was rarely by  $2.75 \pm 0.25$  times.

The mice who expressed seizure provoke with the challenging dose of PTZ (65 mg/kg), did not show any significant movement and almost frizzed for more than 5 minutes.

The same was with the positive group (valproate 100 mg/kg + PTZ 65 mg/kg), which showed some mice crossing some squares after seizure provoked but most of them did not even cross the center.

However, EH (100 mg/kg) showed the significant decrease in exploration behavior comparing them with the controlled group by  $p < 0.05$  as they have attenuated seizure provoke. While the dose of EH (200 mg/kg), open field was not influencing and did not show any significant difference.

| Groups        | The number of<br>square cross<br>$\bar{X} \pm \text{SEM}$ | The number of<br>center cross<br>$\bar{X} \pm \text{SEM}$ |
|---------------|-----------------------------------------------------------|-----------------------------------------------------------|
| Control       | 19.00± 5.261                                              | 2.75 ± 0.25 <sup>c</sup>                                  |
| PTZ           | —                                                         | —                                                         |
| 100 mg EH+PTZ | 4.333±2.028                                               | 1.67 ± 0.33 <sup>c</sup>                                  |
| 200 mg EH+PTZ | 15.43± 4.466                                              | —                                                         |
| PTZ + VPA     | 8.000±4.000                                               | —                                                         |

**Table 3.** Comparison parameters of Open-field test among four different experimental groups

c:  $p < 0.05$

#### 4.3. Effect of *Epilobium hirsutum* on Rota rod

In our current studies, testing the motor coordination of the experimental mice by using Rota rod did not give significant results with the anticonvulsion plant of the *Epilobium hirsutum*. Almost all the groups had the same result, falling twice within the 5 seconds. Which was an indication of CNS depression and neurotoxicity and uninfluenced of the EH on muscular relaxation as an anticonvulsion agent.

#### 4.4. Biochemical results of oxidant/antioxidant stress inducers

Capturing the brain for the biochemical assay was an efficient indicator for brain oxidant/antioxidant mechanism that induced by PTZ kindling with a lethal dose (65 mg/kg) on experimental animals. Oxidization stress caused by (ROS) reaction evaluated by measuring MDA level, which considered index for lipid peroxidation evaluation by

PTZ inducer. The high level of MDA was an indication of brain tissue injury caused by (ROS). However, in our experimental, PTZ group showed the significant increase  $p < 0.01$  of MDA level comparing it with the control group,  $p < 0.05$  comparing them with EH (100 mg/kg) group, and  $p < 0.05$  comparing it with positive group (PTZ + Valproate). As Table 4 and Figure 12 elucidate.

Superoxide dismutase (SOD) that has two isoforms forms (Mn-SOD (MnSOD) resides in the mitochondria, Copper-zinc-SOD is constitutive in lysosomes, nucleus, and between membrane internal and external mitochondria and peroxisomes), which catalyze the conversion of superoxide anion into  $H_2O_2$  and  $O_2$  that prevents the oxidative stress-induced cellular damage (Kiasalari *et al.*, 2013). In our study superoxide dismutase (SOD) activity in PTZ group were the significant decrease  $p < 0.001$  comparing it with the controlled group. However, (SOD) in EH (100 mg/kg) group showed the significant increase by  $p < 0.05$  comparing it with PTZ group. Both groups of EH (200 mg/kg) and (PTZ + Valproate) showed the significant decrease in (SOD) enzyme level comparing it with controlled group  $p < 0.01$ ,  $p < 0.05$  respectively as Table 4 and Figure 14 elucidate.

In studying glutathione peroxidase (GSH-Px) in PTZ group showed the significant decrease by  $p < 0.01$  comparing it with the controlled group; however, (GSH-Px) in (200 mg EH + PTZ) showed the significant decrease by  $p < 0.05$  comparing it with the controlled group. Both groups of EH (100 mg/kg + PTZ) and the positive (PTZ + Valproate) groups reported decreased in (GSH-Px), Table 4, as well as Figure 13, elucidate the explained results.

In studying glutathione (GSH) enzyme in PTZ group showed the significant decrease in its level by  $p < 0.01$  comparing it with both controlled group and the positive group (PTZ + Valproate). However, in (EH 200 mg/kg) showed the significant decrease by  $p < 0.05$  comparing it with the controlled group, Table 4 and Figure 16 elucidate.

Catalase concentration in the brain is much lower than known antioxidant enzymes (SOD, GSH-PX). However, it mainly found in the mitochondria and

peroxisomes that catalyzes the conversion of hydrogen peroxide to water and oxygen. In studying the catalase activity (CAT) in PTZ group was decrease comparing it with the rest of the groups  $1.49 \pm 0.060$ ; however, all of (EH 100 mg/kg), (EH 200 mg/kg) and (PTZ + Valproate) showed a lower level of (CAT) comparing them with the controlled group as Table 4 and Figure 15 elucidate.

**Table 4.** Antioxidant enzymes (SOD, GSH-Px and CAT) activities, MDA and GSH in control, PTZ, 100 mg EH+PTZ, 200 mg EH+PTZ and PTZ + VPA groups in brain tissue samples

| Groups                   | SOD<br>(IU/mg pt)<br>$\bar{X} \pm \text{SEM}$ | GSH-Px<br>(IU/mg pt.)<br>$\bar{X} \pm \text{SEM}$ | CAT<br>(IU/mg pt.)<br>$\bar{X} \pm \text{SEM}$ | MDA<br>(nmol/mg pt.)<br>$\bar{X} \pm \text{SEM}$ | GSH<br>( $\mu\text{mol/g pt.}$ )<br>$\bar{X} \pm \text{SEM}$ |
|--------------------------|-----------------------------------------------|---------------------------------------------------|------------------------------------------------|--------------------------------------------------|--------------------------------------------------------------|
| <b>Control</b>           | 282.12 $\pm$ 4.08 <sup>a,b,c</sup>            | 0.40 $\pm$ 0.021 <sup>b,c</sup>                   | 1.66 $\pm$ 0.074                               | 1.29 $\pm$ 0.038 <sup>b</sup>                    | 21.11 $\pm$ 0.36 <sup>b,c</sup>                              |
| <b>PTZ</b>               | 235.11 $\pm$ 4.09 <sup>a,c1</sup>             | 0.27 $\pm$ 0.015 <sup>b</sup>                     | 1.49 $\pm$ 0.060                               | 1.51 $\pm$ 0.027 <sup>b,c,c1</sup>               | 17.10 $\pm$ 0.49 <sup>b,b1</sup>                             |
| <b>100 mg<br/>EH+PTZ</b> | 259.48 $\pm$ 7.37 <sup>c1</sup>               | 0.32 $\pm$ 0.014                                  | 1.55 $\pm$ 0.134                               | 1.33 $\pm$ 0.034 <sup>c</sup>                    | 19.12 $\pm$ 0.63                                             |
| <b>200 mg<br/>EH+PTZ</b> | 247.81 $\pm$ 4.43 <sup>b</sup>                | 0.29 $\pm$ 0.027 <sup>c</sup>                     | 1.53 $\pm$ 0.078                               | 1.43 $\pm$ 0.040                                 | 18.42 $\pm$ 0.83 <sup>c</sup>                                |
| <b>PTZ + VPA</b>         | 253.37 $\pm$ 5.26 <sup>c</sup>                | 0.32 $\pm$ 0.021                                  | 1.58 $\pm$ 0.114                               | 1.32 $\pm$ 0.045 <sup>c1</sup>                   | 20.84 $\pm$ 0.60 <sup>b1</sup>                               |

**a**  $p < 0.001$ , **b, b1**  $p < 0.01$ , **c, c1**  $p < 0.05$  (Similar letter fields show significance at that letter level).

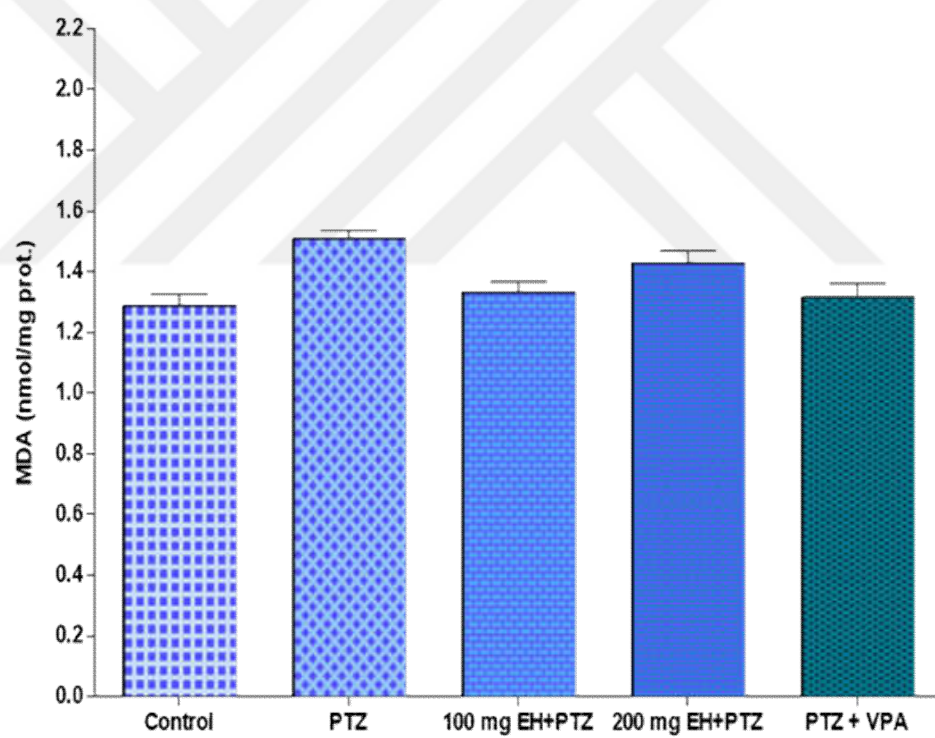


**Figure 12.** MDA evaluation level in (nmol/mg pt.) anoug the 5 groups

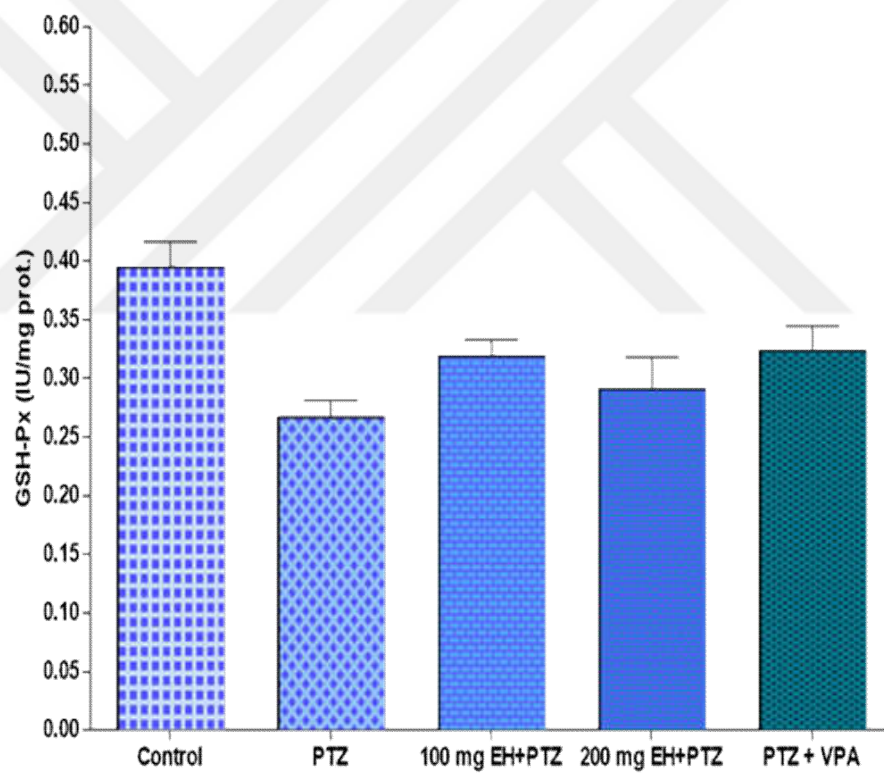


**Figure 13.** GS-HPX evaluation level in (IU/mg pt.) among the 5 groups

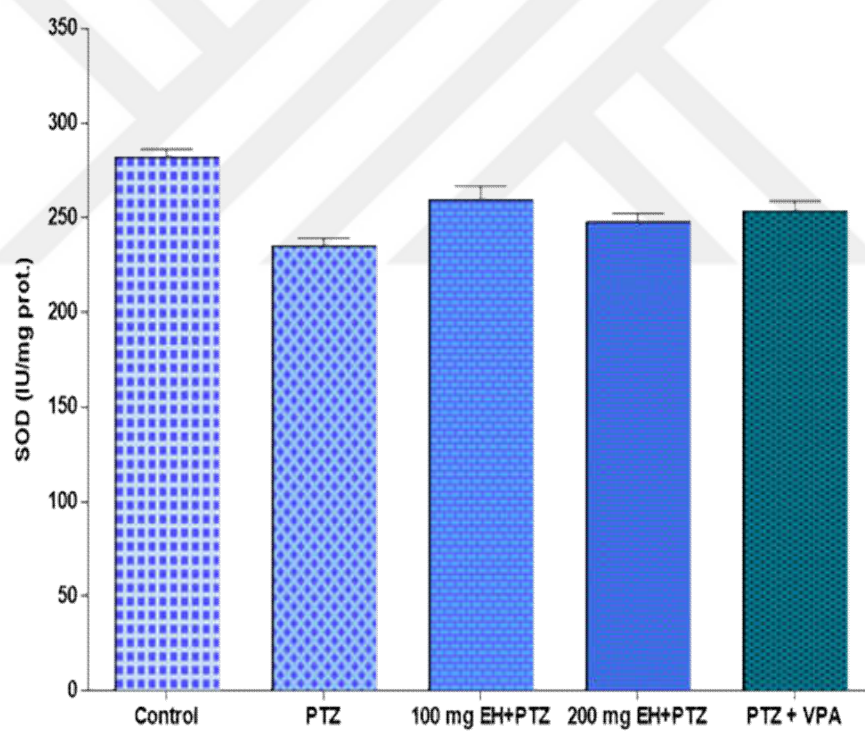


**Figure 14.** SOD evaluation level in (IU/mg pt) among the 5 groups

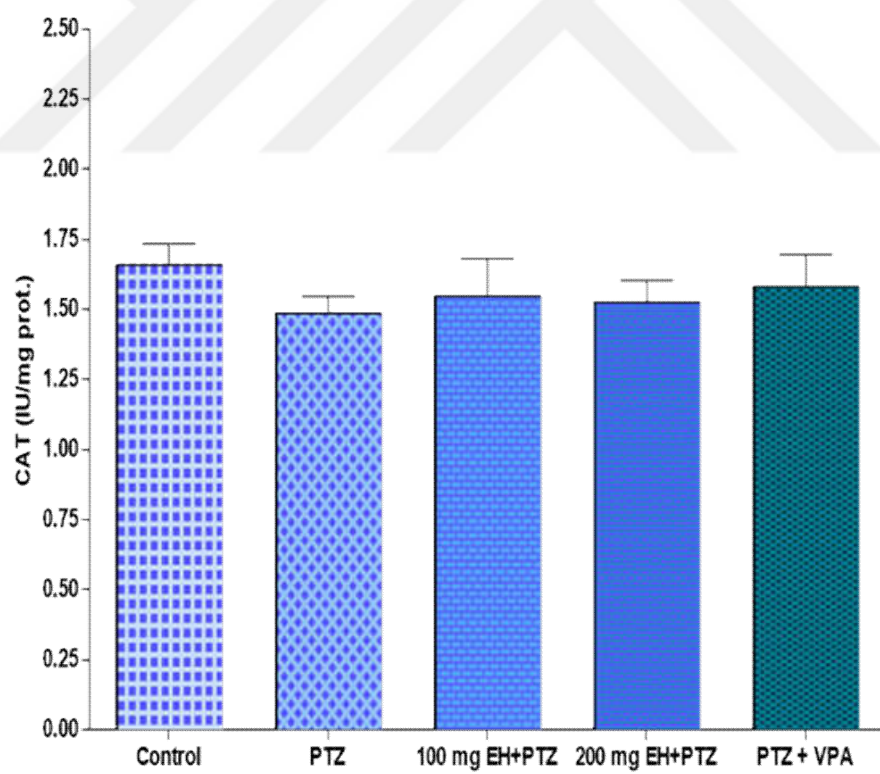


**Figure 15.** CAT evaluation level in (IU/mg pt.) among the 5 groups

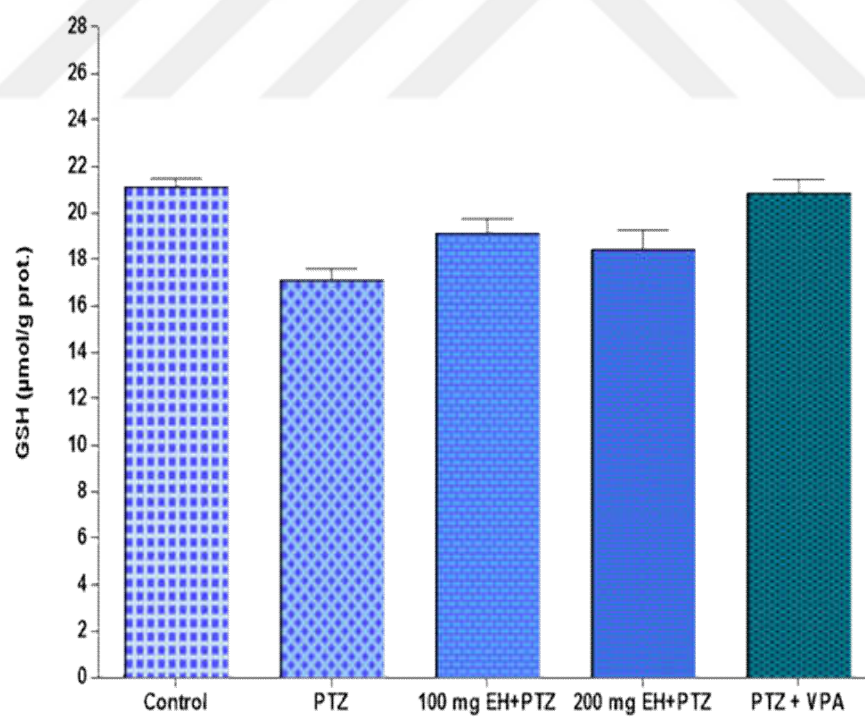


**Figure 16.** GSH evaluation level in ( $\mu\text{mol/g pt.}$ ) among the 5 groups

## 5. DISCUSSION

Before pharmacy science developed, and since the earlier time of the human known, folk depend on medical plants and their sources of treatment in manifold health problems such as disorders of CNS, from anti-depress, improve the mood, relaxation, re-memorizing, and epilepsy, which considered the most controversial neurological disorder of imbalance between excitatory and inhibitory neurotransmitters (Ratcliffe, 2006).

Until the recent time, scientists have not found safe treatment with full seizure control. They have done continual researchers on finding effective therapeutic agents with less side effects.

Folk played a fundamental role in guiding the scientists by offering them data from the ancient and their trends toward the use of traditional therapy, which based on their predecessor's knowledge of using the plant for health purpose. This new interest, gave born of new science known ethnopharmacology, which based on collecting data from the folk and their usage of the plant in health wellness, then producing these data to pharmacologist to investigate the plant active continents and come up with an effective agent with less toxicity effect and gain a beneficial economic value (Patwardhan, 2005).

*Epilobium hirsutum* (EH) is an herbaceous plant, which has used from the ancient as medical care for inflammation, prostate, gastrointestinal, and menstrual disorder (Kiss *et al.*, 2011; Stolarczyk *et al.*, 2013; Vitali *et al.*, 2006); however, in our present study, investigating the anti-convulsion effect of lyophilized extracts of *Epilobium hirsutum* was established successfully by PTZ a seizure inducer. Moreover, testing for the muscular relaxation effect and locomotors activity evaluated using Rota rod test and open field test, respectively.

From previous studies done, we have known the active chemical components in *Epilobium hirsutum* like flavonoids, phenolic acids, steroids, and tannins. Those compounds lighted our research as they showed to have an antioxidant effect that suppresses the damage caused by PTZ (Kiss *et al.*, 2006; Stolarczyk *et al.*, 2013). However, the mechanism behind the anticonvulsion effect cannot be elucidated by single research done; it demands further researchers in determining the active plant components and which one is in its highest concentration that wins to suppressing seizure provoke.

Studying oxidative stress of CNS accomplished by using PTZ kindling model, which is a chemical kindling seizure inducer, used in rodent model after single or sub-convulsive doses. It blockers chloride channel of GABA A receptor, caused local injury of brain tissue, an increase of the hyperactivity of glutamic receptors (NMDA) and causing calcium ions entering into the nerve cells and liberation of free radicals (Corda *et al.*, 1992; Costa, 1994; Ilhan *et al.*, 2006).

As AEDs work by binding to GABA receptor, they enhance the GABAergic inhibitory neurotransmission by attenuating seizure provoke. As a result, in our present study, we have used the positive group a drug of valproate (100 mg/kg) (one of the AEDs, which is known to have antioxidant effect by scavenger the free radical components and produce a neuroprotective effect, by this, safe brain's neurotic cells from injury) (Khalili *et al.*, 2011). However, its broad-spectrum usages against the partial and general seizures and different neurology and psychiatry causes such as bipolar disorders, panic disorders, and migraine, made it nominated for most of PTZ kindling examinations (Ilhan *et al.*, 2006).

As the brain is highly rich with a dipodic membrane of unsaturated free fatty acids and poor in antioxidant enzymatic defense system, it makes it more vulnerable for aerobic metabolism and susceptible to oxidative stress that end with destroying of its membrane and ban its function (Ji and Leichtweis, 1997). Neuronal DNA also noticed to alternate its function, which is not repaired in the brain tissue (Katyal and McKinnon, 2008).

The reactive oxygen species (ROS) of ( $O_2^-$ , OH, NO, and  $ONOO^-$ ) usually develop when an aerobic metabolism is taking place. They are endogenous sources of mitochondrial homogeneous system subversion or of enzymatic or autooxidation reactions that form imbalance between (ROS) and cellular antioxidant defense system. Studying the influence of PTZ biochemically showed to increase the elevation of those (ROS) and diminish in antioxidant enzymes (Ilhan *et al.*, 2005).

In our current study, we have used the experimental animals in their close to understand the abnormality causes of seizure in human. However, the extracted plant of EH was given for 7 days, at the both doses of (100 and 200 mg/kg; i.p) with separated groups. From the first day of administration, showed a significant decrease in their normal activities.

On the tested day or (the seventh day) PTZ (65 mg/kg; i.p) was administrated to the all groups, except the controlled to study the plant defendability against seizure. both dose of (100 and 200 mg/kg; i.p) showed seizure attenuating by significantly delay in the onset of convulsion and have short duration of convulsion when it compared with PTZ group and the positive group of valproate. This was an indication of plant medical prosperity and their potent pharmacologically components with the less toxicity and economic demand. However, the mortality showed at the dose of 100 mg/kg (14%), which considered being less comparing with the completely experimental animals.

Moreover, the extracted plant of EH showed to have more the potent anticonvulsant effect than the AEDs of valproate (100 mg/kg; i.p), which did not prevent seizure provoke when they compared experimentally. The same result was seen in studying the antioxidant effects of nigella sativa oil against pentylentetrazol-induced kindling in mice and ginkgo biloba extract (Ilhan *et al.*, 2005; 2006). This result was explaining the effect of the extracted plant in pretreatment process of the male mice for 7 days, and before the administration of the acute phase of PTZ (65 mg/kg), in increasing the antioxidants enzymes, which showed to have an effective role in scarfing free radicals and produce neuroprotective activity in the present of PTZ seizure inducer.

Studying the anticonvulsive effect of the extracted plants have usually cared with different methods, and with the same aim, proving the anticonvulsive effect of the extracted plant using the epileptic induced animals. For example studying the anticonvulsive effect of hydroalcoholic extract of *ferula assa foetida* gum using pentylentetrazole induced kindling in male mice, showed significant reduction in the remaining 5 phases of seizures by the different administrated doses (25 mg, 50 mg, 100 mg) during a period of 22 days of sub-consultative dose PTZ of 35 mg/kg (Kiasalari *et al* 2013). However, the challenged dose of PTZ (75 mg/kg; i.p) was administrated at the 24 or the last day, to study tonic-clonic seizures or perhaps death.

Biochemically, PTZ influence the glutamate receptors (NMDA) by opening its channel and induce calcium ions entering into the nerve cells; however, studying the biochemical assay of family members of opioid plant, reported to have analgesic effects through stimulation of opioid receptors in the central nervous system, which in turn inhibits calcium entry into nerve cells and provoke the seizure. Thus, *ferula* family thought to have the same mechanism of action of stimulation action of opioid receptors in the post-synaptic neurons (Fazly *et al.*, 1997).

Evaluating brain antioxidant enzymes in *ferula assa foetida* gum extract, showed the significant increase in (SOD) antioxidant enzymes while decrease in (MDA) (a lipid peroxidation marker) and (NO) molecule that increased with acute seizures and progression of PTZ.

In our current study, we had limited evaluating oxidative stress of (MDA), as an index of lipid peroxidation, which showed to be a significant decrease in EH at the doses of (100 and 200 mg/kg; i.p) when it compared with PTZ group. Although valproate did not show its effect theoretically, in the biochemical assay gave a clear elucidation of the antioxidant effect of valproate in the brain tissue of the epileptic mice.

Evaluation of MDA in PTZ group was a sign for phospholipid membrane damaging of the brain tissue, diacylglycerols, free fatty acids (FFAs), and lipid peroxides (Costa, 1994).

The formation of superoxide radicals, which have unpaired electrons in their outer shell, made them highly reactive to various molecules such as, changes in DNA bases, different proteins (receptors and enzymes) and cause lipid membrane rupture, which mainly indicated by measuring malondialdehyde (MAD) level.

However, in normal physiological conditions, the brain injured tissue caused by free radicals, used to be protected by antioxidant defense system such as enzymatic (glutathione peroxidase, superoxide dismutase, and catalase), or non-enzymatic (glutathione and uric acid), and dietary (vitamins E, A and C, quinones, b-carotene, albumin, bilirubin, and flavons), which convert most (ROS) to water from (Wilson, 1997).

In epilepsy, most of these oxidation products, produced during seizure provoke that lead to tissue injury. However, and as most of the antioxidants defend enzymes in the brain are in small quantity, they lose their function against the potent of (ROS). Chronic disease like Alzheimer's, Parkinson's, anoxia and ischemia of brain and heart as well as arteriosclerosis, rheumatism, and cancer, showed almost the same deduction (Ilhan *et al.*, 2005; 2006).

In our current study, we have evaluated the antioxidant non-enzyme GSH, which showed an increase in both doses of the extracted plant (100 and 200 mg/kg) comparing it with PTZ group. This mechanism was enough to convince us the antioxidant effect that exists in the extracted plant in the present of PTZ inducer. While in the positive group, showed its highest concentration comparing it with the two doses of the extracted plant.

On the other hand, evaluating the antioxidant enzyme of (GSH-Px), in our experiment, showed to be increased in the both doses of the extracted plants when they

were compared with PTZ; however, in the positive group of valproate showed higher concentration comparing it with the doses of the extracted plant.

While (SOD), in our experiment, showed its higher concentration in the both doses of the extracted plant when it compared with PTZ group; however, the positive group showed a slight decrease in its level comparing it with EH (100 mg/kg) does.

Evaluating (CAT) enzyme in our experiment, showed its higher concentration in the both treatments doses of the extracted plant when it was compared with PTZ group, while in the positive group showed a slight decrease in its level comparing it with EH (100 mg/kg) does.

In study of (Freitas, 2009), reported that the antioxidant enzymes are not showing any alternation during the 6 h of seizure inducing by pilocarpine injection, while the mice that pretreated with antioxidant agent of lipoic acid 30 minutes before pilocarpine administration, showed 60% of attenuated brain damaging and during the 24 h no mortality have been reported. From this study, we can suggest the pretreatment effect of EH, which was administrated for 7 days, caused an increasing of brain antioxidant enzymes that worked as neuroprotective by scarfing the free radicals produced by PTZ seizure inducer and attenuate convulsion. While the PTZ group failed in proving antioxidant enzymes alternation within the 6 h.

From this variation, we have noticed an increase the antioxidant enzymes in the positive group of valproate in biochemically assay, while in the theoretical examination, showed being decreased in the onset of convulsion while an increase in the duration of convulsion, which gave us many doubt suggestion that ought to study in further research.

Moreover, the biochemical assay as well as the open field test, showed more positive effect with the EH dose of 100 mg/kg than 200 mg/kg, which give as indication of being depended dose (100 mg/kg).

In a hydroalcoholic extract of *Cyperus rotundus* and *Brassica nigra* (Kiasalari *et al* 2012; Khalili *et al.*, 2011), showed the significant reduction in intensity and duration of PTZ induced seizure with the depended doses of 400 mg and 300 mg respectively. While biochemically, both doses showed the significant increase of antioxidant enzymes of SOD, which catalyze the conversion of superoxide to hydrogen peroxides and finally water that safe neurotic cells from oxidation reaction and scratch up of their membrane. However, they have evaluated the endogenous molecule of NO, which played an active role in many physiology functions; in epilepsy is showed synaptic plasticity, neuronal excitability regulation, and seizure activation.

NO is a small endogenous molecule can diffuse simply through the membrane. In epilepsy, it showed to have a contravention role in pro-convulsion effect and protective effect. In a study of (Przegalifiski *et al.*, 1996), reported inhibition of NOS has prolonged seizure duration and increase mortality, as positive feedback activation of NMDA receptor activation. While seizure inducers like PTZ showed to accumulate NO in NMDA receptor, which in turn activates the second messenger of (guanosine 3',5'-cyclic monophosphate (cGMP) that stimulate in NMDA receptor activation or increase in their formation. However, anticonvulsive drugs showed to inhibit this second messenger and by this further activation of NMDA receptor (Lapouble *et al.*, 2002).

While in study of *Desmodium triflorum* (Bhosle, 2013), which was reference for our study, of administration of the extracted plant for seventh days with using the latent dose of PTZ (65 mg/kg; i.p) on the seventh day, they have used diazepam rather than valproate in the positive group, which showed to have completely anticonvulsive effect by suppressing convulsion due to enhancing in GABA mediators when it compared with our positive group of valproate (Mahomed and Ojewole, 2006). Thus, the anticonvulsion effect of *Desmodium triflorum* suggested due to the enhancement of GABAergic neurotransmission. Moreover, the main reason behind *Desmodium triflorum* anticonvulsion activities suggested due to the phytochemical components of alkaloid, steroid, flavonoids, saponin, proteins, and tannins.

Biochemically desmodium triflorum showed the positive effect by increasing the level of non-enzyme of GSH in the present of PTZ induced while significantly decrease in lipid peroxidation by measuring MDA level (Bhosle, 2013). Our study was more interested in testing more antioxidant enzymes such as, CAT, GSH-Px, SOD, and GSH, which gave us the more free indication of explaining the reaction taken in the brain in the present of PTZ inducer. Moreover, we have evaluated epileptic mice behavior of movement and their respond to the external environment by using open field test and muscular relaxation of Rota rod test, which was poorly performed in desmodium triflorum, cyperus rotundus, and brassica nigra (Bhosle, 2013, Kiasalari *et al.*, 2012; Khalili *et al.*, 2011).

In brassica nigra, study the oxidative stress caused by PTZ cared by evaluating the level of NO, OX, ADA beside to MDA; all were an indication of neuron damage by PTZ kindling. However, in studies of ferula assa foetida gum, the study limited evaluating the level of NO and MDA (Ilhan *et al.*, 2006; Kiasalari *et al.*, 2012). While a study of the systemic administration of adenosine ameliorates with PTZ kindling, was evaluated by testing CAT level, adrenal ascorbic acid, and GSH as an indication of brain defend the system that was a significant increase in their level comparing them with PTZ group (Kumar *et al.*, 2016).

As long as epilepsy affects the temporal lobe of the brain, where memories and thinking taking place, seizure provokes reported to impair memory function by quickly forgotten information (Shulman and Barr, 2002). On the other hand, when seizure affects the frontal lobe, they lose the ability of organization, focus, and attention. Depression, restless was also seen with chronic epileptic patients, which suggested due to the formation of the chemical changes with every seizure provoke (Erdogan *et al.*, 2004).

In our experimental mice were held from their tail after seizure provoke to test their ability of exploration, and normal interaction with the environment around, almost all the groups except the controlled group and extracted dose of EH (100 mg/kg) showed immobility behavior and freezing state.

Testing the mice for their locomotors movement and exploration behaviors after seizure provoked were more worthy with EH doses of (100 mg/kg), which showed decreases in their locomotors movement when they compared with the controlled group in their response to the new environment they were putting in after seizure provoke. This was enough to explain the ability of the plant extract of EH at the dose of (100 mg/kg) to produce treatment with seizure free and provide normal interaction with the environment around and with less anxiety and emotional disorder that saw in the epileptic mice.

Different studies showed different results of anticonvulsive plants by testing the neuropharmacological continents of the extracted plant using open field. In studying the anticonvulsive effect of ferulic acid derivatives: Isopentyl ferulate showed, no significant decrease of mice movement behavior comparing them with a positive group of diazepam at the dose of (25 and 50 mg/kg), despite of that, they were more close to controlled group movement, which was an indication of uninfluenced of open field test with that extracted plant (Machado *et al.*, 2015). While different doses of (15 and 30 mg/kg) of the same plant, using (YLS-1<sup>a</sup> Multi-autonomous Activity Instrument) model, showed significantly reduced the locomotors activity (Tu *et al.*, 2012).

The same reported in studying the anticonvulsant effect of  $\beta$ -caryophyllene, which showed no significant change comparing them with the controlled group (Correia *et al.*, 2016).

While, studying the ethanolic effect of the extract indigofera tinctoria as well as eugenol, using experimental rats, showed a slight decrease in rat movement behavior, however, it considered not efficient enough when compared with the controlled group (Asuntha et al., 2010; Goyal and Sasmal, 2009).

However, study the anticonvulsant activities of the alcoholic extract of *Zizyphus nummularia* leaves, showed to have significant antianxiety effect by decrease

the number of crossing squares of the mice and increase the time spent in center square (Goyal and Sasmal, 2009 ).

As Rota rod test used to verify animals skeleton muscles relaxation and their balance performance on Rota rod stand, drugs that act on peripherally neuromuscular junction/muscle fibers and reduce the muscle tone and/or cause paralysis like diazepam (Sharma *et al.*, 2012), were widely used in evaluating animal muscle performance, when a new investigated agent.

In our experiment, testing animal balance performance after seizure provoked was no effective change in the motor coordination performance, they showed almost the same result of falling twice during the first 5 seconds of the tested time 300 seconds.

This indication is telling us that the anticonvulsive effect of the extract plant was not due to muscle relaxation and was not influenced by the extracted plant components.

However, our test of anticonvulsive effects of extracted plant EH using Rota rod test was not different from the previous tests performed. The effect of an alcoholic extract of capparidaceae, isopentyl ferulate,  $\beta$ -caryophyllene, showed to decrease the duration of animal staying on the stand of the Rota rod when they were compared with controlled and positive groups, which was indication of mice behavioral debilitation in CNS depression effects and neurotoxicity (Correia, 2016; Goyal and Sasmal, 2009; Machado *et al.*, 2015).

This was the same with methanol-methylene chloride root bark extract of *annona senegalensis* and its ethyl-acetate fraction, which showed poor performance of the mice on the horizontal Rota rod during the 60 min when they compared with the controlled group. (Okoye *et al.*, 2013).

Studying the anticonvulsion of *figus benghalensis* extract on the Rota rod was earlier fail of the mice when they compared with the stander group of diazepam (Sharma *et al.*, 2012).

The opposite was true with *indigofera tinctoria* extract, which showed to attenuated neurotoxicity effect of the extracted plant and being able to maintain equilibrium for more than 120 seconds (Asuntha *et al.*, 2010).

From our present research, further research ought to be done, using sub-convulsion doses of PTZ (35 mg/kg) with diazepam, which might give more explained results for understanding the different mechanism of EH.

Moreover, as our study carried in vivo, in vitro study suggested also. We have studied two doses (100 and 200 mg/kg; i.p) different doses might give more results that are convincing.

As we have collected the plant from the coastal line of Van Lake, different areas of different climates may show more interesting results of the different concentration of different active components in EH extracted plant.

Moreover, in our experimental, we have used the lyophilized method in plant extraction; study the plant with different extraction methods may improve the active components.

Studying with bigger animal like rats, rabbits or monkeys may facilitate biochemical assay and break the gatekeeper of most researchers done; studying the enzymes by taking blood tests might give different unexplained results or even gives useful elucidations (Sudha *et al.*, 2001). Never the less, our experiment was the first to study the EH on CNS disorder of epilepsy with positive results using different tested apparatus, which was poorly utilized in the similar previous researchers.

As a result, in the PTZ-induced epilepsy model, the lyophilized extract of *Epilobium hirsutum* (EH) reduced the behavioral onset of convulsion and duration of convulsion. In addition, healing in some biochemical parameters was observed.

However, more detailed results can be obtained by using different experimental seizures or epilepsy models.

## SUMMARY

**Dzhafar S. S., Investigation the Effect of Penthylenetetrazole Inducing Epilepsy Model Using Extraction of *Epilobium hirsutum*. Van Yüzüncü Yıl University, The Health Sciences Institute, Department of Pharmaceutical Professional Sciences, MSci, Thesis, Van, 2017.**

We aimed to reveal the pharmacological effects of the lyophilized extract of *Epilobium hirsutum* (EH) on epilepsy in our study using epilepsy model induced by (pentylene-tetrazole) PTZ. In our study, 40 mice were used totally which were divided into 5 groups (n=8). Our groups were comprised of Control (SF), positive control (PTZ 65 mg/kg and PTZ 65 mg/kg and valproate 100 mg/kg, i.p.), and plant EH (PTZ 65 mg/kg+EH 100 mg/kg, i.p. and PTZ 65 mg/kg+EH 200 mg/kg, i.p.) groups. EH was applied during 7 days and PTZ (65 mg/kg, i.p) was administered to the all groups except the Control group and seizures were evaluated. Open field and Rota rod tests were used after PTZ administration in order to evaluate motor coordination, behavior of exploration and muscle relaxation. Subsequently, the animals were sacrificed and their brains were removed for the evaluation of biochemical parameters. Onset of convulsion was significantly ( $p<0.05$ ) extended in EH (100-200 mg/kg) groups compare to PTZ group. Seizure duration was decreased significantly ( $p<0.01$ ) in EH (200 mg/kg) groups compare to PTZ group. Reduction in locomotor activity was occurred in open field ( $p<0.05$ ) whereas no significant difference was detected between all groups in Rota Rot test compare to PTZ group. MDA level was decreased significantly ( $p<0.05$ ) in EH (100 mg/kg) groups compare to PTZ group. GSH levels were significantly detected as high in EH (100 and 200 mg/kg) groups compare to control group. GSH-Px values were significantly ( $p<0.05$ ) detected as high in EH (200 mg/kg) groups compare to control group. SOD activity was significantly ( $p<0.01$ ) detected as high in EH (100 mg/kg) groups compare to PTZ group. CAT activities were detected as high in PTZ and EH groups compare to PTZ group.

Consequently, it was observed that the lyophilized extract of *Epilobium hirsutum* (EH) reduced the behavioral onset and duration of convulsions and ameliorated some biochemical parameters in PTZ-induced epilepsy model. However, we are in opinion of detailed results can be obtained in case of using different seizure or epilepsy models.

**Keywords:** Epilepsy, Pentilentetrazole, *Epilobium hirsutum*, Mice, Oxidative stress

## ÖZET

**Dzhafar S. S., Pentilentetrazol İle Oluşturulan Epilepsi Modelinde *Epilobium hirsutum* Ekstraktının Etkisinin Araştırılması. Van Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, Eczacılık Meslek Bilimleri Anabilim Dalı Farmakoloji Bilim Dalı, Yüksek Lisans Tezi, Van, 2017.**

Çalışmamızda, pentilentetrazol (PTZ) ile oluşturulan epilepsi modelinde *Epilobium hirsutum*'un liyofilize ekstresinin (EH) epilepsi üzerine farmakolojik etkilerini ortaya koymayı amaçladık. Araştırmamız da 5 grup ve her grupta 8'er fare olmak üzere toplam 40 fare kullanıldı. Gruplarımız; Kontrol (SF), pozitif kontrol (PTZ 65 mg/kg ve PTZ 65 mg/kg + valproat 100 mg/kg/i.p) ve bitki EH (65 mg/kg +100 mg/kg ve 65 mg/kg + 200 mg/kg/i.p) gruplarından oluşturuldu. Farelere 7 gün boyunca EH verildi ve 7. gün, kontrol grubu hariç tüm gruplara PTZ (65 mg/kg/i.p) uygulandı ve nöbet olup olmadığı değerlendirildi. PTZ uygulanmasından sonra, motor koordinasyon, arama davranışı ve kas gevşemesini değerlendirmek için açık alan ve Rota Rot testi kullanıldı. Bu testlerden hemen sonra, biyokimyasal parametreleri değerlendirmek için hayvanlar sakrifiye edildi ve beyin dokuları çıkarıldı. PTZ grubuna göre EH (100-200 mg/kg) gruplarında nöbetlere başlama süresi anlamlı olarak uzadı ( $p<0.05$ ). EH (200 mg/kg) grubunda PTZ grubuna göre nöbet süresi kısaldı ( $p<0.01$ ). Açık alan testinde, PTZ grubu kontrol grubu ile karşılaştırıldığında anlamlı olarak lokomotor aktivitede azalma meydana geldi ( $p<0.05$ ), bununla birlikte Rota Rot testinde tüm gruplar arasında anlamlı fark bulunamadı. PTZ grubu ile karşılaştırıldığında EH (100 mg/kg) grubunda MDA seviyesi anlamlı olarak azaldı ( $p<0.05$ ). EH (100 mg/kg ve 200 mg/kg) gruplarında GSH seviyesi kontrol grubuna kıyasla anlamlı olarak yüksek bulundu. Kontrol grubuyla karşılaştırıldığında GSH-Px değerleri EH (200 mg/kg) grubunda anlamlı olarak yüksek bulundu ( $p<0.05$ ). EH (100 mg/kg) grubuna göre SOD aktivitesi PTZ grubuna göre yüksek bulundu ( $p<0.01$ ). CAT aktivitesi kontrol grubu ile karşılaştırıldığında PTZ ve EH gruplarına kıyasla yüksek olduğu görüldü. Sonuç olarak, PTZ'ye bağlı epilepsi modelinde, *Epilobium hirsutum*'un liyofilize ekstresinin davranışsal olarak nöbetlere başlama süresini ve nöbet süresini azalttığı, ayrıca biyokimyasal olarak bazı parametreleri iyileştirdiği görüldü. Bununla birlikte, farklı deneysel nöbet ya da epilepsi modeli kullanılarak da daha ayrıntılı sonuçlar elde edilebileceği kanaatindeyiz.

**Anahtar Kelimeler:** Epilepsi, Pentilentetrazol, *Epilobium hirsutum*, Fare, Oksidatif stres

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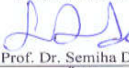
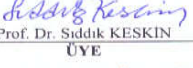
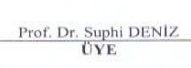
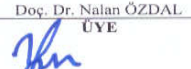
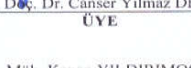
## CURRICULUM VITAE

Sara Sami DZHAFAR with the Bulgarian citizenship was born in Aden- Yemen in 1985. I had studied my primaries and high schools in the city of Aden-Yemen. I had graduated from Aden University of Pharmacy in 2010. I started working in pharmacy science 2008; however, in 2011 I joined working in Medecins Sans Frontieres organization (MSF) as a Pharmacy Store Manager in Sana'a Yemen. In 2015, I registered in the health Institute of Van Yuzuncu Yil University in Van -Turkey for a Master degree program in the department of pharmacology.



## **ATTACHMENTS**

**ATTACHMENT 1. The Acceptance Letter form Experimental Animal Ethics Committee of Van Yuzuncu Yil University.**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                                                                                                                                                                                          | <p><b>T.C.</b><br/> <b>YÜZÜNCÜ YIL ÜNİVERSİTESİ</b><br/> <b>HAYVAN DENEYLERİ YEREL ETİK KURULU</b><br/> <b>ARAŞTIRMA KESİN SONUÇ ONAY BELGESİ</b></p> <p><b>YUZUNCUYILUNIVERSITY (TURKEY)</b><br/> <b>ANIMAL RESEARCHES LOCAL ETHIC COMMITTEE</b><br/> <b>RESEARCH FINAL REPORT APPROVAL CERTIFICATE</b></p> |
| Araştırmacının Adı                                                                                                                                                                                                                                                                                                                                                                                                                                        | Pentilentetrazol İle Oluşturulan Epilepsi Modelinde <i>Epilobium Hirsutum</i> Ekstraktının Etkisinin Araştırılması.                                                                                                                                                                                          |
| Title of the Research                                                                                                                                                                                                                                                                                                                                                                                                                                     | Investigation the effect of Pentilentetrazol inducing Epilepsy model using extration <i>Epilobium Hirsutum</i>                                                                                                                                                                                               |
| Araştırmacı(lar) Investigator(s)                                                                                                                                                                                                                                                                                                                                                                                                                          | Yürütücü / Chief investigator : Yrd. Doç. Dr. Oruc ALLAHVERDİYEV<br>Yardımcı Araştırmacı(lar) / Co-investigator(s): Sara Sami DZHAFAR                                                                                                                                                                        |
| Araştırmacının Başlama Tarihi / Research Starting Date:                                                                                                                                                                                                                                                                                                                                                                                                   | 06.04.2017                                                                                                                                                                                                                                                                                                   |
| Araştırmacının Bitiş Tarihi / Research Completion Date:                                                                                                                                                                                                                                                                                                                                                                                                   | 25.07.2017                                                                                                                                                                                                                                                                                                   |
| Proje Süresi / Total Time of Project:                                                                                                                                                                                                                                                                                                                                                                                                                     | 12 ay/12 months                                                                                                                                                                                                                                                                                              |
| Proje No / Project Number:                                                                                                                                                                                                                                                                                                                                                                                                                                | TYL-2017-5873                                                                                                                                                                                                                                                                                                |
| Araştırmayı Destekleyen Kuruluş (varsa) / Funding institution(s) (if available):                                                                                                                                                                                                                                                                                                                                                                          | Yüzüncü Yıl Üniversitesi Bilimsel Araştırma Projeleri Başkanlığı/Yuzuncu Yil University, Scientific Research Projects                                                                                                                                                                                        |
| Destek Şekli ve Miktarı / Type and amount of funding:                                                                                                                                                                                                                                                                                                                                                                                                     | 3736 TL                                                                                                                                                                                                                                                                                                      |
| <p><b>Karar:</b><br/> Yukarıda bilgileri verilen araştırma projesinin kesin sonuç raporu Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 24/08/2017 tarih ve 2017/08 sayılı kararı ile kabul edilmiştir.</p> <p><b>Decision:</b><br/> Final report of the research project detailed above was approved by Yuzuncu Yil University Animal Researches Local Ethic Committee in the session held on 24/08/2017 (decision number 2017/08).</p> |                                                                                                                                                                                                                                                                                                              |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>BAŞKAN/CHAIR</b><br/> <br/> Prof. Dr. Semiha DEDE</p>                                                                                                                                                               |
| ÜYE                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <p><b>ÜYE</b><br/> <br/> Prof. Dr. Fazıl ŞEN</p>                                                                                                                                                                          |
| Prof. Dr. N. Tuğba BİNGÖL                                                                                                                                                                                                                                                                                                                                                                                                                                 | <p><b>ÜYE</b><br/> <br/> Prof. Dr. Atilla DÜRMÜŞ</p>                                                                                                                                                                      |
| Yrd. Doç. Dr. Ferda KARAKUŞ                                                                                                                                                                                                                                                                                                                                                                                                                               | <p><b>ÜYE</b><br/> <br/> Yrd. Doç. Dr. Oruc ALLAHVERDİYEV</p>                                                                                                                                                             |
| Yrd. Doç. Dr. Yıldırım BAŞBUĞAN                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>ÜYE</b><br/> <br/> Vet. Hek. İsmail Hakkı BEHÇET</p>                                                                                                                                                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>ÜYE</b><br/> <br/> Prof. Dr. Suphi DENİZ</p>                                                                                                                                                                      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>ÜYE</b><br/> <br/> Doç. Dr. Nalan ÖZDAL</p>                                                                                                                                                                       |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>ÜYE</b><br/> <br/> Yrd. Doç. Dr. Cansel Yılmaz DEMİR</p>                                                                                                                                                          |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>ÜYE</b><br/> <br/> Zır. Müh. Kenan YILDIRIMOĞLU</p>                                                                                                                                                               |

**ATTACHMENT 2. The Acceptance Letter for Results of the Thesis by Experimental Animal Committee in Van Yuzuncu Yil University.**



T.C.  
YÜZÜNCÜ YIL ÜNİVERSİTESİ  
HAYVAN DENEYLERİ YEREL ETİK KURULU  
ARAŞTIRMA KESİN SONUÇ ONAY BELGESİ

YUZUNCUYIL UNIVERSITY (TURKEY)  
ANIMAL RESEARCHES LOCAL ETHIC COMMITTEE  
RESEARCH FINAL REPORT APPROVAL CERTIFICATE

|                                                                                                                                                                                                                     |                                                                                                                                       |                                                     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|
| Araştırmanın Adı                                                                                                                                                                                                    | Pentilentetrazol İle Oluşturulan Epilepsi Modelinde <i>Epilobium Hirsutum</i> Ekstraktının Etkisinin Araştırılması.                   |                                                     |
| Title of the Research                                                                                                                                                                                               | Investigation the effect of Pentilentetrazol inducing Epilepsy model using extration <i>Epilobium Hirsutu</i>                         |                                                     |
| Araştırmacı(lar)<br>Investigator(s)                                                                                                                                                                                 | Yürütücü / Chief investigator : Yrd. Doç. Dr. Oruc ALLAHVERDİYEY<br>Yardımcı Araştırmacı(lar) / Co-investigator(s): Sara Sami DZHAFAR |                                                     |
| Araştırmanın Başlama Tarihi / Research Starting Date: 06.04.2017                                                                                                                                                    |                                                                                                                                       |                                                     |
| Araştırmanın Bitiş Tarihi / Research Completion Date: 25.07.2017                                                                                                                                                    |                                                                                                                                       |                                                     |
| Proje Süresi / Total Time of Project: 12 ay/12 months                                                                                                                                                               |                                                                                                                                       |                                                     |
| Proje No / Project Number: TYL-2017-5873                                                                                                                                                                            |                                                                                                                                       |                                                     |
| Araştırmayı Destekleyen Kuruluş (varsa) / Funding institution(s) (if available): Yüzüncü Yıl Üniversitesi Bilimsel Araştırma Projeleri Başkanlığı/ Yuzuncu Yil University, Scientific Research Projects             |                                                                                                                                       |                                                     |
| Destek Şekli ve Miktarı / Type and amount of funding: 3736 TL                                                                                                                                                       |                                                                                                                                       |                                                     |
| <b>Karar:</b><br>Yukarıda bilgileri verilen araştırma projesinin kesin sonuç raporu Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 24/08/2017 tarih ve 2017/08 sayılı kararı ile kabul edilmiştir. |                                                                                                                                       |                                                     |
| <b>Decision:</b><br>Final report of the research project detailed above was approved by Yuzuncu Yil University Animal Researches Local Ethic Committee in the session held on 24/08/2017 (decision number 2017/08). |                                                                                                                                       |                                                     |
|                                                                                                                                                                                                                     | <b>BAŞKAN/CHAIR</b><br><br>Prof. Dr. Semiha DEDE                                                                                      |                                                     |
| <b>ÜYE</b><br>Prof. Dr. Fazıl ŞEN                                                                                                                                                                                   | <b>ÜYE</b><br><br>Prof. Dr. Siddık KESKİN                                                                                             | <b>ÜYE</b><br>Prof. Dr. Suphi DENİZ                 |
| <b>ÜYE</b><br>Prof. Dr. N. Tuğba BİNGÖL                                                                                                                                                                             | <b>ÜYE</b><br><br>Doç. Dr. Atilla DÜRMÜŞ                                                                                              | <b>ÜYE</b><br>Doç. Dr. Nalan ÖZDAL                  |
| <b>ÜYE</b><br><br>Yrd. Doç. Dr. Ferda KARAKUŞ                                                                                                                                                                       | <b>ÜYE</b><br>Yrd. Doç. Dr. Oruc ALLAHVERDİYEY                                                                                        | <b>ÜYE</b><br><br>Yrd. Doç. Dr. Canser Yılmaz DEMİR |
| <b>ÜYE</b><br><br>Yrd. Doç. Dr. Yıldırım BAŞBUĞAN                                                                                                                                                                   | <b>ÜYE</b><br><br>Vet. Hek. İsmail Hakkı BEHÇET                                                                                       | <b>ÜYE</b><br>Zir. Müh. Kenan YILDIRIMOĞLU          |