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VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
INSTITUTE OF HEALTH SCIENCE

APPLICATION OF ATROPINE IN FASTED ANIMALS AND CONVULSIONS CAUSED BY FEEDING INVESTIGATION OF THE EFFECT OF GLIBENCLAMIDE, MINOXIDIL, CAFFEINE, AND USNIC ACID

Taha Safauldeen Taha Alhajjar

DEPARTMENT OF PHARMACY PROFESSIONAL SCIENCES
(PHARMACOLOGY)

MASTER THESIS

SUPERVISOR

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ACCEPTANCE AND APROVAL PAGE

This thesis entitled ''*Application Of Atropine In Fasted Animals And Convulsions Caused By Feeding Investigation Of The Effect Of Glibenclamide, Mınoxidil, Caffeine, And Usnic Acid*'' prepared and submitted by Taha Safauldeen Taha Alhajjar at Van Yüzüncü Yıl University Department of Pharmacy Professional Sciences (Pharmacology) has been accepted unanimously by the examination committee in as a Master of Science thesis.

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Yüksek Lisans/Doktora tezi olarak hazırlayıp sunduğum *"Aç Hayvanlarda Atropin Uygulanması Ve Yem Verilmesi Ile Oluşan Konvülsiyonlara, Glibenklamid, Minoksidil, Kafein Ve Üsnik Asidin Etkisinin Araşrılması"* başlıklı tezim; bilimsel ahlak ve değerlere uygun olarak tarafımdan yazılmıştır. Tezimin fikir/hipotezi tümüyle tez danışmanım ve bana aittir. Tezde yer alan deneysel çalışma/araştırma tarafımdan yapılmış olup, tüm cümleler, yorumlar bana aittir. Bu tezdeki bütün bilgiler akademik kurallara ve etik ilkelere uygun olarak hazırlanıp, bu kural ve ilkeler gereği, çalışmada bana ait olmayan tüm veri, düşünce ve sonuçlara atıf yapılmış ve kaynak gösterilmiştir.

Yukarıda belirtilen hususların doğruluğunu beyan ederim.

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ABBREVIATIONS

AEDS : Antiepileptic drugs

AMPA : α- amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

BZD : Benzodiazepine

Ca^{+2} : Calcium Ion

CAT : Catalase

CBZ : Carbamazepine

Cl^{-} : Chloride Ion

CLB : Clobazam

CNS : Central nervous system

CZP : Clonazepam

EEG : Electroencephalography

ESL : Eslicarbazepine acetate

ESM : Ethosuximide

FBM : Felbamate

GABA : Gamma amino butyric acid

GBP : Gabapentin

GSH : Glutathione

GSH-Px : Glutathione peroxidase GSH-Px

H_2O_2 : Hydrogen peroxide

I.P. : Intraperitoneal

ILAE : International League Against Epilepsy

K^{+} : Potasium Ion

KATP : ATP-sensitive potassium channel

LCM : Lacosamide

LEV : Levetiracetam

LTG : Lamotrigine

MDA : Malondialdehyde

Mg^{+2} : Magnesium Ion

Na^{+} : Sodium Ion

NMDA : *N*-methyl- D-aspartate (NMDA)

NO	: Nitric Oxide
OXC	: Oxcarbazepine
PB	: Phenobarbital
PER	: Perampanel
PGB	: Pregabalin
PHT	: Phenytoin
PRM	: Primidone
PTZ	: Pentylenetetrazol
RTG	: Retigabine
SOD	: Superoxide dismutase
SV2A	: Synaptic vesicle protein 2A
TGB	: Tiagabine
TPM	: Topiramate
USA	: United State of America
VGB	: Vigabatrin
VPA	: Sodium valproate
WHO	: World Health Organisation
ZNS	: Zonisamide

1. INTRODUCTION

Epilepsy is considered as a popular neurological disorder which is characterized by a disorder in electrical activity located in brain area and is well-known as epileptic seizures (Kreutzer et al, 2016). The electrical activity disruption affects different parts of the normal brain functions like consciousness, awareness, sensation, movement, and behavior. One of the most important epileptic symptoms is seizure attacks that could last for few seconds or few minutes and during this period of time the person may lose consciousness, subsequently, patients return to normal, which take into consideration the stop-stage of seizure episodes (Fisher et al., 2005).

World Health Organization (2017) described epilepsy as a series of involuntary motilities in one part of the human organism (partial) or whole of the body (generalized) with or without loss of awareness. According to WHO, the diagnosis of the patient, epilepsy occurs when the patient has a minimum of two reflex (or unprovoked) seizures in more than 24 hours apart one reflex (or unprovoked) seizure. The possibility of additional seizures comparable to the usual recurrence risk (minimum 60%) after two of unprovoked seizures, may appear late with the personification of an epilepsy syndrome (Fisher, 2014). Epilepsy can emerge from a short duration of inattention or muscle shakes to strong and for a longer duration of involuntary movements (Cooper and Gosnell, 2015).

It has been reported that around sixty-five millions of people suffer from epilepsy (Moshe et al., 2015) worldwide, with 8-10% lifetime risk of epileptic seizure (Zimet et al., 1988). Nearly 90% of them are reported to be in the growing regions and globally up to 3.4 millions of them are in the United States (Schachter et al., 2014; Zach and Kobau, 2017), while 415,000 are in England (Broadbent et al, 2006). Ngugi et al. (2010) stated that 2.4 million people are diagnosed every year with epilepsy, making it one of the important neurological illnesses (Connor and Davidson, 2003).

Epilepsy, when compared with breast cancer disease in women and lung cancer in men, would result in 10% of the population to have a minimum one seizure in their lifetime and part of them will suffer from epilepsy (Hesdorffer et al., 2011). Lishman

has reported that epilepsy is not always diagnosed with the occurrence of only one seizure (Cramer et al., 1998).

Epilepsy is defined as a symptom of neurological defect more than a disease, and remains to be held in recent instructions (Broadbent et al., 2006). There are different types of epilepsy, seizures, and causes of epilepsy, although such causes might not continually be identifiable. Whatever causes that could be identified, whether they are birth complications, brain damage, infections, tumors, neurodegenerative disorders, metabolic disorders or cerebrovascular disease, drugs or toxins can contribute to the development of epilepsy (Cramer et al, 1998). People older than 59 years of age were diagnosed with epilepsy because of the presence of a tumor with a rate of 19%, while alcohol addiction was found as one of the factors to cause seizures in people between 30 to 39 years old.

This study aims at exploring the possible anticonvulsant effect of caffeine, minoxidil, glibenclamide, and usnic acid by using experimental male mice and atropine seizure inducer. The locomotor activity tests, as well as Rota rod tests, supported our research by providing additional information on animal locomotor behavior and muscular relaxation. Biochemical assay has supported the current research which is considered as an asset to evaluate the brain defense system in the presence of seizure inducer of atropine.

Caffeine is a combined competitive antagonist of adenosine A1 and A2A receptors. It inhibits gamma-aminobutyric acid (GABA), by modulating GABA-A receptors, while the stimulation of Adenosine A1-receptor lead to inhibition of the release of dopamine and glutamate. The importance of stimulation of the central A1 receptor is it’s anticonvulsant effects.

Minoxidil is considered as a strong arteriolar vasodilator (powerful vasodilator), since it acts as a potassium channel opener which is present in the cells of the smooth muscle of the peripheral (Gilmore et al., 1970; Charme et al., 1973; Editorial, 1973). Several studies have shown that both minoxidil and diazoxide inhibit calcium uptake through the cell membrane (Chidsey and Gottlieb, 1974).

Glibenclamide acts by blocking ATP sensitive potassium channels and has a possibility of modulating the potassium channel, which might have a potential activity against convulsion (Body,1988).

Usnic acid (UA) possesses pharmacological effects including antioxidant, healing, analgesic, anticonvulsant, antiemetic, anti-asthmatic, antimicrobial, anti-inflammatory, antiviral, and antibiotic properties, but also exhibits adverse effects including liver harmfulness and contact allergy (Cocchietto et al., 2002; Ingolfsdottir, 2002; Shukla et al., 2010; Raizada et al., 2014; Yousuf and Choudhary, 2014; Luzina and Salakhutdinov, 2016).

Medical agents cause various side effects, tolerances, drug interactions and economic demands, which made many researchers looking for alternative therapies with a positive reflection on human health. An agent for seizure control needs to has a potent antioxidant effect and has to prevent the generation of free radicals, which in turn controls the hyperactivation of the neuron by the organizing of neuron the neurotransmitters. Thus, different studies have investigated new compounds with antioxidant effects to present a role in controlling seizures provoked in experimental animals.

In recent studies, it has been shown that rat and mice treated with antimuscarinics such as atropine, scopolamine, biperiden (Enginar and Nurten 2010) and the selective M_1 receptor antagonist pirenzepine (Bacank et al., 2017) after fasting for two days or less (Enginar et al. 2009) were found to develop convulsions soon after being allowed to eat ad libitum. Food deprivation itself, but not its hypoglycemic consequence, seems to be critical in the development of seizures (Enginar et al., 2005). Fasted of food for 48 h produces changes in binding of [3H] glutamate in the brain, implying that neuroadaptive changes occur during fasting (Enginar et al., 2003). It has been suggested that all of the complicated effects occur during the eating of the solid food only, not (slurry or fluid feeding) including the (chewing and swallowing movements, smelling and tasting) and stimulation of the amygdala by repetitive oral and masticator movements are considers as a triggering factor for development of convulsions (Nurten et al. 2009). On the other hand, bearing some similarities in triggering factors and manifestations of the seizures in patients with eating-evoked

epilepsy, convulsions in fasted animals may provide insight into the mechanism of this rare and partially controlled form of reflex epilepsy (Senanayake 1994; Seneviratne et al., 2003 Guimaraes et al. 2005; Striano et al. 2012).

Atropine (Richards, 1990) and scopolamine (Witkin et al., 2014) are nonselective M1 and M2 muscarinic receptor antagonists, while pirenzepine (Bacank et al., 2017) and biperiden (Burke, 1986; Syvalahti et al.,1987; Witkin et al., 2014) possess high M1 selectivity. Nurten and Enginar (2006) have suggested that the reason underlying in the convulsive activity in fasted animals is a decreasing in cholinergic transmission due to the blockade of postsynaptic muscarinic (M1 possibly M2 subtypes) receptors.

This study aims to evaluate the effects of atropine and different dose of caffeine, minoxidil, usnic acid, and glibenclamide on the development of convulsions triggered by food intake in antimuscarinic-treated fasted animals. For this purpose, atropine which is a non- selective M1 and M2 muscarinic receptor antagonist (Richards 1990) was used to reveal its efficacy on the development of convulsions in mice after 24 h of fasting. The dose strengths used in the study were selected depending upon the drug's affinity compared with those of atropine (Nurten and Enginar 2006), and glibenclamide (Lahmann et al.,2015), minoxidil (Chen et al., 2017), caffeine (Tchekalarova et al.,2013), usnic acid (Asma'a and Sarah.,2014). Moreover, the locomotor activity test, as well as Rota rod test, supported the current research by providing additional information about animal locomotor behavior and muscular relaxation. Biochemical assay has supported the current research which is considered as an asset to evaluate the brain defense system in the presence of seizure inducer of atropine.

2. GENERAL INFORMATION

2.1. Epilepsy:

The word epilepsy is derived from the Greek word Epilepsia, Epi: above, Lepsis: to hold, hold and shake, thus the meaning of epilepsy is being caught suddenly. It is a kind of disease caused by repeated involuntary seizures emanated from neuronal hyperactivity in the brain (Ettinger, 1994). WHO defines epilepsy as a chronic brain disease that develops due to various etiology and recurrent seizures due to the excessive discharge of cerebral neurons (Haslam, 2007).

The neurons, which are the anatomical areas or pathways from which unusual epileptic discharges originate, determine the clinical appearance of the seizure. A seizure or convulsion is not adequate to indicate the presence of epilepsy. It is a result of increased excitability of nervure cells, and occurs when the instability between inhibition-excitation is disrupted and repeated (Engel, 2013).

Generally, the occurrence of first convulsions is not considered as epilepsy. In order to diagnose epilepsy, seizure should be repetitive and maintain this feature for years. Head trauma, hemorrhages in the brain, some infections in the brain (such as meningitis, encephalitis or abscesses), tumors may be the cause of convulsions. WHO estimates that the average prevalence of epilepsy activity is 8.2 per 1000 in the general population (Ransom and Blumenfeld, 2007). The epileptic seizure describes abnormal and excessive neuronal activity as a result of temporary morbidness of brain function and the chronic case of repetitive seizures is known as epilepsy.

2.2. Epidemiology:

In Europe, epilepsy is estimated to affect about 2.6 to 6 million people each year (Gustavsson et al., 2011), and almost 170,000 persons in the United States experience the first seizure, half of them have no more seizures (Krumholz et al, 2015; Hauser et al., 2017).

Epilepsy is one of the chronic electrical disorder of brain, that affect up to 50 million of the world population (Shin et al., 2011; Allahverdiyev, 2017), mostly in the

underdeveloped regions. Approximately, 5 and 8 per 1000 persons are affected by epilepsy in the developed areas (Moshe et al., 2015), while 30 and 50 persons per 100 000 are affected by epilepsy worldwide (WHO, 2016).

The disorder affects men more than the women, and the frequency of epilepsy is higher in young people than in elderly. Epilepsy is associated with a brief life span and about 30% of patients with do not respond to treatment (Kwan and others 2010).

The genetic factors that cause epilepsy have become more acknowledgeable in the last few decades (Italiano et al., 2016). There is a higher incidence of occurrence because of genetic disorders in the same families (Covanis, 2005). The risk of having epilepsy within the general population is 3%, but it increases at about 20% in siblings of those with generalized epilepsy (Waltz and Stephani, 2000).

The advance of epilepsy is higher in the first year of life because of genetic factors. The prevalence of disease in people who experienced seizures or used to take antiepileptic drugs to reduce seizures in the past 5 years, estimated about 0.5 - 1 percentage (Hauser et al., 1993). In general, the percentage of people suffering from a non-febrile seizure at any time in their life is 5- 6% (Sander, 2003).

The prevalence of epilepsy in the United States has been reported to be around 6.5 per 1000 people of the population. The organization of epilepsy estimates that almost three million of the Americans suffer from epilepsy (England and others, 2012). The lowest prevalence of epilepsy has been found in Japan as 1.5 per 1,000. Some studies made in Turkey have found that 4.5 per 1,000 of the population in urban areas suffer from epilepsy while 8.8 per 1,000 are in the rural areas (Aziz et al., 1997; Velioglu et al., 2010).

Epilepsy can occur at any age and it affects children at the age of 6-9 years old, more commonly in girls. However, 71% of cause of epilepsy are believed to be supernatural (Aziz et al., 1997; Çalısır, 2006).

2.3. The mechanism of epileptic action:

In general, the mechanism of epileptic seizure depends on the concept of unbalance between the excitatory and inhibitory neurotransmitters. The increase or the decrease in the excitatory or inhibitory or the imbalance between two of them due to excessive state of the repeated action potential lead to an increase in the synaptic excitatory neurotransmissions that function like an opening channel for Na^{+}, Ca^{+2} ions, and glutamate, or a decrease in inhibitory neurotransmission like K^{+}, Cl^{-} and GABA(Bromfield et al., 2006).

The initiate of an action potential leads to quick variations of neuron membrane permeability toward Na^{+} /K^{+}/Ca^{+2} ions, which come throughout the voltage-gated ion channels by closing and opening mechanism. The opening of the voltage-gated channel in the presynaptic causes the increase in concentration of extracellular Ca^{+2}ions leading to cross inside the cell body of the neuron. This, in turn, affects the vesicles by opening and releasing their content to the cleft space to bind with the postsynaptic receptors by ligand-gated mechanism, and open the way for Na ions to be inside and to change the voltage rate from 70 VM to - 60 - 55 VM (thresholds) by causing membrane depolarization of postsynaptic (more positive inside and negative outside) (Bromfield et al., 2006; Katzung et al., 2012).

The most important receptors that affect epilepsy are NMDA, AMPA, GABA, Na^{+}, and T- type Ca^{+2} channels while glycerin is one of the most important neurotransmitters affecting the above receptors. Glycerin binds to NMDA receptors, glutamate binds to NMDA and AMPA receptors. However, barbiturate, GABA and benzodiazepine bind to the GABA receptor site, also serotonin, acetylcholine, endocannabinoid have been reported as a cause of epilepsy (Werner and Coveñas, 2011; Büget et al., 2016). Moreover, the opening and the closing phenomenon has an important role in controlling the function of the brain or formation of a brain malformation by generating electrical imbalance and propagate epilepsy disorder (Katzung et al., 2012).

Glutamates are one of the inotropic receptors (AMPA), as well as (NMDA) and kainate receptors, that have an important role as an excitatory effect of the postsynaptic

membrane. In the normal situation the (AMPA) receptor is characterized as having a weak sensitive to glutamate stimulation by opening the channels and allowing some ions like Na^{+} ion but not the Ca^{+2} ion and bring the K^{+} ions out. The weak sense of AMPA to stimulate glutamate lead to the release of a small number of glutamate and causing depolarization of the membrane (Bromfield et al., 2006).

During the weak stimulation or resting period, the small ions enter and exit (NMDA) receptor channel, which can be blocked through the extracellular Mg^{+2} ion. However, it is removed by higher action potential or rather, or by increasing the amount of glutamates and give an empty place for Na^{+} and Ca^{+2} ions to enter and make an action potential (Katzung et al., 2012).

In epilepsy the Mg^{+2} offset becomes permanent, and the Ca^{+2} influx continuous, which leads to an increasing depolarization and phosphorylation of the more NMDA reproduction on the postsynaptic region that usually ends with death or neuronal harm, under the immoderate neuronal activation. Figure 1 illustrates the general mechanism of GABA in epilepsy (Bromfield et al., 2006; Katzung et al., 2012).

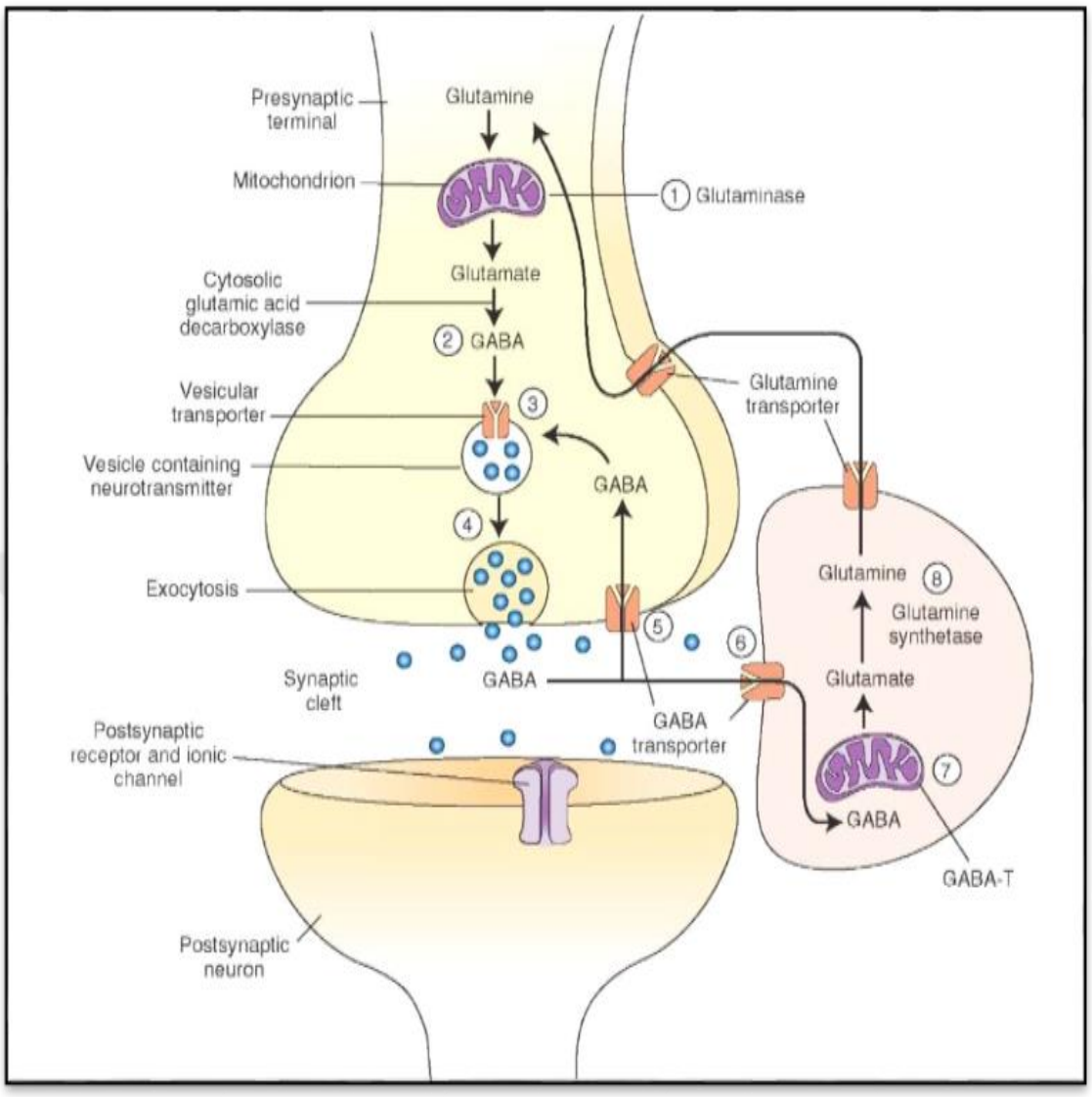


Figure 1. The General Mechanism of GABA in Epilepsy

2.4. The Structure of Brain

The brain is an organ with a vital importance, protected by the rigid bone of the skull. The human brain consists of four important parts: diencephalon, cerebrum, cerebellum, and brain stem. The cerebral hemisphere is the bigger part of the brain made up of about 83% from whole-brain mass. The cerebral hemisphere is formation of five lobes: frontal lobe (sensation), parietal lobe (numbness and tingling), occipital lobe (visual distribution and hallucination), temporal lobe (feeling) and insular lobes (Emotions) (Boor et al., 2016).

The human brain is controlling the functions of the body by gripping the neurons' activities which are specialized nerves that produce electrical impulses in all parts of the central nervous and peripheral systems. To achieve their functions in controlling cognitive activities, sensory perception and skeletal muscle contractions, all the neurons in brain must work in consonance. The brain's electrical activity can be measured by an electroencephalogram (EEG).

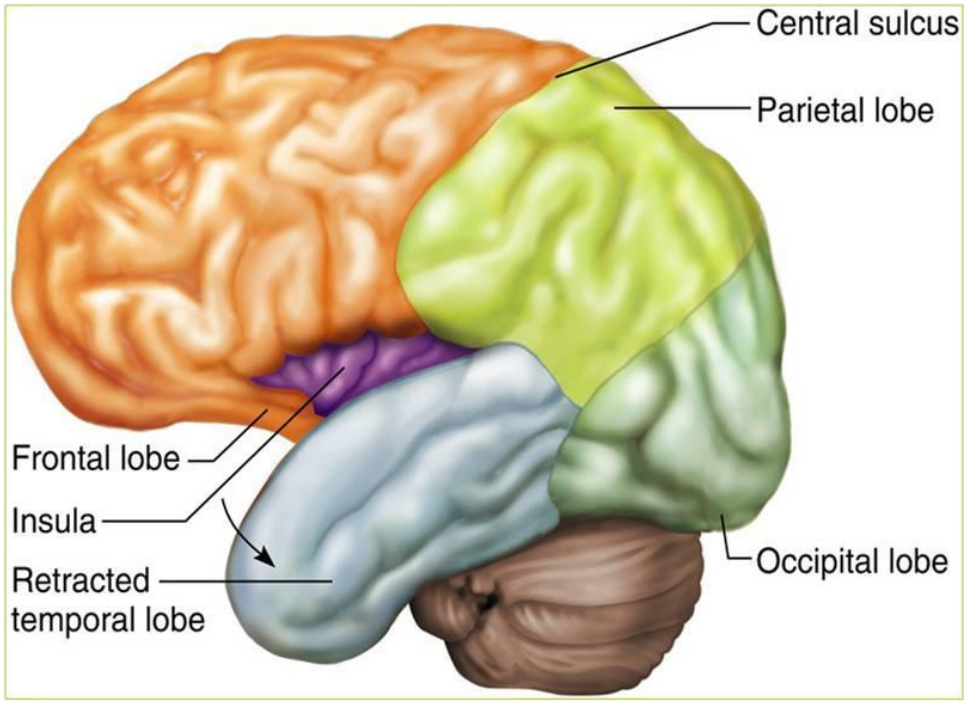


Figure 2. Brain Structure

2.5. Epileptic seizure:

Epileptic seizures are signs of the symptoms resulting from disturbance of electrical activity in the brain. The seizures are described as symptoms that refer to episodic, excessive and disorderly neuronal activity in the brain (Fisher et al., 2005; Falco-Walter et al., 2018). There are numerous kinds of epilepsy and over forty unique kinds of seizure could affect individuals in different ways. Boss and Huether (2017) indicated that there are various seizures classified according to clinical demonstrations, area of origin, EEG examinations or therapy response. Cooper and Gosnell (2015), on the other hand, said that the seizures could be classified based on characteristics, incidence, and clinical signs. All the different types of seizures classified by the ILAE

and ILAE (2017) is adopted in this study. ILAE system subdivides seizures into two broad types, mainly partial (or focal) and generalized seizures. Partial seizures are located in one region of the brain or inside networks of one hemisphere (Burnham, 2002a; ILAE, 2017). Generalized seizures affect the both hemispheres (Burnham, 2002a).

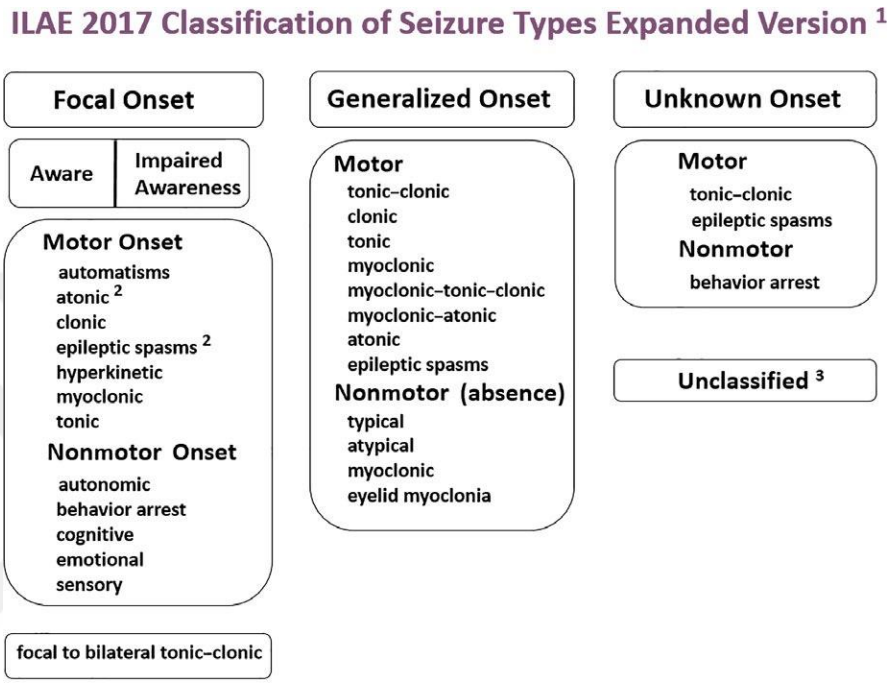


Figure 3. ILAE Classification of Seizure Types 2017

2.6. The classification of seizures

2.6.1. Focal- onset seizures (Partial-onset seizures):

The first universal type of seizure defined by the ILAE (2008) is also called a localized seizure because it is localized in the specific area of the brain. This type starts suddenly and shortly which lasts typically from 60 to 120 seconds.

In the localized seizure the electrical disturbances are focused in just one part of the brain involving neurons in the area of the (cerebral hemisphere) of the brain. According to the types of symptoms and signs, showing the seizure activity area, it can transform into a generalized seizure, which affects the whole brain.

The partial seizures are divided into a simple partial seizure, which affects a small portion of the temporal lobe and can be converted into a complex Partial seizure, which is called also (aura) and this type of epilepsy effects the whole brain.

2.6.1.1 Simple focal seizures:

This type of epilepsy affects the person without causing the loss of conscious (Blundell, 2006); (VanMeter and Hubert, 2014). In this case, the seizures affect a small region in the brain, namely the small part of the temporal lobe, such as the amygdala and hippocampus of the brain and the person does not lose the consciousness. There are two types: sensory seizures (change in any one of the senses) like feelings of anger, fear, happiness, sadness, or nausea, sensations of feeling or movement, alternation in smelling, hearing, seeing, tasting, sensory illusions or hallucinations, dissociation from the environment or self, and remembering events in detail, and autonomic seizures (changes in automatic function (changing in heart beating rate or sweating), psychic seizures (changes in thinking or feeling), Simple focal seizures may start suddenly and shortly which lasts typically from 60 to 90 seconds and the residual weakness may last for 15-30 minutes (Bromfield et al., 2006).

2.6.1.2. Complex partial seizures (with impairment of consciousness)

This type of epilepsy differs from simple partial seizures to unequal degrees of impairment of awareness (Brodie et al., 2005). Complex partial seizures typically last less than 3 minutes with an impaired consciousness, and complex partial seizures last longer than simple partial seizures. This type of seizures usually begins in the temporal lobe of brain and might affect the frontal lobe of brain or limbic system (Van meter and Hubert 2014). It usually cause sharp and fast change in consciousness, malformation of thinking with partially coordinated motor activity. It may affect the people at any age with a longer duration than that of absence seizures, and the patient experiences abnormal behaviors such as lip-smacking, clapping of hands, inappropriate repetitive movements, selecting something from a shop shelf without remembering the activity. Sometimes it could be intoxicated, and result in antisocial behavior and hallucinations which lead to the lose of the person's ability to interact normally with the environment around (Hubert and VanMeter, 2014; Gosnell and Copper, 2015; Huether and Boss,

2017). Paralysis may be visible in one part of the body for minutes, hours or even more (Roffman and Stern, 2006).

2.6.1.3. Focal seizures developing to secondarily generalized Seizures:

These seizures result from developments in complex focal seizures that affect more than one region of the brain to become secondarily generalized seizure or tonic-clonic seizure of legs stiffen, finger rubbing, smacking, chewing or swallowing, trunk, and arms. As it is difficult to recognize the difference between primarily generalized seizure and the secondarily partial seizure, electroencephalogram (EEG) and neuroimaging test (TC or MRI) have been used in figuring out the sort of the seizures (Bromfield et al., 2006).

2.6.2. Generalized-Onset Seizures:

This type of seizures include the disturbance of electrical activity which effects the whole or large area of the brain and characterized by loss of the consciousness.

2.6.2.1 Absence seizures (formerly called petit mal):

Absence seizure is the most common type of generalized seizure which is characterized by weak awareness and responsiveness that take time (20-30 seconds) (Jones et al., 2011). It can be further classified (typical, atypical) with special features but the differences between atypical and typical absence became easy by using EEG device, while absence seizures can occur in many syndromes such as childhood absence epilepsy (CAE) and juvenile myoclonic epilepsy (JME) (Helbig et al., 2008).

Typically, the absence seizure begins from childhood at the age of 4–14 and resolve by puberty age but could persist into adulthood with normal development. It is more common in children and characterized by short period of losing consciousness, staring with unresponsiveness to outside spoken stimuli, slight twitching movements of the lips or head nodding, eye blinking, and hyperventilation. The attack may take place up to hundreds of times a day, with a duration from 10 seconds to 30 seconds, but in children, patients show normal activity with more slowly or with mistakes, loss of the muscle tone of the head, trunk or limbs have also been reported, loss of conscious that

last more than 30 seconds up to few minutes with little confusion after reconquest of consciousness. The typical absence seizures occur due to abnormal electrical activity in the thalamus cortical network and brain abnormalities that were present at the time of birth (congenital) or from a head injury or trauma, or complications of kidney or liver. However, this type of seizures may continue until adulthood (Slaght, 2002; Goldenberg, 2010). Atypical absence seizures are less common than typical absences, and could occur at any age, associated with some of neurological impairment (Farwell et al., 1985).

2.6.2.2. Myoclonic seizures:

In this type of seizures, the durations are short, the patient stays awake and is able to think clearly. The attack in this type affects the arms, upper legs, neck, and shoulder, and may show a slight of jerking of muscle or some of the muscles. These seizures are associated with both typical and atypical absences but more commonly without loss of awareness (Chadwick, 2003). It is difficult to recognize the loss of consciousness because it lasts very short, less than one second (Goldenberg, 2010).

2.6.2.3. Tonic seizure:

In this type of seizures, the durations are short, and the patient falls to the floor, suddenly muscles tension is greatly increased, the extremities are pulled towards the body, and the face may turn in blue. Loss of consciousness may take several seconds to more than one minute and is associated with myoclonic symptoms (Goldenberg, 2010).

2.6.2.4. Clonic seizures:

Clonic seizures can occur during the sleep, and characterized by flexion of neck and waist, while arms and legs of patient start to jerk rapidly and after few times of the attack the jerking slows and stops. The duration of attack is 5-20 seconds and is more common in people of abnormalities (Goldenberg, 2010).

2.6.2.5. Generalized tonic-clonic seizures:

This type of seizures can occur spontaneously or following to simple seizures (VanMeter and Hubert, 2014). It consist of two main phases: tonic phase and clonic

phase, where the tonic phase takes place first, it is short and the patient shows quick loss of consciousness, falls to the floor, suddenly muscles tension is increased and extremities pulled towards the body and the face of patient may turn in blue, lack of salivation and difficulty in swallowing, biting the lip and the tongue, causing bleeding and bladder incontinence taking several time from 30-20 seconds (Goldenberg, 2010). In the clonic phase, arms and legs of the patient start to jerk rapidly after few times of the attack the jerking (rhythmic jerking movements), which progressively slow down before cessation. These seizures last for a few minutes (Burnham et al., 2007). EEG readings exhibit generalized, highly fast effect in both hemispheres (Blumenfeld, 2012).

2.6.2.6. Atonic seizures:

This is an uncommon seizure, indicating the loss of body tone, and characterized by loss of muscle strength which leads to falling, followed by loss of awareness for one or two minutes. This type of seizures begins in childhood and may continue until adulthood (Copper and Gosnell 2015).

2.6.3. Status epilepticus:

Status epilepticus (SE) is described as the repeated seizures without full return of awareness between the seizures or any repeating seizures that may last up to half an hour or more, while some types of seizures can develop into the status epilepticus (Kerr et al., 2017; Bautista, 2013). This condition is risky for the life of patient and needs an urgent attention due to activity of seizure may last more than 30 minutes which leads to damage to the brain or die. This type is more common in people with at 60 years old or more.

2.6.4. Non-epileptic seizures:

In this type, the cause of seizures is not caused by the electrical activity in the brain and can be divided into two main types: psychogenic seizures and organic non-epileptic seizures.

2.7. AEDs according to seizure type:

AEDs are among the common main treatments for patients with epilepsy, and are considered as the first-line treatment for people with epilepsy, where potassium bromide is used for controlling seizures. The decision to starting of AED therapy and choosing the drugs should be implemented after checking the health, lifestyle, pregnancy conditions of the patient as well as the activity and type of seizures and which area of the brain is affected. According to these factors, the agents chosen for the treatment can be considered 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. Table 1 summarizes AEDs according to seizure type (Bromfield et al., 2006).

Using the AED therapy aims at disposing of seizures, decreasing or obtaining a minimum degree to a possible degree. It also aims at avoiding the adverse effects associated with therapy and help the patients maintain their physical activities, and a typical way of life. (Bazilet al., 2005).

Anti-epileptic drugs can be classified according to their essential mechanisms of action as follows:

- Blocking of voltage-gated channels (sodium or calcium).

- Interfering with excitatory glutamate transmission.

- Enhancing inhibitory gamma-aminobutyric acid (GABA).

Seizure Type	Drugs
Generalized Tonic-Clonic seizures	Carbamazepine, Lamotrigine, Oxcarbazepine, Valproate
Tonic or atonic seizures	Valproate
Absence	Ethosuximide, Lamotrigine, Valproate
Myoclonic seizures	Levetiracetam, Valproate, Topiramate
Focal seizures	Carbamazepine, Lamotrigine, Levetiracetam, Oxcarbazepine, Valproate
Partial Seizures	Carbamazepine, Phenytoin, OxcarbazepineValproic Acid
Atypical Absence Myoclonic, and Atonic Seizures	Valproic acid, Lamotrigine, Topiramate

Table 1. AEDS According to Seizure Type

2.8. The General Treatment of Epilepsy:

The first step of the treatment of epilepsy is a full diagnose of the type of epilepsy, whether it is psychological or physiological. psychological, physiological and mortality of the epilepsy patients consider as the reason counts to sudden unexplained death in epilepsy (SUDEP). The diagnosis is giving the reason for epilepsy, if possible, with some of the epilepsy patients (Kerr et al., 2017), however, before starting the treatment, the patient must be diagnosed to determine the type of seizures, to easily control the seizure.

2.8.1. Medical (pharmacological) treatment:

The antiepileptic drugs are considered as first choice in medical treatment for epilepsy, while they are useful in controlling or decreasing seizures in approximately 2-

3 of epilepsy patients. General mechanism of action of antiepileptic drugs is decreasing effect of the electrical activity of brain through inhibiting neuronal depolarization via blocking voltage-gated channels (blocking calcium or sodium channels), increasing the function of potassium channels and blocking glutamate excitation neurotransmitter, enhancing inhibitory gamma-aminobutyric acid (GABA) (Kwan et al., 2001; Bui et al., 2015).

Brown (2016) has indicated that the antiepileptic drugs act to prevent GABA transport, inhibit GABA metabolism or inhibit the excitatory effect, especially the glutamate neurotransmitter

There is a broad spectrum of useful AEDs which are present in various forms: parenterals, capsule, tablet, and syrup for infants and babies. However, when one scheme a plan for treatment, the type and severity of symptoms, combined medication, past response to drugs should be taken into consideration (Nice, 2016).

Medical (pharmacological) treatment is globally considered as the first choice of treatment for epilepsy patients. The epileptic seizures of most patients can be controlled with anti-epileptic (AED) drugs. Overly, these drugs and it is side effects, are bound with the life-time of the patients (Perucca, 2012). However, 30% of epilepsy patients will have some seizures even with the use of anti-epileptic drugs (Eadie, 2012). Besides, patients with AED drug-resistance use surgery as epilepsy therapy.

Voltage-gated sodium channels control the action potential through opening or closing channels which leads to allowing or disallowing the sodium ions passage across the neuronal membrane. PHT, CBZ, LTG, and OXC act by blocking the quickly inactivated state of the sodium channel, while LCM and ESL acetate act by blocking the slowly inactivated state of the sodium channel. Some of the drugs have multiple mechanisms like VPA, FBM, TPM, ZNS by effecting the sodium channels. Sodium channel blockers work during high-frequency repeated action potentials without side effects on the physiological neuronal activity when they are administered at therapy dosing. They act by preventing the repeated action potentials in epileptic focus, and the prevalence of seizure (Lason et al., 2011; Baulac et al., 2017).

The high calcium voltage channels control the release of neurotransmitter from the presynaptic nerve through commanding the amount of penetrated calcium ion which pass through neuronal membrane, PGB and TPM are voltage calcium channel blockers but GBP and PGB block the channel by binding to α2δ subunit. While VPA, ESM and ZNS act on (T-type) calcium channel which plays a pathological role in the absence of seizure (Lason et al., 2011).

The GABA is considered as one of CNS principal neurotransmitter which inhibits the neurotransmitters and is divided into three types of receptors (GABA-A, B, C). Stimulation of inhibitory neurons releases inhibitory neurotransmitter molecules such as GABA- A which bind to the receptor in the postsynaptic cell membrane this leads to an increase in the entrance of such ions like potassium (K^+) and chloride (Cl^-); the influx of Cl^- or efflux of K^+ cause a weak hyperpolarization. GABA-A receptor presents an activity by forming a quick inhibitory effect in postsynaptic potentials, controlling the activity of seizures and inhibiting the spread of seizure activity. PB and BZD are antiepileptic drugs that act on activating the GABA-A receptor while VGB and TGB enhace the GABA by blocking the GABA aminobutyric acid reuptake into the presynaptic also inhibiting GABA aminotransferase. VPA has multiple mechanisms of action and one of those mechanisms is to enhance the inhibitory effect of GABA (Lason et al., 2011).

Glutamate is considered one of the principal CNS neurotransmitters with the excitatory effect which is suggested to inhibit the formation and diffusion of the seizures (Greenwood and Valdes, 2016). It stimulates several receptors, such as (NMDA), (AMPA), and kainate receptors. The stimulation of these receptors causes the release of excitatory neurotransmitters such as glutamate and acetylcholine which binds to the receptors on the postsynaptic. The receptor is contain of channels that control the calcium and sodium ions influx, and outflux of potassium ions (Bromfield et al, 2006; Greenwood and Valdes, 2016). LCM and FBM show antagonistic activity towards the glycine-binding site on NMDA receptors. PER is a selective antagonist of the AMPA receptor, while TPM has an inhibitory effect on kainate receptors. PB has an inhibitory effect on AMPA receptors.

Muscarine potassium channels type Kv7 (KCNQ) are responsible for controlling excitatory of the neuronal. RTG is a first-in-class potassium channel opener (Lason et al., 2011).

The synaptic vesicle protein 2A

This is a general CNS protein distributer, which modulates the secretion of neurotransmitters, especially glutamate. LEV and brivaracetam are selectivity binding action to synaptic vesicle protein 2A (Gao and Li, 2016).

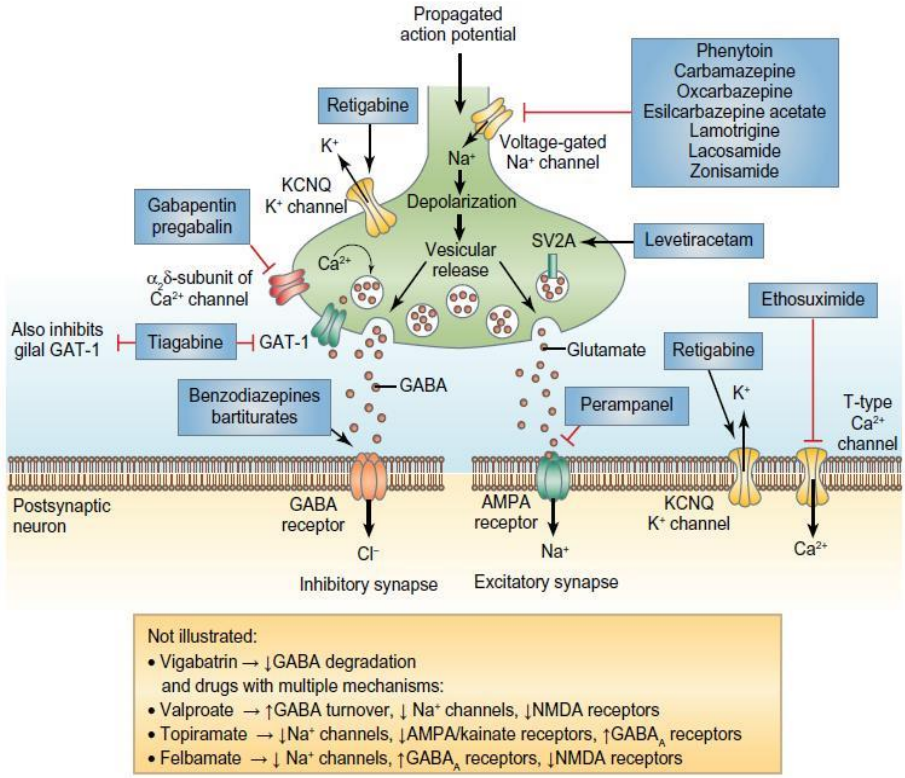


Figure 4. Mechanism of Action of Different Antiepileptic Drugs

2.8.1.1. Barbiturates: phenobarbital and analogues:

These are one of the oldest antiepileptic drugs. The barbituric acid derivatives considered as the primary treatment for status epilepsy as it enhances the inhibitory effect of GABA neuron as a primary mechanism of action. Barbiturates include also mephobarbital(N-methylphenobarbital), primidone (deoxyphenobarbital) and both are

metabolized to phenobarbital in the body (Bialer, 2012). 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 α3-continent, rather than that, it can give opposite effect (Greenfield, 2013). Phenobarbital and its derivatives differ with phenytoin in sedative side effects (Vajda and Eadie, 2014).

2.8.1.2. Hydantoins: phenytoin and derivatives:

Phenytoin is one of the oldest drug used as an anti-seizure therapy by blocking the sodium voltage channels, the chemical structure of which is affiliated with barbiturates. The important advantage for phenytoin is to produce an effective treatment for focal seizure, status epilepticus, and generalized tonic-clonic seizures without causing sedation as a side effect. In this context, fosphenytoin is a prodrug that is quickly converted into phenytoin in the blood in a minute and can be administrated intramuscularly. (Bialer, 2012) (Meldrum and Rogawski, 2007; Stafstrom, 2007).

2.8.1.3. Ethosuximide:

The first drug that used in the treatment of absence seizures is ethosuximide which binds to the T-type Ca^{2+} channel and stops the entrance of Ca^{2+} as a mechanism of action which is utilized in controlling the seizure in absence epilepsy (Coulter et al., 1989). Ethosuximide consists of succinimide which have not anticonvulsant activity, but by introducing ethyl and methyl groups at the position 3 of the succinimide nucleus originates the ethosuximide (Bialer, 2012).

2.8.1.4. Benzodiazepines:

Benzodiazepines are antiepileptic drugs which are used in emergency situations or in acute seizures as well as in particular conditions, such as status epilepticus, like (diazepam, clonazepam, and lorazepam which bind to benzodiazepine sites causing the opening of Cl^{-} ion channels and leading to hyperpolarization of the neurotic membrane, either in some pediatric syndromes like (nitrazepam) (Sohal et al., 2003; Vajda and Eadie, 2014). Clobazam was approved in Australia, Europe, and Canada and in 2011 and approved in the USA for the treatment of seizures especially with Lennox-Gastaut syndrome in adults and children younger than 2 years old of age (Chong and Lerman,

2016). However, benzodiazepines cause certain side effects like sedation, tolerance, memory problems, hyperactivity and withdrawal effects (Vajda and Eadie, 2014).

2.8.1.5. Carbamazepine, oxcarbazepine and eslicarbazepine acetate:

Carbamazepine (CBZ) is the most popular drug, prescribed as antiepileptic for the treatment of focal and generalized tonic-clonic seizures (Brodie et al., 2003). Chemically, it is nearly related to imipramine as a tricyclic compound and used in the treatment of bipolar depression (Bialer, 2012). Oxcarbazepine is related to an iminostilbenes family which are analogs of carbamazepine (Walker and Patsalos, 1995), and is a prodrug that is quickly reduced to the 10-monohydroxy (MHD) which acts as blocking the sodium channels (Gschwind and Seeck, 2016; Krasowski and Mcmillin, 2014). The main antiepileptic action of carbamazepine and oxcarbazepine is blocking the voltage-gated sodium channels (Tecoma, 1999).

Eslicarbazepine acetate is a prodrug that is quickly metabolized and converted into S-licarbazepine (Tatum, 2013), and considered as a blocker of sodium voltage channel (Soares and Almeida, 2007). The European Medicines Agency approved eslicarbazepine in 2009 to be used as a treatment for adults with partial-onset (with or without secondary generalization) seizures (Elger et al., 2007; Elger et al., 2009; Ben-Menachem et al., 2010; Halasz et al., 2010).

2.8.1.6. Valproic acid and derivatives:

Valproic acid and derivatives chemically possess a similar structure to short-chain fatty acids (Silva et al., 2008; Zhang et al., 2013). This is an antiepileptic drug with a wide spectrum and globally considered as one the most widely prescribed AED (Nakashima et al., 2015), and a drug of choice for the treatment of partial and primary generalized epilepsy since 1970 (Williams et al., 2002; Nanau and Neuman, 2013). It is also recommended for patients with seizures which are difficult to diagnose, and the most important side effect of the drugs is teratogenicity (Vajda and Eadie, 2014). In addition, valproate is an anti-epilepsy inhibitory drug that increases GABA in the brain by inhibiting GABA degradation, improving GABA synthesis and inhibition of glutamatergic transmission which controls the sodium and potassium channels (Welch

et al., 1975; Löscher and Schmidt, 1980; Cutrer and Moskowitz, 1996; Cutrer et al., 1997; Stahl, 2004; Emilio, 2005).

2.8.1.7. Gabapentin, pregabalin, vigabatrin and tiagabine:

The chemical structure of gabapentin is 1-(aminomethyl) cyclohexane acetic acid, it is related to the neurotransmitter gamma- aminobutyric acid (GABA), structurally (Marson et al., 1997; Morris, 1999; Goldenberg, 2010). Gabapentin has been licensed in the UK as adjunctive therapy for epilepsy since 1993 (Pitkanen, 2005) and approved in Canada which was prescribed more than 3.9 millions, making it the most common drug in Canada in 2015. According to some studies and the FDA recommendations, gabapentin has been proven as adjuvant therapy for focal seizure with/without secondary generalization in 2016 (Honarmand et al., 2011; Krasowski and Mcmillin, 2014). However, gabapentin mechanism of action is still not fully understood (Bruni, 1998; Beydoun et al., 1998). In human body gabapentin is not metabolized into GABA, and does not degrade or inhibit GABA reuptake but act through binding to the subunit α2δ of voltage calcium channels (Pfizer, 2016). On the other hand the α2δ -1 and 4-subunit protein of voltage-gated calcium channels presynaptic showed to be the key lock of gabapentin and pregabalin (Stahl et al., 2013). Pregabalin binds to α2δ subunit of calcium channels in the CNS, while it is considered as a potent analog of gabapentin (Bialer, 2006) and used much more for the management of diabetic peripheral neuropathy, fibromyalgia, and postherpetic neuralgia more than for the treatment of seizure disorders but have been proved to show effect in focal-onset seizures (Schulze-Bonhage, 2013; Krasowski and Mcmillin, 2014). Vigabatrin is a synthetic derivative of GABA (Krasowski, 2010) and vigabatrin may produce serious irreversible visual deficits in any concentration (Ben- Menachem, 2014; Tatum, 2013). Tiagabine is a inhibitor of the reuptake of GABA neurotransmitters through binding to GABA aminobutyric acid receptor and induce the Cl^{-} ion influx, by prolonging their extracellular existence. While vigabatrin also is an inhibitor of converting enzyme of 4-aminobutyrate aminotransferase (GABA transaminase) which is responsible for the inactivation of succinic semialdehyde and glutamate and which lead to increasing the level of GABA in the brain GABA (Rogawsk And Cavazos, 2014).

2.8.1.8. Lamotrigine, zonisamide and topiramate:

Lamotrigine is related to the class of phenyltriazines and considered as one of the oldest antiepileptic drugs, but structurally is not relating to any of the antiepileptic drugs. The drug was used before in the management of epilepsy with pregnant women and supposed to be one of the best options for treatment but was discontinued due to the rash as side effect (Yasam et al., 2016). Lamotrigine was used to treat epilepsy (Goldsmith et al., 2003) and widely used in partial, secondary generalized tonic-clonic seizures, and Lennox-Gastaut syndrome (Stefan and Feuerstein, 2007). Lamotrigine blocks presynaptic sodium channels that reduce neurotransmitter release by stabilizing the presynaptic neuronal membrane (Cheung et al., 1992; Cunningham and Jones, 2000). Zonisamide is a benzisoxazole derivative with a sulphonamide side chain and has a wide spectrum of action used in both focal and generalized seizures treatment through inhibition of both Na^{+} andCa^{+2} channels, and were used in patients of all ages in Japan, Korea, USA and Europe (Emilio, 2005; Cox et al., 2014). Topiramate exhibits a broad spectrum of activity, and considered as one of the sugar derivatives, manufactured from D-fructose and acetone (Bialer, 2012). It is characterized by the capacity to resist different types of seizures (Kaminski et al., 2014) through different mechanism of actions blocks voltage-gated sodium channels, inhibits (AMPA) and kainate glutamate receptors and enhancement of GABA at some subtypes of GABA-A receptors (Follett, 2004). However, the undesirable adverse effects of the drug do not show a good tolerability profile, lasting weight loss, and cognitive slowing potential (Gschwind and Seeck, 2016).

2.8.1.9. Felbamate and stiripentol:

Felbamate is used for treating focal seizures in adults as well as in the Lennox-Gastaut syndrome through binding to NMDA site rather than the glycine and stopping the Na^{+} influx (Sohal et al., 2003). The use of the drug is limited because it causes aplastic anemia and severe liver failure as a serious side effect (Krasowski and Mcmillin, 2014). Stiripentol is an aromatic allylic alcohol that has been approved in Europe and USA for the treatment of severe myotonic epilepsy (Dravet syndrome) in infants (Krasowski and Mcmillin, 2014; Verrotti et al., 2016).

2.8.1.10. Levetiracetam and brivaracetam:

The chemical structure of levetiracetam is a heterocyclic amide related to piracetam (Bialer, 2012). It is considered as a first-choice therapy for the status epilepticus (Gschwind and Seeck, 2016) by inhibiting the excitatory neurotransmitter release through binding with synaptic vesicle protein SV2A (Lynch, 2004; Kaminski et al., 2012). In this context, brivaracetam was discovered and approved in February 2016 for using in the treatment of focal-onset seizures in epilepsy cases above 16 years old (Mula, 2016a).

2.8.1.11. Rufinamide, lacosamide, retigabine and perampanel:

Rufinamide is an anticonvulsant agent, available as an adjunctive treatment used in combination with other drugs in the therapy of seizures especially in children for Lennox-Gastaut syndrome at the age of 4 years or more, Rufinamide consider as triazole derivative (Ben-Menachem, 2014). Lacosamide is an effective amino acid that was developed specially for using against against non-convulsive and convulsive status epilepsy in patients older 16 years old (Gschwind and Seeck, 2016). It acts through the inactivation or blocking of sodium channels which leads to inhibition of repeated neuronal firing (Meldrum and Rogawski, 2007; Stafstrom, 2007; Hoy, 2013; Huang et al., 2015). While Lacosamide has been approved in the therapy of focal onset seizures (Stephen and Brodie, 2011). Retigabine or ezogabine is known as carbamic acid ethyl ester derivative in the United States (Tatum, 2013). Due to the adverse effects, the use of retigabine was limited to the patients who already taking the drug and showing good response without side effects (Ben- Menachem, 2014). Perampanel is an AED that was recently approved (PER) and effective in the treatment of myoclonic seizures and Lennox-Gastaut syndrome, including gradual myoclonus epilepsy (Goldsmith and Minassian, 2016). Besides, it is one of the selective AMPA receptor antagonist which is used for the treatment of epilepsy (Gschwind and Seeck, 2016; Satoru and Shin-ichiro, 2019).

2.9. General information about experimental material

2.9.1. Atropine:

Atropine, a tertiary amine, belonging to belladonna alkaloids is present in a number of natural plants of the nightshade family like Jimson weed, deadly nightshade and mandrake (Brust, 2004). It has been reported that the isolation of atropine was first made in 1833 (Roberts, 1998). Atropine was used in it's salt from to form that produced from reaction with sulfuric acid (atropine sulfate) and is present in the model list of essential medicines as a necessary medication required in the health system (WHO, 2014). The atropine has been used as an essential component in the pharmceutical formulations for various indications including eye drops, solution for intravenous injection, and nerve intoxication medication. Pharmacologically, atropine exhibits high affinity to the muscarinic receptors, known as cholinergic blockers because is considered as a competitive antagonist of acetylcholine by preventing the acetylcholine from binding to corresponding binding site on muscarinic receptors. Moreover, atropine prevents the acetylcholine from binding to the muscarinic receptors which are present on the muscle of the heart, sweat glands, ganglia of the peripheral, smooth muscle, and CNS especially when administered at high dose it is capable of penetrating through the blood-brain barrier (McDonough and Shih, 2007; Fusek et al., 2015). Atropine is a primary medication and drug of choice in the treatment of insecticide intoxication and organophosphate. Furthermorei it has been used for long duration of time. Insecticide intoxication and organophosphates are toxic chemicals substances which has a biological effect acting by inhibiting acetylcholinesterase enzyme, breaking down the acetylcholine neurotransmitter and increasing acetylcholine level in the synapses muscarinic, nicotinic, and cholinergic, with overstimulation of the cholinergic system (Geyer et al., 2009).

Some clinical and animal studies have shown that high selective antagonists for muscarinic acetylcholine receptors AChR may be beneficial in the medication for neurodegenerative CNS disorders comprising dystonia, Parkinson's disease and epilepsy (Bymaster and Felder, 2002; Bymaster et al., 2003c; Bymaster et al., 2003b;

Katzenschlager et al., 2003; Wess et al., 2007; Fisher, 2008b; Langmead et al., 2008a; Conn et al., 2009b).

However, Donders recommended atropine as a treatment in patients with myopia (Donders, 1864), however, before that time atropine had been used as a pupillary dilator to diagnostic the posterior segment of the eye short time treatment to enhance the vision in condition of cataracts and to promote mydriasis during the ophthalmic surgery (Jones, 1856).

2.9.2. Caffeine:

Caffeine (1,3,7-trimethylxanthine), is natural alkaloid of plant origin and belongs to purine alkaloids, and universally is the most common and widely consumed CNS stimulant (Nehlig, 1999). Caffeine is present in some of our daily beverage products including tea, cola drinks, energy drink's, chocolate candy, cocoa and the heights dose in coffee (Gilbert et al., 1976; Fredholm et al., 1999; Kaufman and Sachdeo, 2003). It is also present in some of antiviral drugs, analgesics and appetite stimulants (Gilbert et al., 1976; Nehlig et al., 1992; Bernstein et al., 2002; Jankiewicz et al, 2007).

Caffeine possess multiple mechanisms depending on the administered dose: 1) inhibition of phosphodiesterase enzyme which results in decreasing of cyclic adenosine monophosphate responsible for vasodilation of smooth muscle, 2) stimulation of ryanodine receptor which is responsible for the release of intracellular calcium, 3) inhibition of GABAA receptor, 4) blocking adenosine receptors, 5) at concentrations of 500 uM caffeine could inhibit glycine receptors (Daly, 2007). Caffeine penetrates through placental and blood-brain barriers (Ikeda et al., 1982; Tanaka et al., 1984; Arnaud, 1993).

Some clinical and animal studies have reported that caffeine can prevent seizures in a patient with epilepsy. However, the seizures may occur after the intake of toxic doses of caffeine (Banner and Czaijka, 1980; Babu, 2011) or intake caffeine for a long period (Antonaci et al., 1996; Kaufman and Sachdeo, 2003; Mackow et al., 2016).

The importance of activation of the central A1 receptor is sedation, anxiolytic, and anticonvulsant effects) (Londos et al., 1980). The A2 receptor is a Gs protein-coupled stimulation of receptor that might result in activation of adenylyl cyclase and lead to the release of neurotransmitters including acetylcholine, noradrenaline, dopamine, and glutamate (Daly et al., 1983; Sebastiao and Ribeiro, 1996).

2.9.3. Minoxidil:

Minoxidil, a piperidine-pyrimidine derivate, was used at the beginning of the 1970s as an anti-hypertensive agent. Hypertrichosis was a well-known side effect of minoxidil and enhance hair growth for male type baldness (Zappacosta, 1980), and it is also used in females to prevent hair loss and enhance hair growth (Cotterill and Unger, 1987; Rossi et al., 2012; Herskovitz and Tosti, 2013).

It generally acts as a potassium channel opener in kidney cells and human prostate cancer cells (Abdul and Hoosein, 2002; Schwab et al., 1993). A number of studies have demonstrated that minoxidil supresses the development of malignant cells in the prostate cancer (Hsu et al., 1994)

According to Gu et al. (2013) minoxidil acts to increase the permeability of blood-brain tumor barrier in the rat.

The impact of minoxidil in Ca^{2+} homeostasis in prostate malignant cells is indistinct. Moreover, minoxidil is considered as a strong arteriolar vasodilator (powerful vasodilator) as it acts as a potassium channel opener present on the cells of the smooth muscle of the peripheral artery producing a high decrease in blood pressure without orthostatic hypotension, and with no adverse effects that normally associated to adrenergic receptors (Gilmore et al., 1970; Charme et al., 1973; Editorial, 1973).

The anti-hypertensive and the follicular effects of minoxidil are associated with its dynamic metabolic derivate, the minoxidil sulfate (Buhl et al., 1989). FDA has approved minoxidil for the treatment of Androgenetic alopecia (AGA).

2.9.4. Usnic acid:

Usnic acid (UA) is normally found in a lichen species, and considered as the most important secondary metabolite of lichens Usnicacid (UA) (Maciąg et al., 2014). It was firstly isolated in 1844 as crystalline with yellow color by the German scientist Knop, while usnic acid (UA) was synthesized chemically from methylphloroacetophenone (Komiya and Shibata, 1969).

Several studies on lichen and UA have shown pharmacological effects as antioxidant, healing, analgesic, anticonvulsant, antiemetic, anti-asthmatic, antimicrobial, anti-inflammatory, antiviral, and antibiotic properties, but it causes some adverse effects including liver harmfulness and contact allergy (Cocchietto et al., 2002; Ingolfsdottir, 2002; Shukla, 2010; Yousuf, 2014; Raizada et al., 2014; Luzina and Salakhutdinov, 2016).

Usnic acid has been advertised as a food product in the USA to help in weight loss since it cause an increase in fat digestion and basal metabolic rate (Guo et al., 2008).

Usnic acid as potassium salt inhibits the secondary growth of malignant cells in colorectal cancer (Yang et al., 2018).

Animal Studies showed, on the homogenates of liver and rat, they got two actions depending on usnic acid concentration, at 1μM, an increase in oxygen consumption with a present of several substances. At (8-30μM) concentrations, the uptake of phosphate decreased although the fall in oxygen consumption (Johnson et al., 1950; Pramyothin et al., 2004).

Usnic acid has been reported to show a good effect in the therapy for diseases, however, followed by liver disorder (hepatotoxicity) when taken as a dietary medication to lose weight (Maciąg et al., 2014).

Odabasoglu has stated that the antioxidant effect in indomethacin-induced ulcers in rat's stomach is because of it’s ability to perform peroxyl radical scavenging, reduce hydroxyl radicals and to decrease the synthesis of nitrite (Odabasoglu et al., 2006)

The analgesic and antipyretic actions of UA were evaluated by some studies on mice. These studies used usnic acid at low dose of 100 mg/kg for oral administration; UA exhibited an analgesic effect similar to acetic acid, while on high oral dose at 300 mg/kg, an antipyretic effect especially after lipopolysaccharide-induced hyperthermia (Neff et al., 2004).

2.9.5. Glibenclamide:

Glibenclamide is a potent second-generation sulfonylurea antidiabetic medication that enhances the control of glucose through acting on insulin secretion (Luzi and Pozza, 1988). Moreover, it acts by blocking or modulating ATP sensitive potassium channels, any drug that acts by modulating potassium currents might have potential activity against convulsion. (Body,1988). The ATP-sensitive K+ (KATP) channels are present in numerous cells, including heart myocytes, B cells in the pancreas, muscle cells, and neurons (Hosseinzadeh et al., 2018) and are linked to various physiological functions (Acufla et al., 1995; Bahrami et al., 2018). KATP channels are also present in presynaptic and post-synaptic various areas in the brain.

While, the action of KATP channels are controlled by cell metabolism and which gives way to connecting the electrical activity of a cell to its metabolic state (Manhole, 2004).

Few studies have shown that potassium ATP channels play an outstanding role on seizure arresting in different models (vitro or vivo) (Mattia et al., 1994; Katsumori et al., 1996; Yamada and Inagaki, 2005; Shafaroodi et al., 2007).

Several studies on mice have shown that blocking a subunit of KATP channels cause a decrease in hypoxia and in threshold for generalized seizure (Yamada et al., 2001).

3. MATERIAL AND METHODS

3.1. Material:

Atropine, glibenclamide (Lahmann et al.,2015), minoxidil (Chen et al., 2017), caffeine (Tchekalarova et al.,2013), and usnic acid (Asma'a and Sarah.,2014) were dissolved in saline. Saline and drugs were given intraperitoneally (i.p.) in a volume of 1 ml/kg body weight.

3.2. Experimental animals model:

Swiss albino male mice 8-10 and weighing 25-35 g were housed under standard laboratory condition at least 1 week prior to experimentation and were allowed to both food and water. All studies were approved by the Van Yüzüncü Yıl University local ethics committee on animal experiments (2018/10. 25.10.2018) and were in accordance with the EU directive 2010/63/EU on the protection of animals used for scientific purposes. As shown in figure 5

Figure 5. Animal Model Mouse

3.3. Conducting Experiments

Investigation of the effect of glibenclamide, minoxidil, caffeine, and usnic acid on convulsions caused by atropine administration and food in mice fasted for 24 hours was made.

In our study, the anticonvulsant effects of glibenclamide, minoxidil, caffein, and usnic acid, which were previously determined to be antiepileptic in different epilepsy

models would be investigated for the first time in this model (Pithadia and ark.,2013; Tchekalarova et al., 2013; Ostadhadi and ark.,2018).

After being weighed, mice were moved to clean cages with fresh bedding and were either fed ad lib (fed) or deprived of food (fasted) with free access to water. Twenty-four hours later, at time of testing, fasting animals were reweighed and adminestred with saline solution or glibenclamide at various doses (25, 50 mg/ kg) (Lahmann et al.,2015), minoxidil (25, 50 mg/ kg)(Chen et al., 2017), caffeine (10, 20 mg/ kg) (Tchekalarova et al.,2013), usnic acid (100,300 mg/kg) (Asma'a and Sarah.,2014), and 10 minutes later with saline or atropine at a dose of 2.4 mg/kg was injected. All animals were individually placed in wire mesh cages soon after injections and were given food pellets 20 min later. They were observed for 30 min for the incidence and onset of convulsions. Seizure activity was quantified by staging: (0) no difference; (1) freezing and gustatory movements; (2) forelimb clonus; (3) forelimb clonus with rearing; (4) forelimb clonus with rearing and falling down; (5) generalized convulsions with rearing, falling down, and jumping. A convulsive response was assessed as forelimb clonus with rearing. Onset of convulsions was defined as the time passed before an animal displayed forelimb clonus with rearing after starting to eat. Incidence of convulsions was expressed as the percentage of animals displaying either stage 3, 4 or 5 activity in each group.

Groups:

Control (Serum physiological, 1 ml / kg)

Atropine (2.4 mg / kg)

Glibenclamide (25 mg / kg) + Atropine (2.4 mg / kg)

Glibenclamide (50 mg / kg) + Atropine (2.4 mg / kg)

Minoxidil (25 mg / kg) + Atropine (2.4 mg / kg)

Minoxidil (50 mg / kg) + Atropine (2.4 mg / kg)

Caffeine (10 mg / kg) + Atropine (2.4 mg / kg)

Caffeine (20 mg / kg) + Atropine (2.4 mg / kg)

Usnic acid (100, mg / kg) + Atropine (2.4 mg / kg)

Usnic acid (300 mg / kg) + Atropine (2.4 mg / kg)

3.3.1. 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 complete nervous system as physiological and psychological symptom was seen with most of the neurologic patients (WHO, 2016). In the current study, brain electrical abnormalities were examined, by observing paralysis, muscle weakness, poor coordination, and confusion behavior of the mice. After treatment, animals were immediately placed for Locomotor activity test and the Rota rod test, carried on the tested day.

3.3.2. Locomotor activity test

The locomotor activity device is 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 was used to measure depression, mental activity, exploration, anxiety and locomotor activity just as seizures psychotic emotion. However, an increase in the count of mice movement was considered as central nervous stimulation while a decrease in the number of mice movement was regarded as central nervous depressant activity (Correia et al., 2016) as shown in Figure 6.

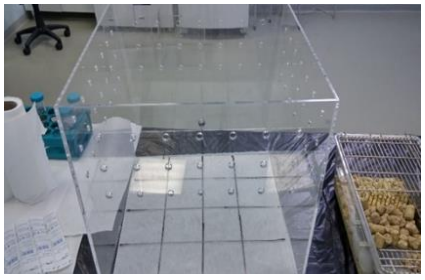


Figure 6. Locomotor Activity Devise

The Procedure

The mice were handled from the base of their tails in the center of the locomotor activity test with the four paws and allowed to explore the apparatus for 5 minutes. Epileptic seizures were observed and square transitions were counted for 30 minutes, according to (Brown et al., 1999; Machado et al., 2015).

3.3.3. Rota rod test

Rota-rod test was used to test the skeleton muscles relaxation as it assesses motor coordination, motor learning, toxicity, sedation, stamina, motor memory, and balances of the mice on the rota rod device. This test 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 (Asuntha et al., 2010) as shown in Figure 7.

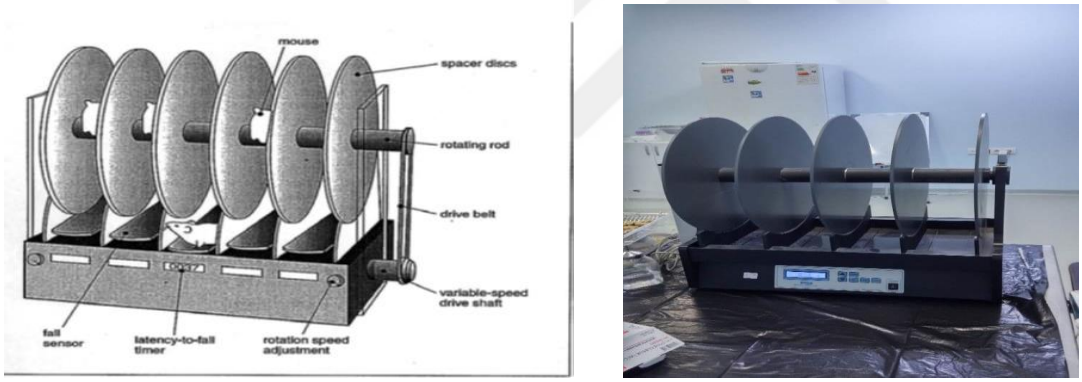


Figure 7. Rota Rod Devise

The Procedure

After treating mice with glibenclamide (25, 50 mg/kg), minoxidil (25, 50 mg/kg), caffeine (10, 20 mg/kg), usnic acid (100,300 mg/kg)10 minutes later saline or 2.4 mg/kg atropine were administrated. The mice were handled from their tails at all times to be placed on the Rota rot stand of 25 mm diameter at the speed of 10 rpm for 3 minutes with changing the gray rubber foam prior with each new group (Machado, 2015). There was one pre-test for the mice to get used to the Rota Rot instrument. Once the mice attain their position, there was an accelerating the rotating from 4 rpm to 10 rpm in 3 minutes. Between one and another mouse, the device was cleaned with water

then with 50% ethanol and the length of the stay on the tracker for every mouse was recorded and the mouse was never been dropped from the instrument.

3.3.4. Sample prepares for biochemical assay

At the end of the observation period, the Locomotor activity test, and Rota rod test. the animal were administrated with anesthesia, the brains were rapidly removed and then washed with cold saline solution two times and kept at -30 ◦C.

The brains were cut into smaller parts by using scissors and were homogenized with 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, BandelinSonupuls) that was 8 min homogenized to bust down the cell continents and extract solution A in this phase. (Ilhan et al., 2004; Kiasalari et al., 2012; Kiasalari et al., 2013; Rahmati et al., 2013).

Nevertheless, the homogenate solution was centrifuged at 9500 rpm for 30 min to insulate 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) (Ilhan et al., 2004; Kiasalari et al., 2012; Rahmati, 2013; Kiasalari, et al., 2013).

3.3.5. Measurement of Oxidative Stress Parameters

The animals were decapitated 60 minutes after drug treatment. Oxidative stress parameters in total brain tissue were examined. The wet weights of tissues were set to 50 mg and hardened by means of liquid nitrogen and powdered in a porcelain mortar. Tris/sucrose buffer were added to the tissue transferred to the glass tube at 1/10 (w/v). The tissue samples transferred to glass tubes were numbered and centrifuged at 3,000 rpm for 10 minutes at +4 °C. After centrifugation, malondialdehyde (MDA), nitric oxide, protein carbonyl group and glutathione (GSH) levels, catalase, superoxide dismutase (SOD), activities and protein levels of supernatant were examined.

Lipid peroxidation levels were measured according to the Ohkawa method via thiobarbituric acid (TBA) reaction, the method was based on the color that resulted from the TBA with malondialdehyde reaction at 532 nm, and the spectrophotometer was used to measure the color. It depends on the absorbance of the color (pink-red) which were resulted from the reaction of MDA is one of the most stable lipid peroxidation products with thiobarbituric acid on the spectrophotometric evaluation.

Measurement of Catalase Activity:

CAT was measured according to the method described by (Aebi, 1984), which is based on observation the enzymatic degradation of the H2O2 substrate by catalase at 240 nm (Nader et al., 2017).

Measurement of SOD:

The principle of measurement of SOD enzyme activity, which is considered as an accelerator the dismutation of endogenous and exogenous toxic superoxide radicals formed during oxidative energy generation into water and molecular oxygen, was described by the Sun method (Sun, 1988). This method is based on the computation of the color absorbance by nitroblue tetrazolium (NBT) at 560 nm by superoxide radicals produced by xanthine oxidase in the presence of xanthine (Nader et al., 2017).

Glutathione (GSH) Level Measurement:

GSH analysis was performed according to the method reported by Beutler et al., (1963). All the proteins without a sulfhydryl (-SH) group in the brain tissue homogenate were precipitated. In the clear liquid obtained, the yellow complex formed by the -SH groups with DTNB is measured colorimetrically at a wavelength 412 nm

Nitric Oxide Measurement:

The amount of nitric oxide was measured by (Miranda et al.,2001) “Vanadium-3-chloride-Griess Reaction method” method. The measurement is the conversion of vanadium chloride to nitrate in nitrite at 37 ° C, and diazotization of nitrite with sulfanilamide, a primary aromatic amine in acidic medium, defined as the Griess Reaction, and a colored azo derivative with N- (1-naphthyl) ethylenediamine (NEDD).

Protein Carbonyl Group Measurement:

Determination of the protein carbonyl group was a method developed by (Levine et al., This method was based on the spectrophotometric determination of the color of hydrazone compounds formed by reaction of carbonyl groups formed by oxidation of proteins with 2,4-dinitrophenylhydrazine (Nader et al., 2017).

Measurement of acetylcholinesterase activity: Measurement of acetylcholinesterase activity will be performed according to the method of (Ellman et al.1961) Acetylcholinesterase is an enzyme that catalyzes the cleavage reaction of acetylthiocolin with thiocholine to acetate. AChE activity is determined by measuring the intensity of the yellow color produced by 5-thio-2-nitrobenzoic acid formed by the reaction between thiocholine and DTNB 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (ellman's reagent) on a spectrophotometer at 412 nm wavelength.

3.4. Statistical analysis

The data demonstrated with arithmetic mean and standard deviation were subjected to statistical assessment using SPSS v.16.0 for windows (IBM, NY, USA). The significant difference of tested groups was compared with one-way analysis of variance (ANOVA). Tukey multiple comparison tests used for sub-group comparisons. The statistical probability (p) value less than 0.05 were considered to be significant.

4. RESULT

During the 30-minute follow-up, animals were treated with atropine after 24 hours of fasting, except for the control group.

Groups	***Score 0***	***Score 1***	***Score 2***	***Score 3***	***Score 4***	***Score 5***
Control	0	0	0	0	0	0
Atropin	0	0	2	3	3	1
Glibenclamide (25, i.p)	0	0	3	4	2	0
Glibenclamide (50, i.p)	0	0	2	3	3	1
Minoxidil (25, i.p)	0	1	1	4	3	0
Minoxidil (50, i.p)	0	2	3	3	1	0
Caffeine (10, i.p)	0	0	2	3	3	0
Caffeine (20, i.p)	0	0	3	3	2	1
Usnic acid (100, mg/kg, i.p)	0	0	1	3	3	1
Usnic acid (300, mg/kg, i.p)	0	0	2	3	3	1

Table 2. Effect of Different Dose of Glibenclamide, Caffeine, Minoxidil, Usnic Acid on Atropine Induce Convulsion

Effect of glibenclamide at various doses (25,50 mg/kg, ip) in atropine induced convulsion, pretreatments with 25, 50 mg/kg of glibenclamide did not prevent the development of convulsions. When compared with the atropine treated mice, glibenclamide delayed the seizure onset time, but the drug groups were not statistically significant ($p > 0.05$). As elucidated in Figure 8.

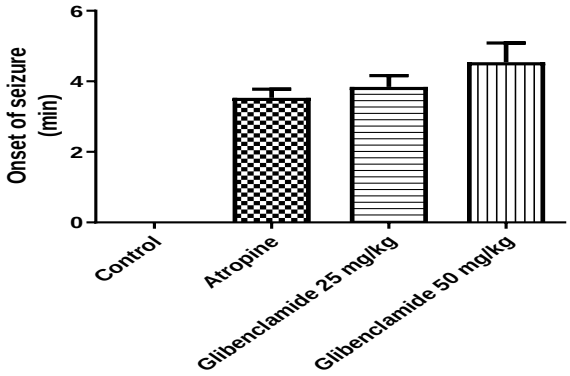


Figure 8. Effect of Glibenclamide in Atropine Induce Convulsion

Effect of caffeine at various doses (10, 20 mg/kg) in atropine induced convulsion, pretreatments with 10, 20 mg/kg of caffeine did not prevent the development of convulsions. When compared with the atropine treated mice, no statistically significant difference was found in the drug groups ($p > 0.05$). As elucidated in Figure 9.

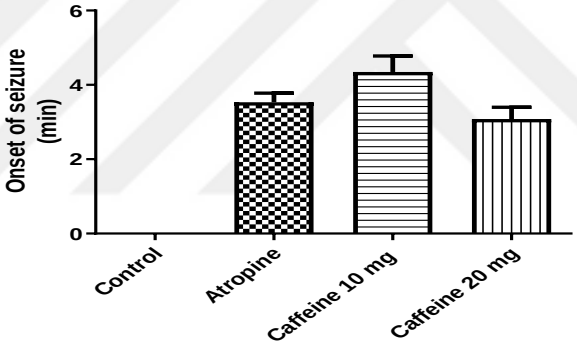


Figure 9. Effect of Caffeine in Atropine Induce Convulsion

Effect of minoxidil at various doses (25,50 mg/kg) in atropine induced convulsion, pretreatments with 25,50 mg/kg of minoxidil did not prevent the development of convulsions. When compared with the atropine treated group, the seizure onset time was significantly higher in the minoxidil + atropine group ($p < 0.05$). However, there was no significant difference found between the groups ($p > 0.05$). As elucidated in Figure 10.

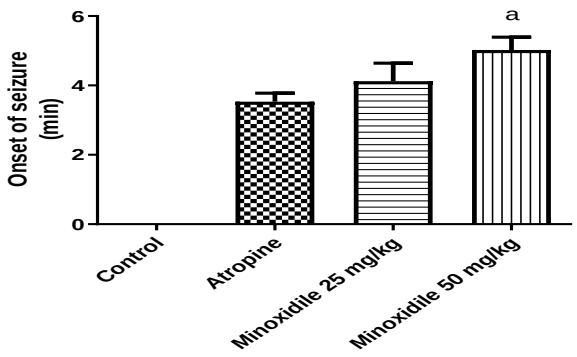


Figure 10. Effect of Minoxidil in Atropine Induce Convulsion

[a] $p<0.05$ when compared with atropine treated control group

Effect of usnic acid at various doses (100, 300 mg/kg) in atropine induced convulsion, pretreatments with 100, 300 mg/kg of usnic acid did not prevent the development of convulsions. When were compared with the atropine treated mice, no statistically significant difference was found in the drug groups ($p> 0.05$). As elucidated in Figure 11.

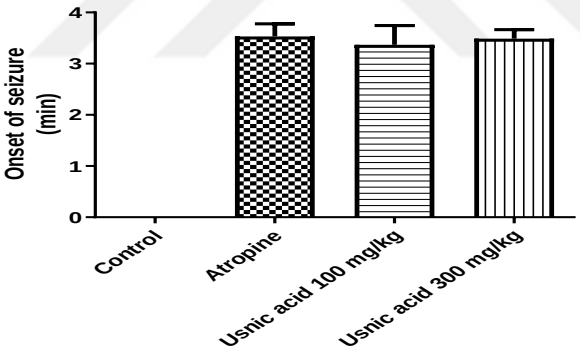


Figure 11. Effect of Usnic Acid in Atropine Induce Convulsion

Results from Rota rod and Locomotor activity tests:

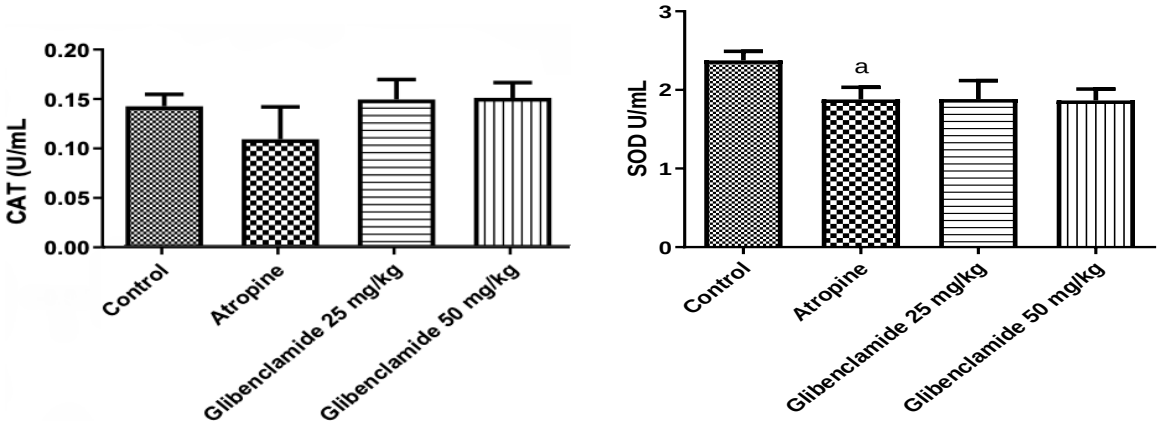
In the current study, testing the motor coordination of the animals by using Rota rod device which did not give a significant result in all drug-treated groups. Which was an indication of CNS depression and neurotoxicity.

In this study, testing the mice for their locomotors movement and exploration behaviors by using a locomotor activity test did not give significant results in all drug-treated groups. They did not exhibit any movement achievement after seizure.

Biochemical result of oxidant/antioxidant stress inducer

Capturing the brain for the biochemical assay was an efficient indicator of brain oxidant/antioxidant mechanism induced by atropine kindling with (2.4 mg/kg) on experimental animals. Oxidization stress caused by (ROS) reaction evaluated by measuring the MDA level, which is considered as an index for lipid peroxidation evaluation by atropine inducer. The high level of MDA was an indication of brain tissue injury caused by (ROS). However, the atropine group significantly higher MDA levels compared the control group ($p<0.05$). When compared with glibenclamide, there was no difference between the groups ($p>0.05$). As elucidated in Figure 12.

In the atropine group, the superoxide dismutase (SOD) levels were significantly lower when compared to the control group ($p <0.05$). When compared with glibenclamide groups, there was no difference between the groups ($p>0.05$). CAT levels were insignificant when the control, atropine, and glibenclamide groups were compared ($p> 0.05$). As elucidated in Figure 12.



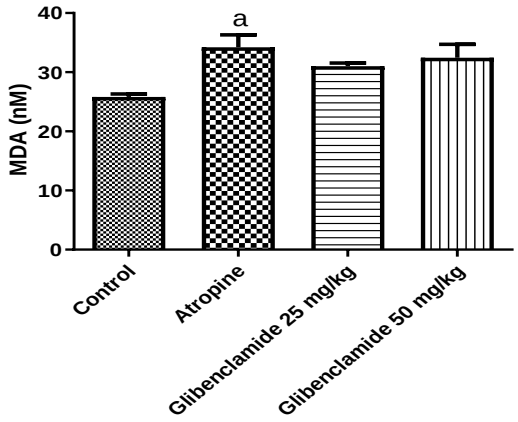
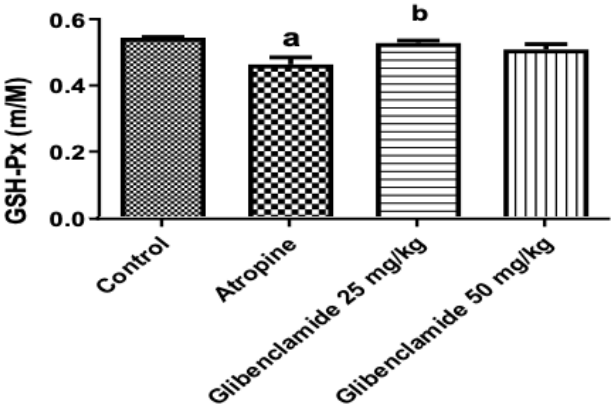
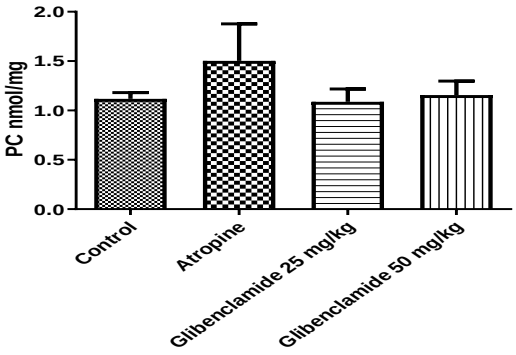


Figure 12. The Level of MDA, SOD and CAT (Glibenclamide + Atropine) in Brain of Mice

[a] $p<0.05$ when compared with control group

The glutathione peroxidase (GSH-Px) levels in atropine group were found to be statistically significantly lower compared to the control group ($p <0.05$) Furthermore, it was statistically significantly increased in glibenclamide + atropine group (25 mg/kg) compared with atropine group ($p> 0.05$). However, PC and NO levels were found not to be statistically significant when the control, atropine, and glibenclamide groups were compared ($p> 0.05$). As elucidatde in Figure 13.





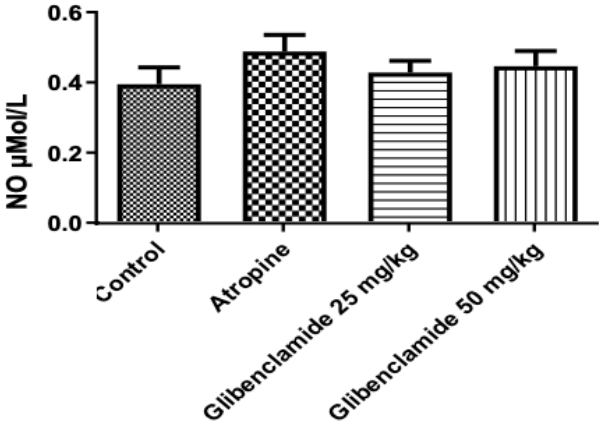


Figure 13. The Level of GSH-PX, PC and NO of (Glibenclamide + Atropine) in Brain of Mice

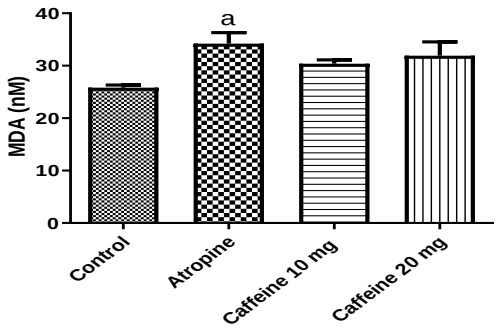
[a] p<0.05 when compared with control group

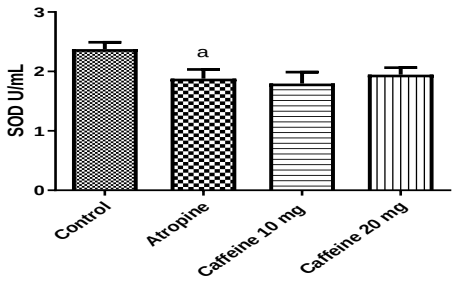
[b] p<0.05 when compared with atropine group

Caffeine

The MDA levels in atropine group were found to be statistically significantly increased compared with the control group (p<0.05). When compared with caffeine groups, there was no difference between the groups (p>0.05). As elucidated in Figure 14.

Superoxide dismutase (SOD) activity in the atropine group were found to be statistically significantly increased when compared to the control group (p <0.05). When compared with caffeine groups, there was no difference between the groups (p>0.05). CAT levels were found not to be statistically significant when the control, atropine, and caffeine groups were compared (p> 0.05). As elucidated in Figure 14.





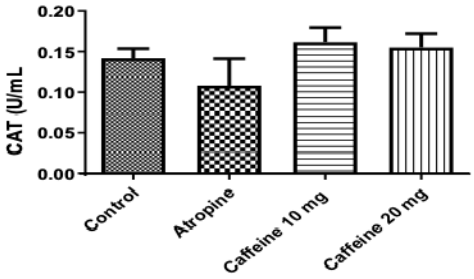


Figure 14. The Level of MDA, SOD and CAT (Caffeine + Atropine) in Brain of Mice.
[a] p<0.05 when compared with control group

The glutathione peroxidase (GSH-Px) levels in the atropine group were found to be statistically significantly decreased compared to the control group (p <0.05). Besides, it was determined to be statistically significantly increased in caffeine + atropine group (10 mg/kg) when compared with the atropine group (p> 0.05). While PC and NO levels were found not to be statistically significant when the control, atropine, and, caffeine groups were compared (p> 0.05). As elucidated in Figure 15.

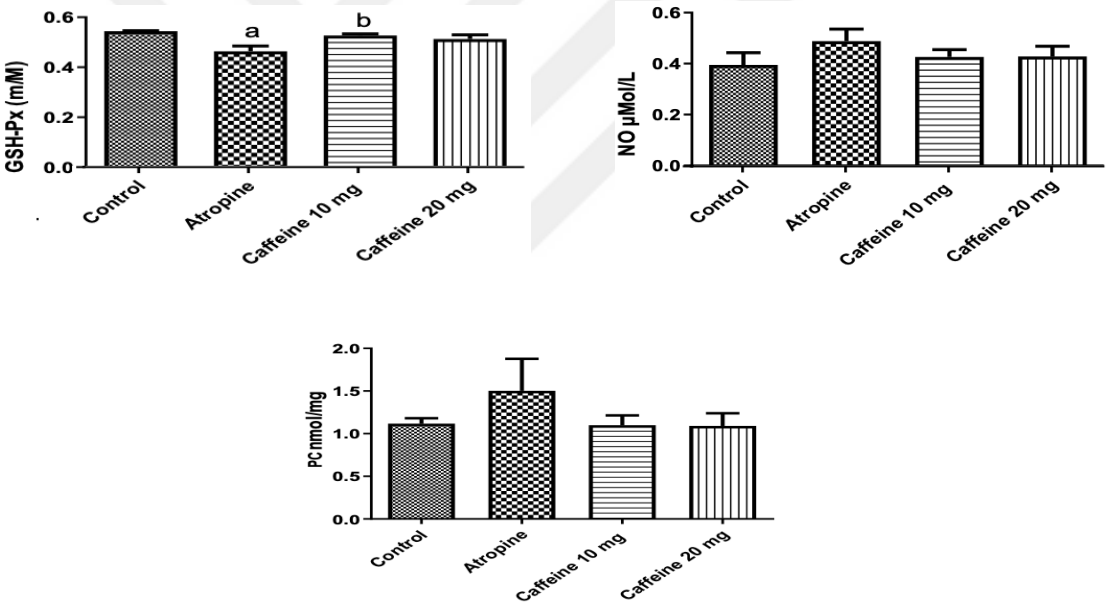


Figure 15. The Level of GSH-PX, PC and NO of (Caffeine + Atropine) in Brain of Mice

[a] p<0.05 when compared with control group

[b]p<0.05 when compared with atropine group

Minoxidil

The MDA levels in atropine group were found to be statistically significantly increased compared with the control group (p<0.05). When compared with minoxidil

groups, there was no difference between the groups (p>0.05). As elucidated in Figure 16.

Superoxide dismutase (SOD) activity in the atropine group were found to be statistically significantly decreased when compared to the control group (p <0.05). When compared with minoxidil groups, there was no difference between the groups (p>0.05). (p> 0.05). In addition, SOD levels in minoxidil 50 mg/kg + atropine group were found to be statistically significantly increased compared to the atropine group (p> 0.05). The CAT levels were found not to be statistically significant when the control, atropine, and, minoxidil groups were compared (p> 0.05). As elucidated in Figure 16.

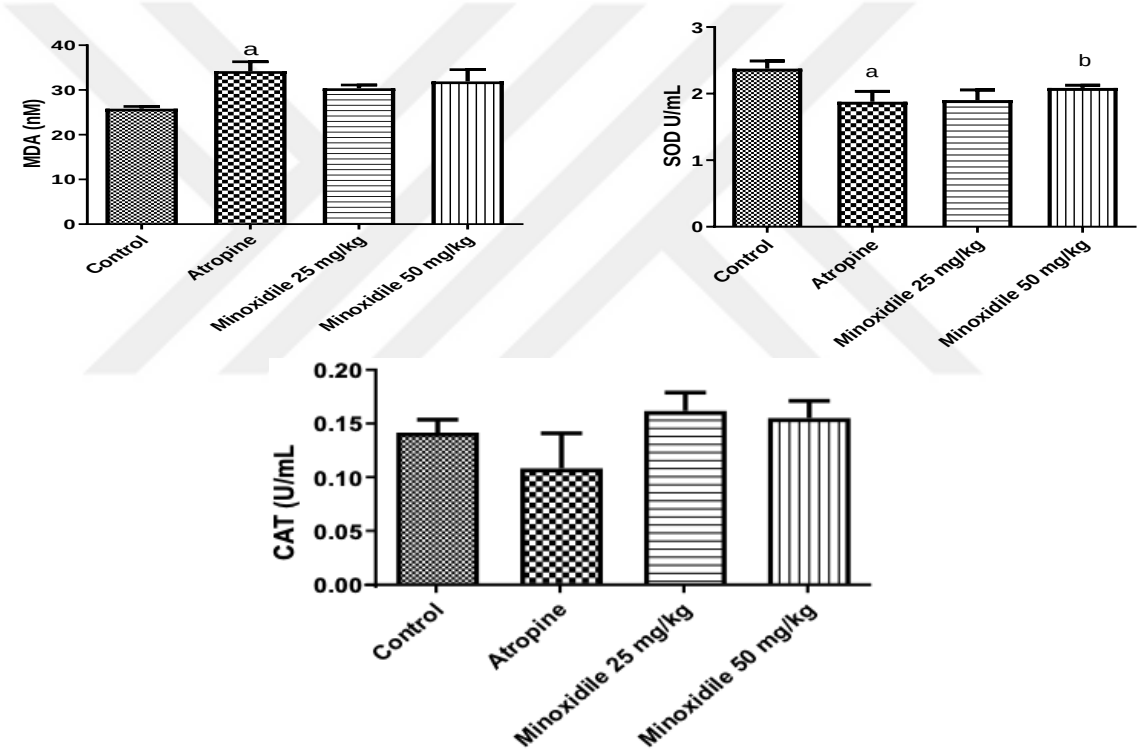


Figure 16. The Level of MDA, SOD and CAT (Minoxidil + Atropine) in Brain of Mice

[a] p<0.05 when compared with control group

[b]p<0.05 when compared with atropine group

In the atropine group, the glutathione peroxidase (GSH-Px) levels were found to be statistically significantly decreased compared to the control group (p <0.05). When

compared with minoxidil groups, there was no difference between the groups (p>0.05). While PC and NO levels were found not to be statistically significant when the control, atropine, and, minoxidil groups were compared (p> 0.05). As elucidate in Figure 17.

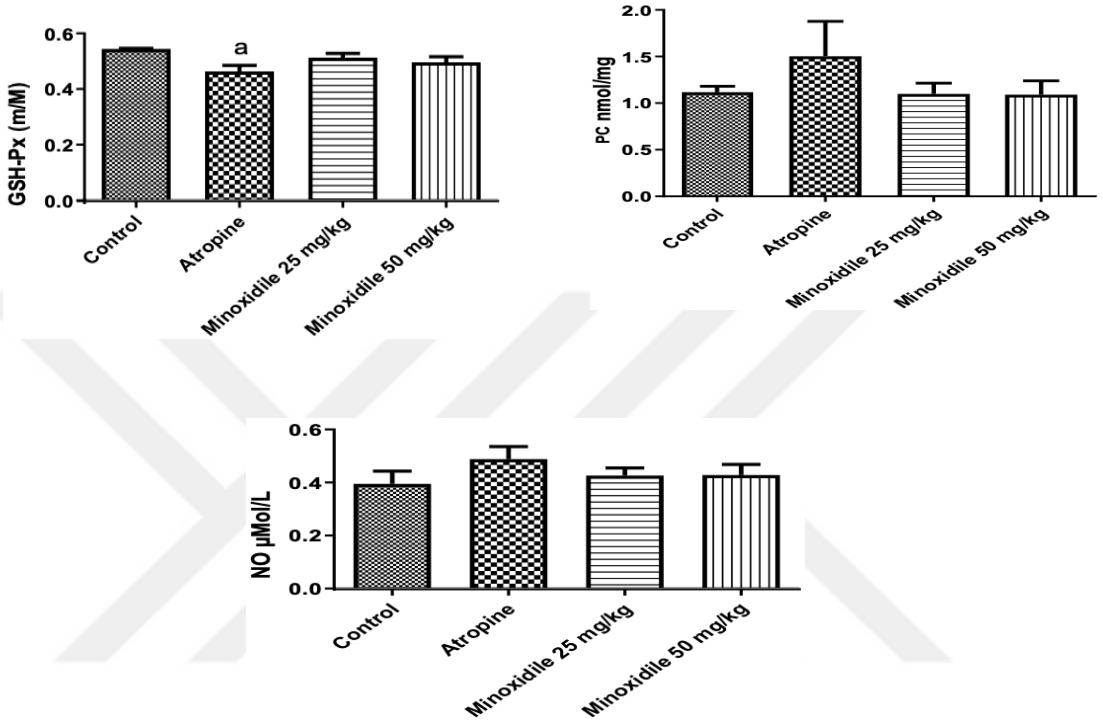


Figure 17. The Level of GSH-PX, PC and NO (Minoxidil + Atropine) in Brain of Mice

[a] p<0.05 when compared with control group

Usnic acid

The MDA levels in atropine group were found to be statistically significantly increased compared with the control group (p<0.05). When compared with usnic acid groups, there was no difference between the groups (p>0.05). As elucidated Figure 18.

Superoxide dismutase (SOD) activity in the atropine group were found to be statistically significantly decreased when compared to the control group (p <0.05). When compared with usnic acid groups, there was no difference between the groups (p>0.05). The CAT levels were found not to be statistically significant when the control, atropine, and, caffeine and usnic acid groups were compared (p> 0.05). As elucidated in Figure 18.

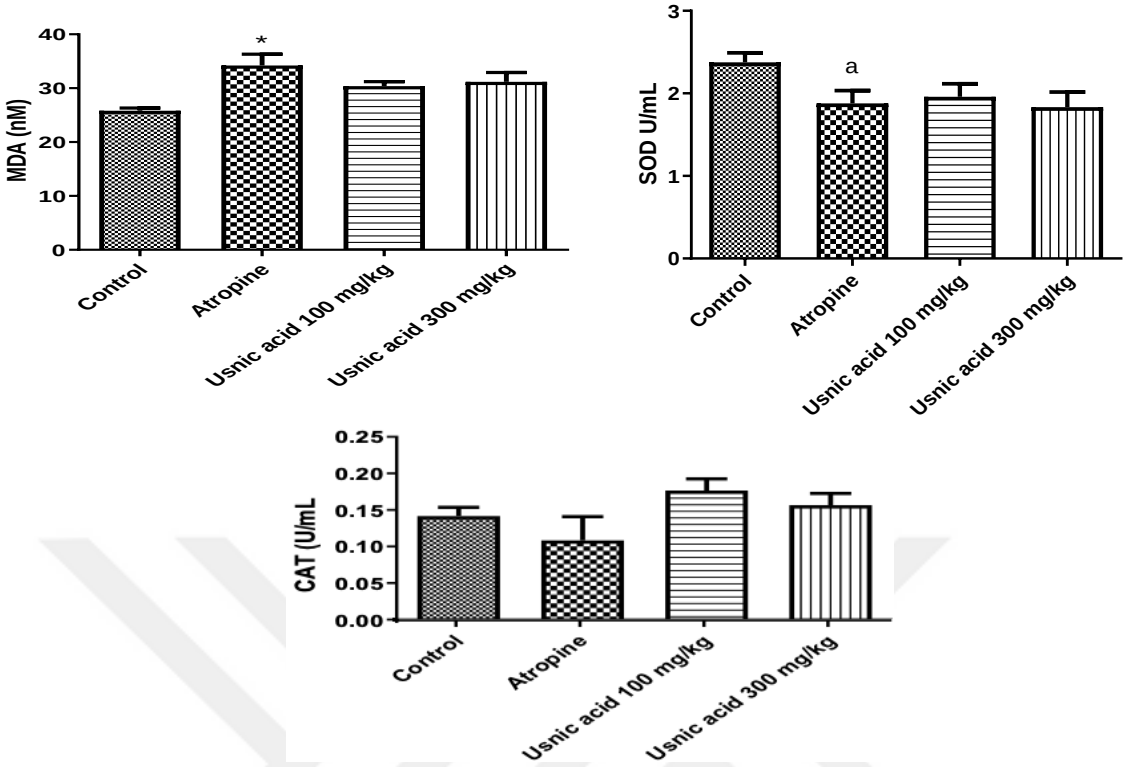
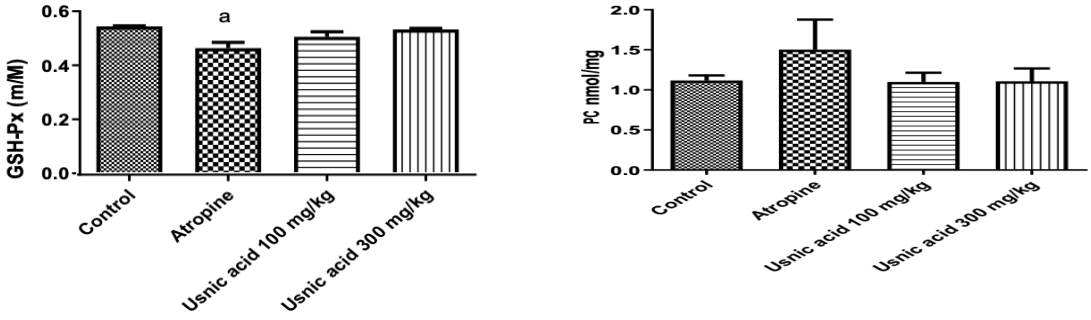


Figure 18. The Level of MDA, SOD and CAT (Usnic Acid + Atropine) in Brain of Mice

[a]p<0.05when compared with control group

In studying glutathione peroxidase (GSH-Px) in the atropine group showed a lower significant compared to the control group (p <0.05). When was comparing with usnic acid groups, there was no difference between the groups (p>0.05). While PC and NO levels were insignificant when the control, atropine, and, usnic acid groups were compared (p> 0.05). While PC and NO levels were found not to be statistically significant when the control, atropine, and, usnic acid groups were compared (p> 0.05). As elucidated in Figure 19.



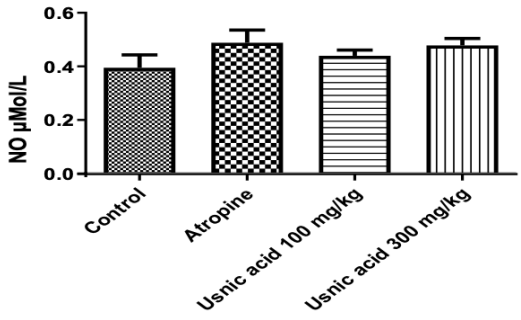


Figure 19. The Level of GSH-PX, PC and NO (Usnic Acid + Atropine) in Brain of Mice

[a] $p<0.05$ when compared with control group

5. DISCUSSION

This study was aimed to evaluate the effects of atropine and different doses of caffeine, minoxidil, usnic acid, and glibenclamide on the development of convulsions triggered by food intake in fasted animals treated with antimuscarinic agents. Accordingly, the non- selective M1 and M2 muscarinic receptor antagonist atropine (Richards 1990) was used to show its efficacy on the development of convulsions in mice after 24 hours of fasting. The scheduled doses used in this study were selected depending upon the affinity of drugs compared with those of atropine (Nurten and Enginar 2006), and glibenclamide (Lahmann et al.,2015), minoxidil (Chen et al., 2017), caffeine (Tchekalarova et al.,2013), usnic acid (Asma'a and Sarah.,2014). Moreover, the locomotors activity test, as well as Rota rod test, provide additional information about animal locomotor behavior and muscular relaxation. Biochemical assay has supported the current research which is considered as an asset to evaluate the brain defense system in the presence of seizure inducer of atropine.

Recent studies have shown that rat and mice treated with antimuscarinics atropine, scopolamine, biperiden (Enginar and Nurten 2010) and the selective M_1 receptor antagonist pirenzepine (Bacank et al., 2017) after fasting up to two days (Enginar et al. 2009) develop convulsions soon after being allowed to eat ad libitum. Food deprivation itself, but not its hypoglycemic consequence, seems to be critical in the development of seizures (Enginar et al., 2005). Fasting the rats for a period of for 48 hours produces changes in binding of [3H] glutamate in the brain, implying that neuroadaptive changes occur during fasting (Enginar et al., 2003). All the complicated outcomes occurring while eating solid food only, not (slurry or fluid feeding) including the (chewing and swallowing movements, smelling and tasting) and stimulation of the amygdala by repetitive oral and masticator movements are considered as a triggering factor for development of convulsions (Nurten et al. 2009). On the other hand, the similarities in triggering factors and manifestations of the seizures in patients with eating-evoked epilepsy, convulsions in fasted animals may provide insight into the mechanism of this rare and partially controlled form of reflex epilepsy (Senanayake 1994; Seneviratne et al., 2003 Guimaraes et al. 2005; Striano et al. 2012).

Atropine (Richards, 1990) and scopolamine (Witkin et al., 2014) are nonselective M1 and M2 muscarinic receptor antagonists, while pirenzepine (Bacank et al., 2017) and biperiden (Burke, 1986; Syvalahti et al.,1987; Witkin et al., 2014) present high M1 selectivity. Nurten and Enginar (2006) have suggested that the decrease in cholinergic transmission due to blockade of postsynaptic muscarinic (M1 possibly M2 subtypes) receptors is the reason behind the convulsive activity in fasted animals.

Using antimuscarinics, (scopolamine, atropine, biperiden pirenzepine), in fasting animals for different periods of time (24, 48 hours) caused development of seizures in a short time after allowing the animal to eat ad-lib (Enginar et al, 1997, 1999, 2003, 2005, 2009; Nurten andEnginar, 2006,2010,2015,2017; Bacanak et al., 2017; Gözüaçık et al., 2019; Büget et al., 2015, 2016). In the present study, mice treated with 2.4 mg/kg of atropine developed convulsion after food intake. The incidence of convulsion was significantly higher in the atropine group compared with the control and drug-treated groups. Pentylenetetrazole, bicuculline, picrotoxin, penicillin, and β-carboline (Da et al., 1998; Kumar et al., 2016) and kainic acid (Zhu et al., 2011) were used in different experimental model studies to prevent GABA neurotransmitters and induce convulsion. In study on animal models by Haider et al., (2017), it has been demonstrated that antimuscarinic administration in animals increased the oxidative stress as a result of increased of MDA level compared with control group. The same result was noticed in the current study as the MDA level was significantly higher compared with the control group. Other studies have reported a significant increase in MDA level, oxidized glutathione (Yadav et al., 2012) and a significant decrease in antioxidant enzymes (Yadav et al., 2012; Budzynska et al., 2014) following the administration of animals with atropine. However, the current study found similar results of MDA level, oxidized glutathione, and antioxidant enzymes compared with control groups. Compared with the other groups, there was no difference found, while PC, NO, CAT and acetylcholinesterase levels were insignificant when the control, atropine and drug-treated groups were compared.

Minoxidil is considered as a strong arteriolar vasodilator (potent vasodilator) as it acts as a potassium channel opener which is present on the cells of the peripheral smooth muscles (Gilmore et al., 1970; Charme et al., 1973; Editorial, 1973). Chidsey

and Gottlieb, (1974) have indicated that both minoxidil and diazoxide inhibit calcium uptake through the cell membrane. Schwab et al., (1993) has shown that minoxidil sulfate is a potassium channel opener in kidney cells. In the current study, minoxidil - a potassium channel opener - was used at dose of 25, 50 mg/kg to investigate its effect as an anticonvulsant in fasting animals for 24 hours, and minoxidil was administered at dose of 25,50 mg/kg + atropine 2.4 mg/kg in mice. The obtained results show the effects of minoxidil on convulsion scores, as shown in table 2, however, minoxidil did not prevent the development of convulsions induced by atropine compared with the atropine group. Chidsey and Gottlieb, (1974) have reported that convulsion onset time was significantly higher as a result of using minoxidil. The current study reached to similar results in minoxidil + atropine group. Several studies have shown that SOD level was significantly higher with a significantly decreasing effect in seizures as a result of using diazoxide, considered as a potassium channel opener in animal models (Arie et al., 2008; Fogle et al., 2016). Thereby, similar results were found in the minoxidil group, considered as a potent potassium channel opener in the current study. Another study on diazoxide has reported a decreasing effect in the seizures induced by dichlorvos (Jazayeri et al., 2013). Shafaroodi et al., (2016) have noticed a link between ATP-sensitive K^+ (KATP) channels and triamterene anti-seizure effect, indicating an increased clonic seizure threshold and latency of seizure which was induced by PTZ. Yıldız et al., (2016) have demonstrated the pinacidil - a KATP channel activator - reduces spike-wave frequency with the epilepsy model induced by penicillin ($p<0.05$).

Glibenclamide acts by blocking ATP sensitive potassium channels and has a possibility of modulating the potassium channel, which might have potential activity against convulsion (Body,1988). The current study demonstrates the effects of glibenclamide on convulsion scores, as shown in table 2, the effect of glibenclamide at dose (25, 50 mg/kg ip) in atropine induced convulsion, pretreatments with glibenclamide did not prevent the development of convulsions. When compared with the atropine treated mice, the latency to convulsions was prolonged, but the drug groups were not statistically significant ($p> 0.05$). In a study by Pithadia et al., (2013), it has been reported that anti-diabetic drug glibenclamide has an anticonvulsant effect in the different models including maximal electroshock seizures (MES) and pentylenetetrazole induced convulsant. It was also indicated in the study that the anticonvulsant effect of

glibenclamide is mediated by the channel of the GABAA/benzodiazepine receptor complex acting by increasing GABA concentration in the brain contrary to the effect of PTZ acting as an antagonist of GABA receptor. Several studies have reported that KATP channels play an important role in the control of seizure threshold in different in vitro or vivo models (Mattia et al., 1994; Katsumori et al., 1996; Yamada and Inagaki, 2005; Shafaroodi et al., 2007). In an experimental study on animal models, it was shown that glibenclamide at dose of 5 mg/kg prevented convulsion induced pentylenetetrazole in mice (Pithadia et al., 2013). This conclusion is similar to the results obtained in the current study where glibenclamide was used at dose of 25, 50 mg/kg which delayed the onset of convulsion. In an experimental study on the liver functions of diabetic rats, it has been reported that GSH-Px level was significantly elevated and this result is in agreement with the results obtained from the glibenclamide 25 mg/kg + atropin group compared with atropine group in the current study ($p > 0.05$).

Caffeine is a mixed competitive adenosine A1 and A2A receptor antagonist. Caffeine inhibits (GABA) (Solinas et al., 2002; Borycz et al., 2007), by modulating GABA-A receptors (Roca et al., 1988; Hossain et al., 2003) while the stimulation of Adenosine A1-receptor inhibits the dopamine and release glutamate. In as study by Londos (1980), the importance of stimulation of the central A1 receptor has shown to generate anticonvulsant effects. In the current study, caffeine was used at a series of doses (10, 20 mg/kg, i.p) to investigate its effectiveness as an anticonvulsant in the fasting mice for 24 hours; the results are shown in Table 2. Effect of caffeine (10, 20 mg/kg) in atropine induced convulsion, and pretreatments with caffeine were not able to prevent the development of convulsions. In this study, administration of caffeine at a dose of 10, 20 mg/kg of caffeine to prevent convulsion was confirmed and prove that some doses of caffeine had no effect against seizures. Various studies have reported that using caffeine at different doses of 60 or 80 mg/kg (Bankstahl et al., 2012), 46.2 and 92.4 mg/kg (Jargiello-Baszak et al., 2016), 20 mg/kg, p.o. (Himmel, 2008), 100 mg/kg (Esmaili and Heydari, 2019) did not significantly change the seizure threshold in different experimental methods which used PTZ and maximal electroshock to induce seizures. Thereby, in the present study similar results were found in the caffeine group at a dose of 10, 20 mg/kg did not prevent convulsion induced by atropine. Luszczki et al., (2006) have reported that caffeine at a dose of 92.4 mg/kg significantly decreased

the threshold for clonic seizures induced by PTZ in mice and this is not in agrrement with the results obtained from current study. In a study by Souza et al., (2013), it has been reported that caffeine supplementation increased glutathione (GSH) content and protected against the increase in the levels of thiobarbituric acid reactive substances (TBARS) and this is similar to obtained results in the current study that the level of glutathione peroxidase (GSH-Px) in caffeine 10 mg /kg + atropine group was significantly increased when compared with the atropine group.

Several studies on lichen and usnic acid (UA) have reported that it's pharmacological effect include antioxidant, healing, analgesic, anticonvulsant, antiemetic, anti-asthmatic, antimicrobial, anti-inflammatory, antiviral, and antibiotic properties as wells some adverse effects including liver damage and allergy (Cocchietto et al., 2002; Ingolfsdottir, 2002; Shukla et al., 2010; Raizada et al., 2014; Yousuf and Choudhary, 2014; Luzina and Salakhutdinov, 2016). Usnic acid was used in the current study at a various dose (100, 300 mg/kg, i.p) to investigate its effectiveness as an anticonvulsant in fasting mice for 24 hours; the results are shown in Table 2. When usnic acid groups were compared with the atropine group, no change was found in seizure onset time and no statistically significant difference was found in the drug-treated groups ($p > 0.05$). Recent studies have indicated that the antioxidant potential index (TRAP) was significantly high with a decrease in the level of nitric oxide generation and hydroxyl radicals, and an increase in the formation of lipoperoxidation in experimental animal model after using usnic acid, as well (Han et al., 2004; Rabelo et al., 2012). However, another study has reported an increase in the level of reactive oxygen species (ROS) that contribute to mitochondrial death after pretreatment with usnic acid in an animal experimental method (Zuo et al., 2015). Han et al., (2004) have observed a decrease in the GSH levels with an increased free radical generation, source of reactive oxygen species after treatment with usnic acid.

As a result, following administration of atropine, the convulsions occurred in mice that were fasted for a period of 24 hours. The effects of evaluated pharmacological agents on the seizure scores were not found to be statistically significant. The pharmacological agents were observed to positively alter some of the biochemical partameters. Moreover, administration of minoxidil with a high dose increased the

duration of seizure onset. In the following studies, the underlying mechanisms can be clarified by using different protocols.

SUMMARY

Alhajjar T. S., Application of atropine in fasted animals and convulsions caused by feeding investigation of the effect of glibenclamide, minoxidil, caffeine, and usnic acid. Van Yüzüncü Yıl University, The Health Sciences Institute, Department of Pharmaceutical Professional Sciences, MSci, Thesis, Van, 2019. Epilepsy is considered a prevalent neurological disorder, characterized by disorder in electrical activity which is located in the brain area and well-known as epileptic seizures. Thisstudy was aimed to establish the pharmacological effect of the glibenclamide, minoxidil, caffeine, and usnic acid, on the atropine inducing convulsions in mice fasted for 24 hours and then allowed to access the food. Materials and Methods: After 24 hours of fasting, animals were divided as control group' drug-treated groups and only atropine treated group. The control group was treated with normal saline solution, the drug-treated groups were administered with glibenclamide at a dose of 25 or 50 mg/kg, minoxidil at dose of 25 or 50 mg/kg, caffeine at a dose of 10 or 20 mg/kg, usnic acid at a dose of 100 or 300 mg/kg, Each group separately and respectively and 10 minutes later (drug-treated groups) were treated with atropine at a dose of 2.4 mg/kg; and lastly, the atropine treated group was injected only with atropine at a dose of 2.4 mg/kg. The animals was allowed to ad libitum feeding and were observed for 30 minutes to evaluated the onset of convulsions the duration and frequency of convulsions. Within the scope of biochemical assays, the obtained tissue samples were examined in terms of oxidative stress and antioxidant activity of enzymes. Results: The convulsions occurred in mice that were fasted for 24 hours following the administration of atropine. The effects of evaluated pharmacological agents on the seizure scores were not found to be statistically significant. The pharmacological agents were observed to positively change some of the biochemical parameters. Moreover, high dose minoxidil was observed to cause an increase in the duration of seizure onset. Conclusion: It can be concluded from this study that the use of the above drugs in the prevention of convulsion resulting from the use of atropine in fasted mice was not effective except for high dose minoxidil which exhibited an effect delaying at the onset time of the seizures.

Key words: Epilepsy, Mice, Atropine, Seizure, Oxidative stress.

ÖZET

Alhajjar T. S., Aç Hayvanlarda Atropin Uygulanması Ve Yem Verilmesi Ile Oluşan Konvülsiyonlara, Glibenklamid, Minoksidil, Kafein Ve Üsnik Asidin Etkisinin Araşrılması.Van Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, Eczacılık Meslek Bilimleri Anabilim Dalı Farmakoloji Bilim Dalı, Yüksek Lisans Tezi, Van, 2019. Epilepsi, beyin bölgesinde yer alan ve epileptik nöbetler olarak bilinen elektriksel aktivitedeki bozukluklarla karakterize edilen yaygin bir nörolojik bozukluk olarak kabul edilmektedir. Bu çalışmada, glibenklamid, minoksidil, kafein ve üsnik asitin 24 saat boyunca aç bırakılan ve daha sonra atropin uygulanarak yemek yemelerine izin verilen farelerde oluşan konvülsiyonlar üzerindeki etkinliğinin incelenmesi amaçlanmıştır. Gereç ve Yöntem: 24 saat açlıktan sonra hayvanlar kontrol grubu, ilaç uygulanan gruplar ve sadece atropine uygulanan gruplar olarak olarak gruplandırılmıştır. Kontrol grubu normal salinle, ilaç uygulanan gruplar sırasıyla, 25 veya 50 mg/kg dozda glibenklamid, 25 veya 50 mg/kg dozda minoksidil, 10 veya 20 mg/kg dozda kafein, 100 veya 300 mg/kg dozda üsnik asitle ve ardından da 10 dk sonra 2,4 mg/kg dozda atropinle muamele edilmiştir. Son olarak, yalnızca atropine uygulanan gruba 2,4 mg/kg dozda atropine enjekte edilmiştir. Farelerin serbest bir şekilde yiyeceğe ulaşması sağlanmış ve nöbet başlagıcından itibaren 30 dk boyunca konvülsiyonların süresi ve sıklığı değerlendirilmiştir. Biyokimyasal değerlendirmeler kapsamında, alınan doku örnekleri oksidatif stres ve enzimlerin antiosidan aktiviteleri yönünden incelenmiştir. Bulgular: 24 saat ardında atropin uygulanan farelerde konvülsiyonlar ortaya çıkmıştır. Değerlendirilen farmakolojik ajanlarının nöbet skorları üzerindeki etkileri istatistiksel olarak anlamlı bulunmamıştır. Farmakolojik ajanların bazı biyokimyasal parametreleri olumlu yönde değiştirdiği gözlemlenmiştir. Ayrıca, yüksek dozda uygulanan minoksidilin nöbet başlangıcı zamanında artışa yol açtığı gözlemlenmiştir. Sonuç: Bu çalışmadan elde edilen bulgulara dayanarak, kullanılan ilaçların 24 saatlik açlıktan sonra atropin uygulanan farelerdeki konvülsiyonların engellenmesinde etkili olmadığı ancak yüksek dozdaki minoksidilin nöbetlerin başlangıcında bir gecikmeye neden olduğu saptanmıştır.

Anahtar Kelimeler: Epilepsi, Fare, Atropin, Nöbet, Oksidatif stress.

GİRİŞ

Epileptik nöbet bir grup kortikal ya da subkortikal nöronun artmış uyarıla bilirliğinden veya senkronize anormal aktivitesinden kaynaklanan bir klinik durumdur. Günümüzde epilepsinin rasyonel bir tedavisi yoktur, uygulanan tedaviler palyatif karakterlidir. Epilepsi tedavisinde temel strateji bazı antiepileptik ilaçlar ile merkezi sinir sisteminde epilepsiye sebep olan normal olmayan elektriksel deşarjların meydana çıkmasının veya yayılmasının önlenmesidir. Epilepsi hastalarının sürekli ilaç kullanmak mecburiyetinde olmaları, ülke ekonomisine önemli bir mali yük oluşturmaktadır. Ayrıca, tedavi süresince hasta uyuncu zamanla azalabilmekte ve özellikle yaşlı ve çocuklarda belirgin olmak üzere antiepileptik ilaçlar uygun şekilde kullanılamamaktadır (Enginar ve Nurten 2010; Soares ve ark., 2017). Epilepsi sendromu ise belli nöbet tipleriyle birlikte ona eşlik eden klinik ve laboratuvar bulgularının tümünü tanımlar. Etyoloji, odağın anatomik yerleşimi, nöbeti tetikleyici etmenler, nöbetlerin başlangıç yaşı, prognoz, tedaviye yanıt ve EEG bulguları sendromun belirlenmesinde önem taşır. Hastalıktan farklı olarak epileptik sendromların ortak bir etiyolojisi ve prognozu yoktur.

Ülkeler arasında bir takım farklılıklar göstermekle beraber epilepsi insidansı genellikle 20-50/100.000, epilepsi prevalansı ise 4-10/1.000 olarak görülmektedir (Büget ve ark., 2016; Allahverdiyev ve ark., 2018). Epilepsinin patofizyolojisi epilepsi hastalığının patofizyolojisi şimdiye kadar tam olarak aydınlatılamamıştır. Ayrıca tüm epilepsi nöbetlerinin patofizyolojisinin aynı olmadığı düşünülmektedir. Epilepside temel mekanizma olarak eksitatör (Glutamat)/inhibitör (gamma aminobütirik asit) dengenin bozulması üzerinde durulmaktadır. Bu iki nörotransmitter arasındaki dengenin gamma aminobütirik asit (GABA) aleyhine değişmesi epilepsinin temel mekanizmalarından biri olarak kabul edilmektedir. Hem deneysel hayvan modellerinde hem de klinikte cerrahi olarak ortaya çıkarılan epileptojenik lezyonlarda benzer anormallikler saptanmıştır (Soukupova ve ark., 2015; Allahverdiyev ve ark., 2018). Fakat bununla birlikte kolinerjik isisteminde epilepsi patofizyoljisinde önemli katkısı vardır (Soares ve ark., 2017).

Aç Hayvanlara Antimuskarinik Uygulanması ve Yem Verilmesi ile Oluşan Konvulsiyonlar

Son dönemler devam etmekte olan bir seri çalışmada antimuskarinik verilen aç hayvanlarda, yem yedikten dakikalar sonra jeneralize olabilen klonik konvulsiyonlar olduğu tespit edilmiştir. Fareler üzerinde yapılan ilk çalışmalarda 48 saat açlık sonrasında skopolamin uygulanması ve yem yenilmesinin konvulsiyon oluşumuna sebep olduğu görülmüştür (Enginar ve Nurten 2010). Antimuskarinik etkili ilaç olan skopolamin yerine atropin veya biperiden verildiğinde de konvulsiyonların meydana çıkması, konvulsiyon oluşumunun skopolamine has olmayıp antimuskarinik mekanizma ile oluştuğu düşünülmüştür. Bununla birilikte konvulsiyonların benzer koşullarda sıçanlarda da meydana gelmesi türe özgü olmadığını ortaya konmuştur. Açlık süresinin konvulsiyonların meydana çıkmasına etkisinin araştırıldığı çalışmada, 2, 3, 12, 18 ve 24 saat devam eden açlık sonrasında da konvulsiyonların meydana çıktığı gözlemlenmiştir. Açlık boyunca meydana gelen hipogliseminin konvulsiyon oluşumuna etkisini araştırmak maksadı ile yapılan araştırmada, açlık süresince hayvanlara glikozlu su verilerek hipogliseminin önlenmesi ile konvulsiyonların oluşumu engellenemediği gösterilmiştir. Konvulsiyonların meydana çıkmasında antikolinerjik verilmesinin ve yemden yoksun kaldıktan sonra yeniden yeme ulaşıp yemenin temel etkenler olduğu gösterilmiştir. Konvulsiyonların 2 saat gibi kısa zamanlı açlık sonrasında da meydana çıkması, yemden yoksun kalmanın sebep olduğu stresin konvulsiyonların oluşmasında etkili olabileceğini düşündürmüştür.

Antimuskarinik uygulanmasından önce glutamaterjik N-metil-D-aspartat (NMDA) reseptörlerinin nonkompetitif antagonisti MK-801 (Enginar ve ark. 1997), dopaminerjik reseptör antagonistleri klorpromazin ve haloperidol (Enginar ve ark., 2003) ve α2-adrenerjik reseptör agonistleri klonidin ve tizanidin (Enginar ve ark., 1999) verilmesi konvülsiyon oluşumunu tamamen önlemiş veya azaltmıştır. Antiepileptiklerden valproat, gabapentin, diazepam, karbamazepin ve levetirasetam konvülsiyon sıklığını azaltıcı veya konvülsiyon başlama süresini uzatıcı etki göstermiş, diğer antiepileptikler ise belirgin bir etki oluşturamamıştır (Büget ve ark., 2016).

Kafein, adenozin A1 ve A2A'in karışık bir yarışmalı reseptör antagonistidir. GABA-A reseptörlerini module ederek gam-amino bütirik asiti (GABA) inhibe eder.

Ayrıca, adenozin A-1 reseptörünün stimülasyonu dopaminin salımının inhibisyonuna yol açarak glutamat salımına neden olur. Merkezi A1 reseptörün stimülasyonun önemi antikonvülsan etkilere yol açmasıdır. Yapılan bir çalışmada, 60 mg/kg ve 80 mg/kg dozda kafeninin akut uygulamasının PTZ ile indüklenen nöbet eşiğini önemli derecede değiştirmediği bildirilmiştir (Bankstahl ve ark., 2012). Başka bir çalışmada, 46,2 mg/kg ve 92,4 mg/kg dozda kafeninin akut uygulamasının farede maksimal elektroşokta prokonvülsan etki göstermediği bildirilmiştir (Jargiello-Baszak ve ark., 2016). Ayrıca, 20 mg/kg P.O. akut kafein uygulamasının yetişkin sıçanlarda nöbet eşiğini değiştirmediği bildirmiştir (Himmel, 2008). Başka bir araştırmadan elde edilen sonuçlardan farklı olarak, 92,4 mg/kg dozda uygulanan kafeinin farelerde PTZ ile indüklenmiş klonik nöbetlerin eşiğini düşürdüğü bildirilmiştir (Luszczki ve ark., 2006). Ayrıca yapılan başka çalışmada 150 ile 200 mg/kg kafein dozunun farelerde klonik nöbetlere yol açtığını bildirmişlerdir (Marangos ve ark., 1981). Esmaili ve Heydari tarafından, 2019 tarafında yapılan bir çalışmada 100 mg/kg dozdaki kafein dozunun nöbet eşiğini değiştirmediği bildirilmiştir. Benzer sonuç Souza ve ark. tarafından yapılan bir çalışmada, kafein tüketiminin glutatiyon (GSH) seviyesini arttırdığı ve tiyobarbütirik asit reakif bileşiklerinin (TBARS) seviyesindeki artışa karşı koruduğunu bildirmişleridir (Souza ve ark.,2013).

Minoksidil periferdeki düz kas hücrelerindeki potasyum kanalılarını açma suretiyle etki etmesi nedeniyle güçlü bir arteriyel vazodilatör olarak kabul edilmektedir (Gilmore ve ark., 1970; Charme ve ark., 1973; Editorial, 1973). Minoksidil ve diazoksitin hücre membranlarından kalsiyum alımını inhibe ettiği gösterilmiştir (Chidsey and Gottlieb, 1974). Bazı potasyum kanal açıcılarının epileptik nöbetlerin azaldığını ortaya koymuşlar (Chidsey ve Gottlieb, 1974). Farklı bir araştırma da 50 mg/kg minoksidil + atropin grubunda SOD düzeyinin atropin grubuna göre anlamlı derecede yüksek olduğunu gösterilmiştir (Arie ve ark., 2008).

Glibenklamidin ATP'ye hassas potasyum kanallarını bloke etmesi ve potasyum kanallarını module etme olasılığıyla konvülsiyonlara karşı potansiyel aktivite gösterebileceği bildirilmiştir (Body,1988). Pithadia ve arkadaşlarının antidiyabetik bir ilaç olan glibenklamidin fenilentetrazol ile indüklenmiş olan konvülsiyonları inhibe ettği bildirilmiştir. Ayrıca, glibenklamidin antikonvülsan etkisinin GABA

A/benzodiazepine reseptör kompleksi kanalı aracılığıyla meydana geldiği bildirilmiştir (Pithadia ve ark., 2013). Bazı çalışmalar KATP kanallarının farklı in vitro ve in vivo modellerde nöber eşiğinin kontrolünü sağlama göz çarpan bir rol üstlendiğini göstermişler (Mattia ve ark., 1994; Katsumori ve ark., 1996; Yamada ve Inagaki, 2005; Shafaroodi ve ark., 2007). Ayrıca farelerde yapılan başka bir çalışma KATP kanallarının alt ünitelerinden birinin bloke edilmesinin hipoksiyi azalttığı, dolayısıyla genelleşmiş nöbet için azalmış bir eşik gösterdiğini bildirmiştir (Yamada ve ark., 2001). Başka bir araştırma 25,50 mg/kg dozunda glibenklamidin konvülsiyonun başlamasına engel olduğu gösterilmiş, benzer bir çalışmayla (Pithadia ve ark., 2013) 5 mg/kg glibenklamidin konvülsiyonu önlediğini tespit edilmiştir. Yapılan araştırmadan elde edilen glutatiyon peroksidaz (GSH-Px) sonuçlarına göre glibenklamid + atropin (25 mg/kg) grubunun atropin grubuyla karşılaştırıldığında daha yüksek değerler elde edilmiştir. Benzer sonuçlata diyabetik sıçanlarda artmış GSH seviyesi bildirilmiş olan başka bir çalışmada da ulaşılmıştır (Ghosh ve ark., 2008).

Liken ve usnik asitle yapılan çalışmalarda antioksidan aktivite, yara iyileştirici, analjezik, antikonvülsan, antiemetic, anti-astmatik, antienflamatuvar, antiviral ve antibiyotik özellikler gibi farmakolojik etkileri gösterilmiştir (Cocchietto ve ark., 2002; Ingolfsdottir, 2002; Shukla ve ark., 2010; Raizada ve ark., 2014; Yousuf ve Choudhary, 2014; Luzina ve Salakhutdinov, 2016). Usnik asidin antioksidan potansiyel indeksini (TRAP) araştıran çalışmalarda önemli derecede yüksek bir antioksidan kapasite sergilediği gösterilmiştir ve ayrıca hidroksil radikallerine karşı etkili olduğunu, nitrik oksit üretiminde azalma ve lipoperoksidasyon oluşumunu aktive ettiği bildirilmiştir (Rabelo ve ark., 2012). Aynı zamanda, usnik asitin mitokondriyal apoptik ölümüne katkı sağlayarak reaktif oksijen türlerinin (ROS) oluşumunu arttırdığı başka bir çalışmayla bildirilmiştir (Zuo ve ark., 2015).

Bu bilgilerin ışığında, çalışmamızda, farklı epilepsi modellerinde antikonvülsan özelliği gösterilmiş glibenklamid, minoksidil, kafein, ve üsnik asidin ilk kez bu modelde antikonvülsan etkileri araştırıldı.

MATERYAL VE YÖNTEM:

Yirmi dört saat aç bırakılan farelerde atropin uygulanması ve yem verilmesi ile oluşan konvülsiyonlara, glibenklamid, minoksidil, kafein, ve üsnik asidin etkisinin araştırıldı. Hayvanlar sabah 09.00'da tartılarak temiz kafeslere alındı ve su alımı serbest olacak bicimde yemden yoksun bırakıldı. 24 saat sonra aç hayvanlara önce serum fizyolojik veya glibenklamid (25, 50 mg/kg, i.p), minoksidil (25, 50 mg/kg, i.p), kafein (10, 20 mg/kg, i.p), üsnik asit (100, 300 mg/kg, i.p) 10 dakika sonra serum fizyolojik veya 2,4 mg/kg atropin uygulandı (Seth ve ark., 2010; Tchekalarova ve ark., 2010; Pithadia ve ark., 2013; Polat ve ark., 2016; Büget ve ark. 2016). Deneyde kullanılacak hayvanlar randomize olarak her grupta 7 hayvan olmak üzere 10 gruba ayrıldı. Uygulamaların hemen ardından, hayvanlar izleme kafeslerine alındı. Yirmi dakika sonra kafeslere yem konuldu ve hayvanların serbestçe yemeleri sağlandı. Tüm hayvanlar 30 dakika izlendi ve konvulsiyon başlama suresi ve sıklığı değerlendirildi (Seth ve ark., 2010; Tchekalarova ve ark., 2010; Pithadia ve ark., 2013; Polat ve ark., 2016; Büget ve ark. 2016).

Grublar;

Kontrol (Serum fizyolojik, 1 mg/kg)
Atropin (2,4 mg/kg)
Glibenklamid (25, i.p), + Atropin (2,4 mg/kg)
Glibenklamid (50 mg/kg, i.p), + Atropin (2,4 mg/kg)
Minoksidil (25, i.p), + Atropin (2,4 mg/kg)
Minoksidil (50 mg/kg, i.p), + Atropin (2,4 mg/kg)
Kafein (10, i.p), + Atropin (2,4 mg/kg)
Kafein (20 mg/kg, i.p), + Atropin (2,4 mg/kg)
Üsnik asit (100, mg/kg, i.p) + Atropin (2,4 mg/kg)
Üsnik asit (300 mg/kg, i.p) + Atropin (2,4 mg/kg)

Nörolojik Küsurların Değerlendirilmesi

Atropin uygulandıktan 45 dakika sonra nörolojik küsurların olup olmadığı önce Lokomotor aktivite cihazı ile ve arkasından hemen Rota Rot cihazı ile değerlendirildi.

Lokomotor aktivite cihazında 10 dakika, farelerin şahlanma, katettikleri mesafe, gezinme, sterotipik hareketleri ve total lokomotor aktiviteleri ölçüldü. Rota Rot aletinin hızı 10 rpm'e ayarlandı ölçüm yapıldı. Farelerin Rota Rot aletine alışabilmeleri için 1 ön deneme yapıldı. Rota Rot aletinde kaldıkları süre kaydedildi ve aletten hiç düşmeyen fare en yüksek değer olan 300 saniye kaldıkları kabul edildi. Davranışsal belirtiler değerlendirildikten 30 dakika sonra sakrifiye edildi, biyokimyasal parametrelere bakılmak üzere kan ve dokuları alındı.

Oksidatif Stres Parametrelerinin Ölçümü

Davranış testleri bittikten hemen sonra hayvanlar dekapite edildi. Total beyin dokusunda oksidatif stres parametreli bakıldı. Yaş ağırlıkları 50 mg olarak ayarlanan dokular, sıvı azot yardımıyla sertleştirilerek porselen havanda toz haline getirildi. Cam tüpe aktarılan doku üzerine 1/10 (w/v) olacak şekilde Tris/sukroz tamponu eklendi. Cam tüplere aktarılan doku örnekler numaralandırılarak 3.000 rpm' de 10 dakika +4 °C'de santrifüj edildi. Santrifüj sonrası süpernatantın malondialdehit (MDA), nitrik oksit, protein karbonil grubu ve glutatyon (GSH) seviyelerine, katalaz, süperoksid dismutaz (SOD) ve asetilkolinesteraz aktivitelerine ve protein düzeylerine bakıldı.

Malondialdehit (MDA) Ölçümü: MDA düzeyi Ohkawa'nın Tiobarbitürik asit reaksiyon metodu ile tayin edildi. Katalaz Ativitesinin Tayini: Katalaz aktivitesi tayini Aebi tarafından tarif edilen yönteme göre yapıldı. Yöntemin esası, H_2O_2 substratının katalaz ile enzimatik yıkılmasının 240 nm de izlenmesidir (Nader ve ark. 2017).

Süperoksid Dismutaz Enzim Aktivitesinin Ölçümü: Oksidatif yolla enerji üretimi sırasında oluşan endojen ve eksojen kaynaklı toksik süperoksit radikellerinin suya ve moleküler oksijene dismutasyonunu hızlandıran SOD enzim aktivitesinin ölçüm prensibi, Sun ve ark. geliştirdiği ve ksantin varlığında ksantin oksidazın açığa çıkardığı süperoksit radikallerinin nitroblue tetrazolium (NBT) ile 560 nm'de absorblanan rengin ölçülmesine dayanır (Nader ve ark. 2017).

Glutatyon (GSH) Düzeyi Ölçümü: GSH analizi, Beutler ve ark.'nın bildirdiği yönteme göre yapıldı. Bu yöntemde beyin dokusu homojenizatındaki sülfidril (-SH) grubu taşımayan tüm proteinler çöktürülmektedir. Elde edilen berrak sıvıda, -SH

gruplarının DTNB ile oluşturduğu sarı renkli kompleks, 412 nm dalga boyunda kolorimetrik olarak ölçüldü.

Asetilkolinesteraz Aktivitesinin Ölçümü: Asetilkolinesteraz aktivitesinin ölçümü, Ellman ve ark.'nın yöntemine göre yapıldı. Asetilkolinesteraz, asetiltiyokolinin tiyokolin ile asetata parçalanması reaksiyonunu katalizleyen bir enzimdir. AChE aktivitesi, tiyokolin ile DTNB arasındaki reaksiyonun sonucunda oluşan 5-tiyo-2-nitrobenzoik asitin verdiği sarı rengin yoğunluğunun, 412 nm dalga boyunda spektrofotometrede ölçülmesi ile belirlenmektedir.

Nitrik Oksit Ölçümü: Nitrik oksit miktarı Miranda ve ark.'nın "Vanadium-3-klorür-Gries Reaksiyonu" yöntemi ile ölçüldü. Ölçüm, Vanadium klorür'ün, 37°C'lik ortamda nitratı nitrite dönüştürmesi ve Gries Reaksiyonu olarak tanımlanan, nitritin asidik ortamda primer bir aromatik amin olan sülfanilamit ile diazotizasyonu ve N-(1-naphthyl) ethylenediamin (NEDD) ile renkli bir azo türevi oluşturması esasına dayanmaktadır. Protein Karbonil Grubu Ölçümü: Protein karbonil grubu tayini Levine ve arkadaşları tarafından geliştirilen bir metot olup proteinlerin oksidasyonu sonucu oluşan karbonil gurupları ile 2,4- dinitrofenilhidrazinin reaksiyona girmesi ile oluşan hydrozone bileşiklerinin renginin spektrofotometrik olarak ölçüldü (Nader ve ark. 2017).

İstatistiksel Analizler

Analizler SPSS for Windows v16.0 paket programında yapıldı. Tüm parametreler için gruplar arasındaki istatistiksel farklılıkların test edilmesinde ANOVA ve alt grup karşılaştırmalarında Tukey çoklu karşılaştırma testleri kullanıldı. Tanıtıcı istatistik olarak ortalama ± standart sapma değerleri verilmiş ve değişkenler için error bar grafikleri çizildi. Elde edilen sonuçlarda $p<0,05$ ise fark veya ilişki istatiksel olarak anlamlı kabul edildi.

BULGULAR

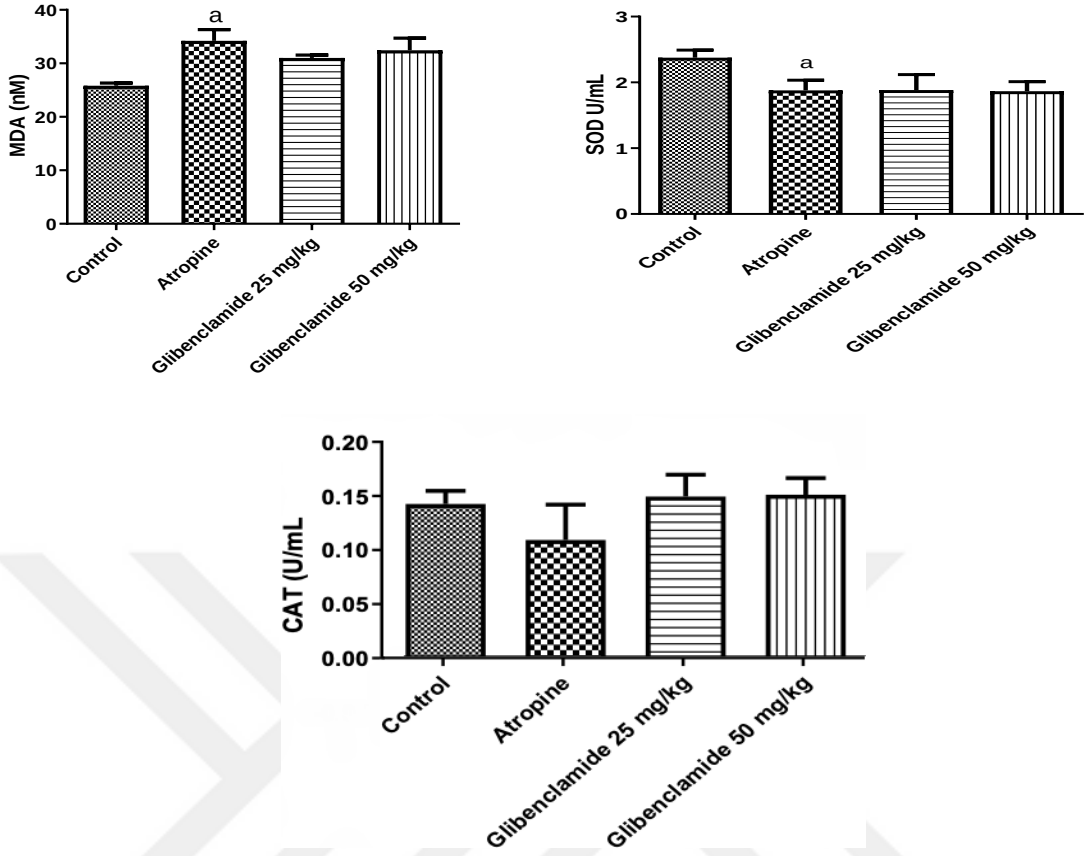
Gruplar	*Skor 0*	*Skor 1*	*Skor 2*	*Skor 3*	*Skor 4*	*Skor 5*
Kontrol	0	0	0	0	0	0
Atropin	0	0	2	3	3	1
Glibenklamid (25, i.p)	0	0	3	4	2	0
Glibenklamid (50, i.p)	0	0	2	3	3	1
Minoksidil (25, i.p)	0	1	1	4	3	0
Minoksidil (50, i.p)	0	2	3	3	1	0
Kafein (10, i.p)	0	0	2	3	3	0
Kafein (20, i.p)	0	0	3	3	2	1
Üsnik asit (100, mg/kg, i.p)	0	0	1	3	3	1
Üsnik asit (300, mg/kg, i.p)	0	0	2	3	3	1

Tablo 1. Farklı dozlarda glibenklamid, kafein, minoksidil ve usnik asit dozlarının atropin bağlı konvülsiyona etkisi.

Atropin grubu kontrol grubu ile karşılaştırıldığında anlamlı olarak atropin grubunda nöbet skorları yüksek bulundu. İlaç gruplarını atropin grubu ile karşılaştırdığımızda istatistiksel olarak sadece yüksek doz minoksidil grubu (50 mg/kg) nöbete başlama süresini uzattığı görüldü (Tablo 1).

Glibenklamid

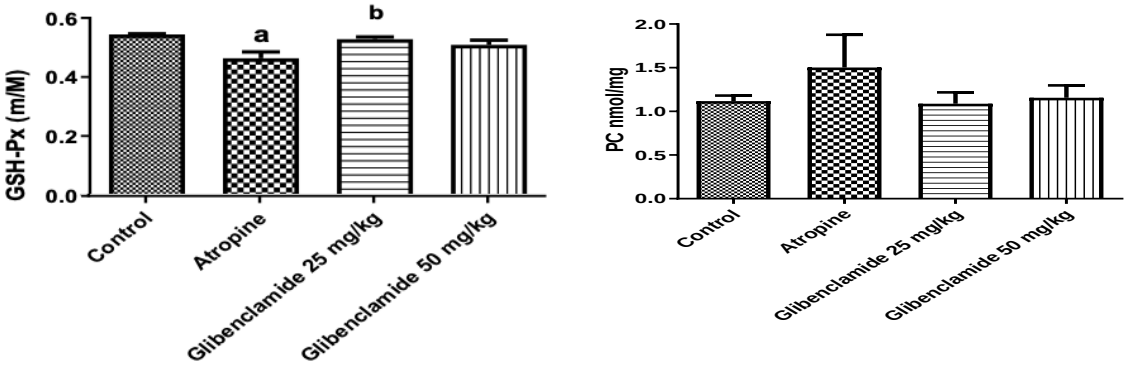
MDA düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda MDA seviyesi anlamlı olarak fazla bulundu ($p<0,05$). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı ($p >0,05$). SOD düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda SOD seviyesi anlamlı olarak fazla bulundu ($p<0,05$). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı ($p >0,05$). CAT düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu ($p >0,05$) (Şekil 1).

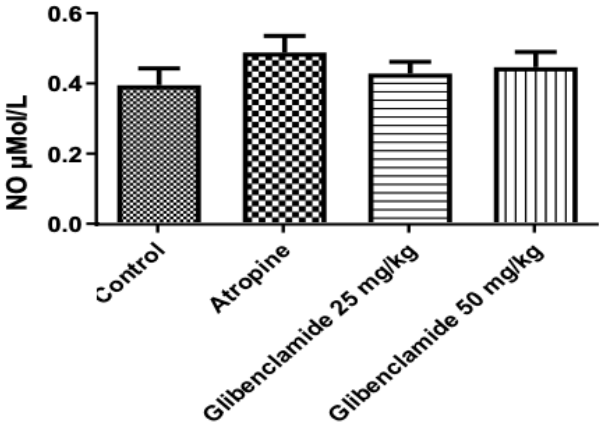


Şekil 1. Fare Beyninde MDA, SOD ve CAT değerleri.

[a] p<0.05 kontrol gurubuna göre

GSH-Px düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda GSH-Px seviyesi anlamlı olarak düşük bulundu (p<0,05). Ayrıca atropine grubu ile karşılaştırıldığında PZT+ glibenclamide 25 mg/kg grubunda anlamlı olarak fazla bulundu (p >0,05). PC ve NO düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu (p >0,05) (Şekil 2).





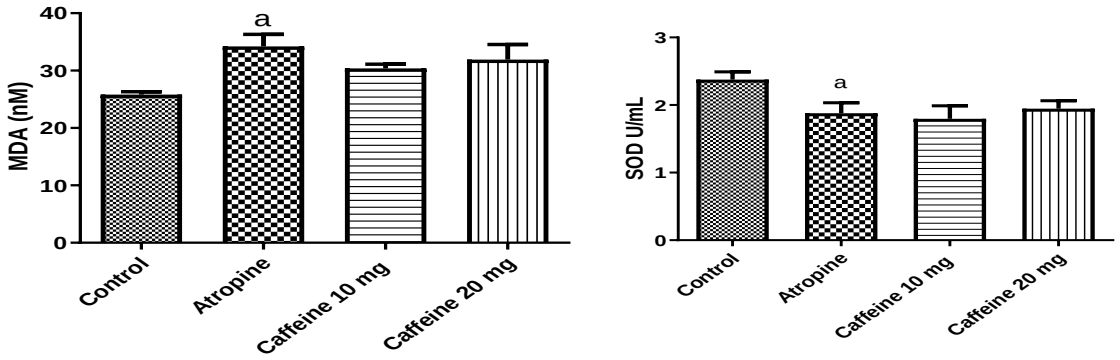
Şekil 2. Fare beyninde GSH-Px, PC ve NO değerleri

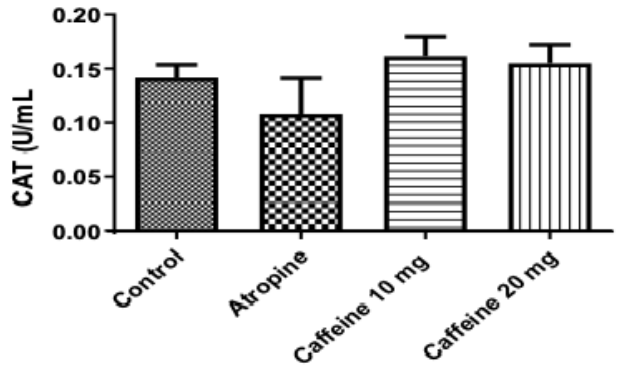
[a] p<0.05 kontrol gurubuna göre

[b] p<0.05 atropin grubuna göre

Kafein

MDA düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda MDA seviyesi anlamlı olarak fazla bulundu (p<0,05). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı (p >0,05). SOD düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda SOD seviyesi anlamlı olarak fazla bulundu (p<0,05). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı (p >0,05). CAT düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu (p >0,05) (Şekil 3).

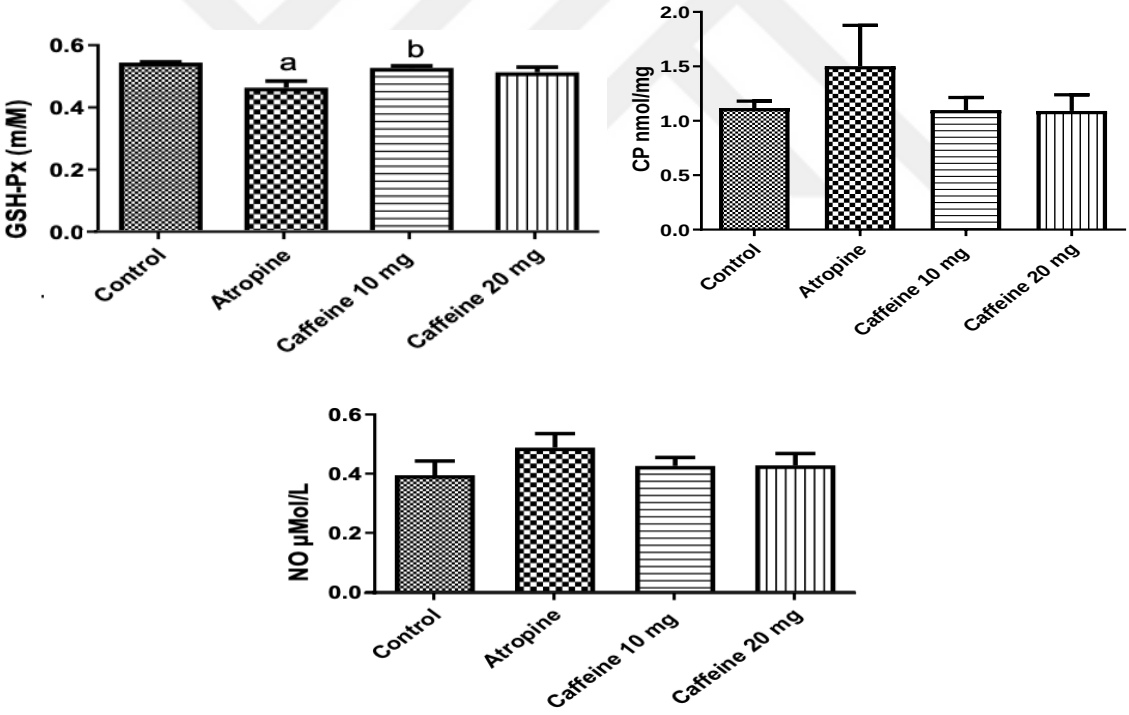




Şekil 3. Fare beyninde MDA, SOD ve CAT değerleri.

[a] p<0.05 kontrol gurubuna göre

GSH-Px düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda GSH-Px seviyesi anlamlı olarak düşük bulundu (p<0,05). Ayrıca atropine grubu ile karşılaştırıldığında atropine+caffeine 10 mg/kg grubunda anlamlı olarak fazla bulundu (p>0,05). PC ve NO düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu (p >0,05) (Şekil 4).



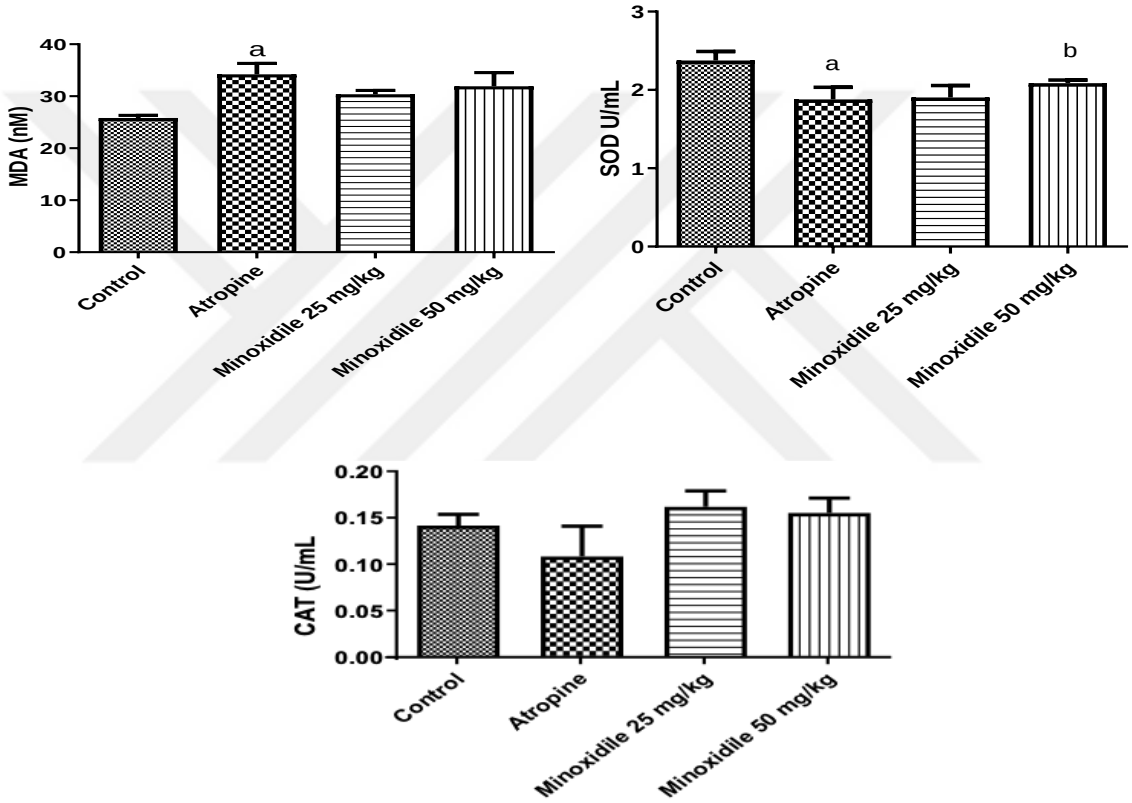
Şekil 4. Fare beyninde GSH-Px, PC ve NO değerleri.

[a] p<0.05 kontrol gurubuna göre

[b] p<0.05 atropin grubuna göre

Minoksidil

MDA düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda MDA seviyesi anlamlı olarak fazla bulundu ($p<0,05$). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı ($p >0,05$). SOD düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda SOD seviyesi anlamlı olarak düşük bulundu ($p<0,05$). Ayrıca SOD düzeyi atropine grubu ile karşılaştırıldığında atropine+minoksidil 50 mg/kg grubunda anlamlı olarak fazla bulundu ($p>0,05$). CAT düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu ($p >0,05$) (Şekil 5).

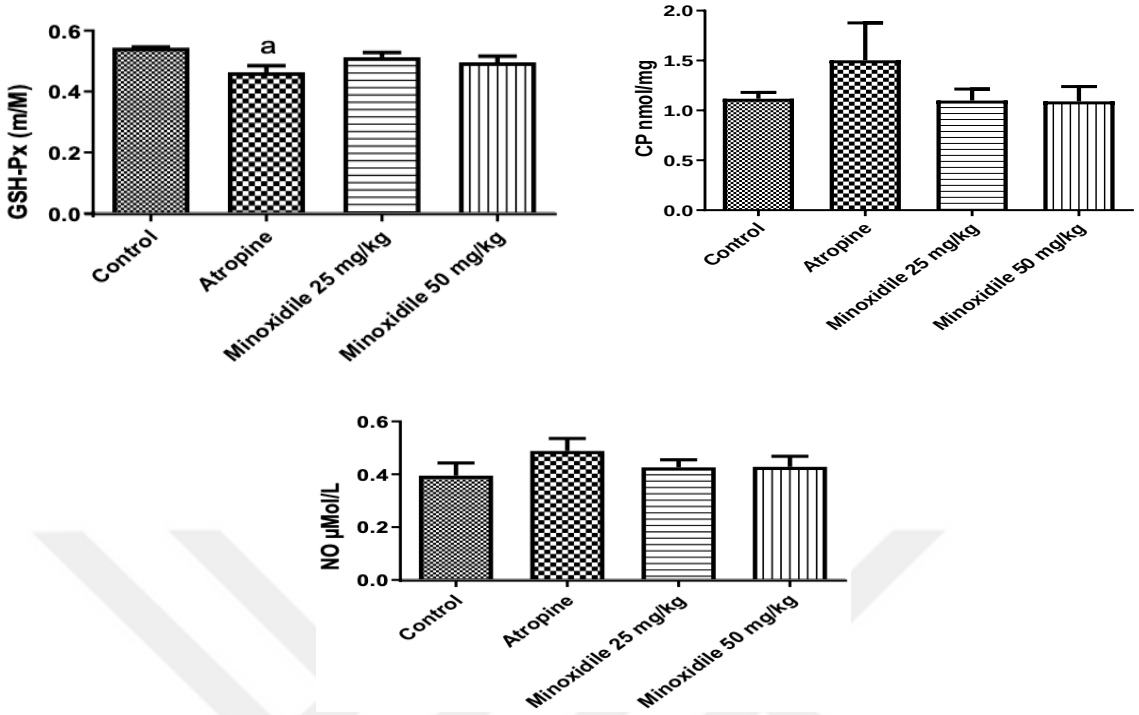


Şekil 5. Fare beyninde MDA, SOD ve CAT değerleri.

[a] $p<0.05$ kontrol gurubuna göre

[b] $p<0.05$ atropin grubuna göre

GSH-Px düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda GSH-Px seviyesi anlamlı olarak düşük bulundu ($p<0,05$). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı ($p >0,05$). PC ve NO düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu ($p >0,05$) (Şekil 6).

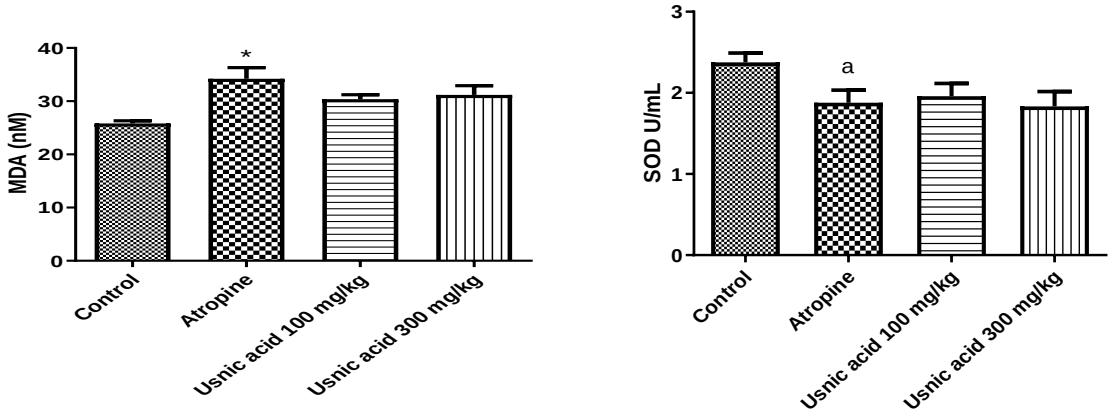


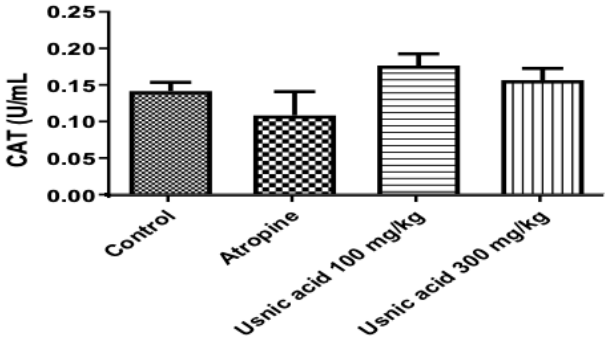
Şekil 6. Fare beyninde GSH-Px, PC ve NO değerleri.

[a] p<0.05 kontrol gurubuna göre

Usnik asid

MDA düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda MDA seviyesi anlamlı olarak fazla bulundu (p<0,05). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı (p >0,05). SOD düzeyi kontrol grubu ile karşılaştırıldığında atropine grubunda SOD seviyesi anlamlı olarak düşük bulundu (p<0,05). CAT düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu (p >0,05) (Şekil 7).

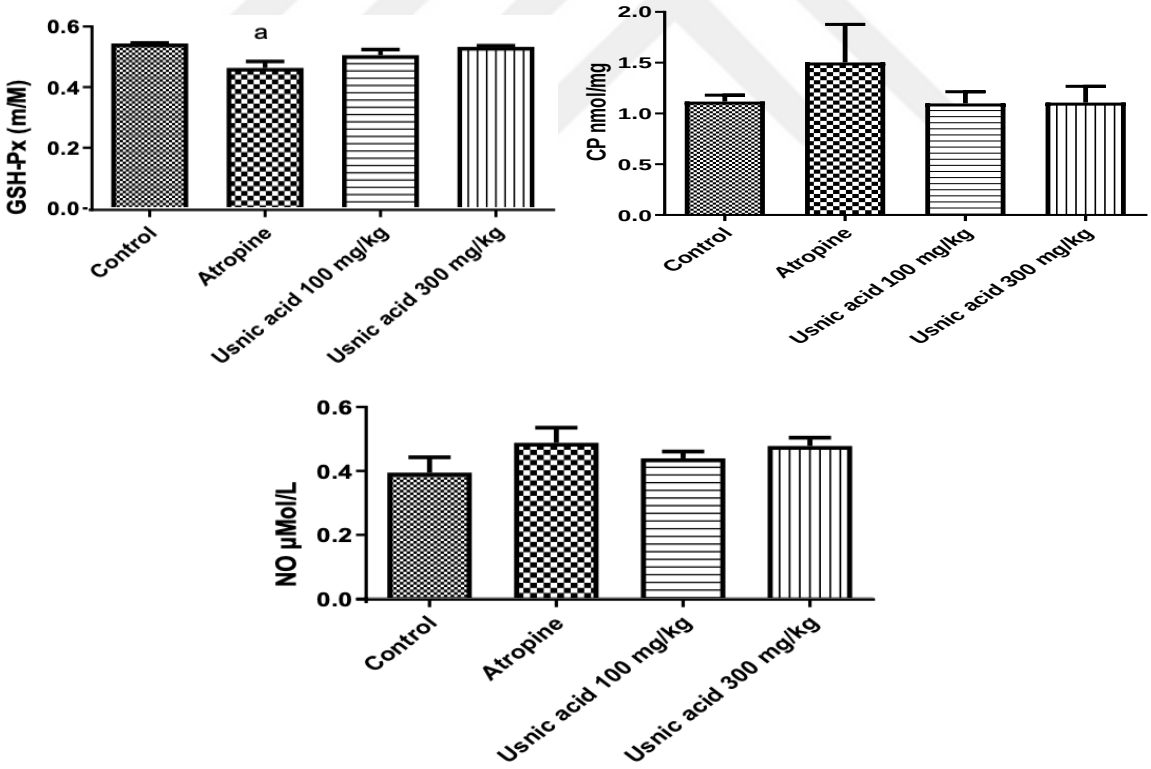




Şekil 7. Fare beyninde MDA, SOD ve CAT değerleri

[a] p<0.05 kontrol gurubuna göre

GSH-Px düzeyleri kontrol grubu ile karşılaştırıldığında atropine grubunda GSH-Px seviyesi anlamlı olarak düşük bulundu (p<0,05). Diğer guruplar karşılaştırıldığında gruplar arasında fark bulunmadı (p >0,05). PC ve NO düzeyleri kontrol, atropine ve ilaç gruplarını karşılaştırıldığında anlamsız bulundu (p >0,05) (Şekil 8).



Şekil 8. Fare beyninde GSH-Px, PC ve NO değerleri

[a] p<0.05 kontrol gurubuna göre

TARTIŞMA VE SONUÇ

Epileptik nöbet bir grup kortikal ya da subkortikal nöronun artmış uyarıla bilirliğinden veya senkronize anormal aktivitesinden kaynaklanan bir klinik durumdur. Günümüzde epilepsinin rasyonel bir tedavisi yoktur, uygulanan tedaviler palyatif karakterlidir. Epilepsi tedavisinde temel strateji bazı antiepileptik ilaçlar ile merkezi sinir sisteminde epilepsiye sebep olan normal olmayan elektriksel deşarjların meydana çıkmasının veya yayılmasının önlenmesidir. Epilepsi hastalarının sürekli ilaç kullanmak mecburiyetinde olmaları, ülke ekonomisine önemli bir mali yük oluşturmaktadır. Ayrıca, tedavi süresince hasta uyuncu zamanla azalabilmekte ve özellikle yaşlı ve çocuklarda belirgin olmak üzere antiepileptik ilaçlar uygun şekilde kullanılamamaktadır (Enginar ve Nurten 2010; Soares ve ark., 2017) Epilepsi sendromu ise belli nöbet tipleriyle birlikte ona eşlik eden klinik ve laboratuvar bulgularının tümünü tanımlar. Etyoloji, odağın anatomik yerleşimi, nöbeti tetikleyici etmenler, nöbetlerin başlangıç yaşı, prognoz, tedaviye yanıt ve EEG bulguları sendromun belirlenmesinde önem taşır. Hastalıktan farklı olarak epileptik sendromların ortak bir etiyolojisi ve prognozu yoktur.

Kontrol grubu hariç tüm gruplarda konvulsiyon oluştuğu görüldü. Bu sonuç diğer gruplar ile karşılaştırıldığında istatistiksel olarak anlamlı derecede farklı bulunmadı. İlaçlar grupların konvulsiyon sürelerini azalttı fakat konvulsiyon skorlarını değiştirmedi. Biyokimyasal parametreler üzerine ilaçların etkisine bakıldığında göreceli olarak olumlu etkileri saptansa da çoğusunun istatistik olarak anlamlı olarak etkili bulunmadı. Davranışsal olarak Rota Rot ve Lokomotor sonuçları istatistik olarak anlamsız bulundu.

Kafein, adenozin A1 ve A2A'in karışık bir yarışmalı reseptör antagonistidir. GABA-A reseptörlerini module ederek gam-amino bütirik asiti (GABA) inhibe eder. Ayrıca, adenozin A-1 reseptörünün stimülasyonu dopaminin salımının inhibisyonuna yol açarak glutamat salımına neden olur. Merkezi A1 reseptörün stimülasyonun önemi antikonvülsan etkilere yol açmasıdır. Yapılan bir çalışmada, 60 mg/kg ve 80 mg/kg dozda kafeninin akut uygulamasının PTZ ile indüklenen nöbet eşiğini önemli derecede değiştirmediği bildirilmiştir (Bankstahl ve ark., 2012). Başka bir çalışmada, 46,2 mg/kg ve 92,4 mg/kg dozda kafeninin akut uygulamasının farede maksimal elektroşokta

prokonvülsan etki göstermediği bildirilmiştir (Jargiello-Baszak ve ark., 2016). Ayrıca, 20 mg/kg P.O. akut kafein uygulamasının yetişkin sıçanlarda nöbet eşiğini değiştirmediği bildirmiştir (Himmel, 2008). Başka bir araştırmadan elde edilen sonuçlardan farklı olarak, 92,4 mg/kg dozda uygulanan kafeinin farelerde PTZ ile indüklenmiş klonik nöbetlerin eşiğini düşürdüğü bildirilmiştir (Luszczki ve ark., 2006). Ayrıca yapılan başka çalışmalar 150 ile 200 mg/kg kafein dozunun farelerde klonik nöbetlere yol açtığını bildirmişlerdir (Marangos ve ark., 1981). Esmaili ve Heydari tarafından yapılan araştırmada, 100 mg/kg dozdaki kafein dozunun nöbet eşiğini değiştirmediği bildirilmiştir. Bizim çalışmamızda 10 mg/kg dozda kafein+atropin uygulamasının bu grupta glutation peroksidaz (GSH-Px) seviyelerinde atropine grubuna göre önemli bir artışa neden olduğu tespit edildi. Benzer sonuç Souza ve ark. tarafından yapılan bir çalışmada, kafein tüketiminin glutatiyon (GSH) seviyesini arttırdığı ve tiyobarbütirik asit reakif bileşiklerinin (TBARS) seviyesindeki artışa karşı koruduğunu bildirmişleridir (Souza ve ark.,2013). Ayrıca, CAT, PC ve NO seviyelerinin kontrol grubuyla karşılaştırıldığında atropin ve kafein gruplarında anlamlı olmadığı tespit edilmiştir ($p > 0.05$).

Minoksidil periferdeki düz kas hücrelerindeki potasyum kanalılarını açma suretiyle etki etmesi nedeniyle güçlü bir arteriyel vazodilatör olarak kabul edilmektedir (Gilmore ve ark., 1970; Charme ve ark., 1973; Editorial, 1973). Minoksidil ve diazoksitin hücre membranlarından kalsiyum alımını inhibe ettiği gösterilmiştir (Chidsey ve Gottlieb, 1974). Bazı potasyum kanal açıçılarının epileptik nöbetlerin azaldığını göstermişler (Chidsey ve Gottlieb, 1974). Farklı bir araştırma da 50 mg / kg minoksidil + atropin grubunda SOD düzeyinin atropin grubuna göre anlamlı derecede yüksek olduğunu gösterilmiştir ($p > 0.05$) (Arie ve ark., 2007). Bizim çalışmada minoksidil + atropin grubunda minoksidilin konvülsiyon başlangıç zamanını istatistiksel olarak önemli derecede arttırdığı tespit edilmiş olup ($p < 0.05$).

Fogle ve ark. tarafından yapılan bir çalışmada bir potasyum kanal inhibitörü olan diazoksitin hayvanlarda nöbetleri önemli derece azalttığı ve ATP61 nöronlarında uyarılmayı azalttığı bildirilmiştir (Fogle ve ark., 2016). Başka bir çalışmada, diklorvos ile indüklenmiş nöbetlerde diazoksitin nöbetleri azalttığı gözlemlenmiştir (Jazayeri ve ark., 2013). Glibenklamidin ATP'ye hassas potasyum kanallarını bloke etmesi ve

potasyum kanallarını module etme olasılığıyla konvülsiyonlara karşı potansiyel aktivite gösterebileceği bildirilmiştir (Body,1988). Pithadia ve ark. antidiyabetik bir ilaç olan glibenklamidin fenilentetrazol ile indüklenmiş olan konvülsiyonları inhibe ettği bildirilmiştir. Ayrıca, glibenklamidin antikonvülsan etkisinin GABA A/benzodiazepine reseptör kompleksi kanalı aracılığıyla meydana geldiği bildirilmiştir (Pithadia ve ark., 2013). Bazı çalışmalar KATP kanallarının farklı in vitro ve in vivo modellerde nöber eşiğinin kontrolünü sağlama göz çarpan bir rol üstlendiğini göstermiştir (Mattia ve ark., 1994; Katsumori ve ark., 1996; Yamada ve Inagaki, 2005; Shafaroodi ve ark., 2007). Ayrıca farelerde yapılan başka bir çalışma KATP kanallarının alt ünitelerinden birinin bloke edilmesinin hipoksiyi azalttığı, dolayısıyla genelleşmiş nöbet için azalmış bir eşik gösterdiğini bildirmiştir (Yamada ve ark., 2001). Benzer bir çalışmayla (Pithadia ve ark., 2013) 5 mg/kg glibenklamidin konvülsiyonu önlediğini tespit edilmiştir. Yapılan araştırmadan elde edilen glutatiyon peroksidaz (GSH-Px) sonuçlarına göre glibenklamid + atropin (25 mg/kg) grubunun atropin grubuyla karşılaştırıldığında daha yüksek değerler elde edilmiştir ($p>0.05$). Benzer sonuçlata diyabetik sıçanlarda artmış GSH seviyesi bildirilmiş olan başka bir çalışmada da görülmüştür (Ghosh ve ark., 2008). Bu sonuçlar bizim sonuçlarla benzer yöndedir.

Liken ve usnik asitle yapılan çalışmalarda antioksidan aktivite, yara iyileştirici, analjezik, antikonvülsan, antiemetic, anti-astmatik, antienflamatuvar, antiviral ve antibiyotik özellikler gibi farmakolojik etki gösterilmiştir (Cocchietto ve ark., 2002; Ingolfsdottir, 2002; Shukla ve ark., 2010; Raizada ve ark., 2014; Yousuf ve Choudhary, 2014; Luzina ve Salakhutdinov, 2016). Usnik asidin antioksidan potansiyel indeksini (TRAP) araştıran çalışmalarda önemli derecede yüksek bir antioksidan kapasite sergilediği gösterilmiştir ve ayrıca hidroksil radikallerine karşı etkili olduğunu, nitrik oksit üretiminde azalma ve lipoperoksidasyon oluşumunu aktive ettiği bildirilmiştir (Rabelo ve ark., 2012). Aynı zamanda, usnik asitin mitokondriyal apoptik ölümüne katkı sağlayarak reaktif oksijen türlerinin (ROS) oluşumunu arttırdığı başka bir çalışmayla bildirilmiştir (Zuo ve ark., 2015). Bizim çalışmamızda usnik asit tüm bulgularda anlamsız bulunmuştur.

Sonuç olarak, yirmi dört saat aç bırakılıp yem verdikten sonra atropin uygulanan hayvanlarda nöbet ortaya çıktı. Kullandığımız farmakolojik ajanlarının nöbet skoru

üzerinde etkileri istatistiksel olarak anlamlı bulunmadı. Biyokimyasal parametrelerden bazılarını kullandığımız farmakolojik ajanlar olumlu yönde değiştirdi. Ayrıca yüksek dozda minoksidil havanların nöbete başlama sürelerini istatistiksel olarak uzattı. Bundan sonra ki çalışmalarda, farklı protokoller kullanılarak alta yatan mekanizmalara açıklık getirilebilir.

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CURRICULUM VITAE

Taha Safauldeen Taha Alhajjar with Iraqi citizenship was born in Mosul city Iraq in 1992. I had studied my primaries in Mosul and high schools in the city of Erbil-Iraq. I had graduated from Ternopil State Medical University in Ternopil city - Ukraine as a pharmacist with the title of Master of Science in pharmacy in 2015. In 2017, I registered as a student at Van YuzuncuYil University in Van -Turkey to study a Master's degree program in the department of pharmacology.

ATTACHMENTS

ATTACHMENT 1. The Acceptance Letter form Experimental Animal Ethics Committee of Van YuzuncuYil University.

T.C.
VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU

ARAŞTIRMA BAŞVURU ONAY BELGESİ

	Araştırmanın Adı	Aç hayvanlarda atropin uygulanması ve yem verilmesi ile oluşan konvülsiyonlara, glibenklamid, minoksidil, kafein, ve üsnik asidin etkisinin araştırılması
	Araştırmanın Yürütücüsü	Dr. Öğr. Üyesi Oruc ALLAHVERDİYEV
	Yardımcı Araştırıcılar	Dr. Öğr. Üyesi Mehmet BERKÖZ Yük. Lis. Öğr. Taha Safauldeen Taha
	Kurumu	Eczacılık Fakültesi
	Araştırmanın Tahmini Süresi	23 Ay
	Kullanılacak Hayvan Türü ve Sayısı	Fare 70 Adet
	Destekleyecek Kuruluş (lar)	Van YYÜ Bilimsel Araştırma Projeleri Başkanlığı
	Başvuru Tarihi	16.10.2018

KARAR BİLGİLERİ	**Karar No:2018/10**	**Tarih:25.10.2018**
	Van Yüzüncü Yıl Üniversinesi Eczacılık Fakültesi öğretim üyesi/elemanı Dr. Öğr. Üyesi Oruc ALLAHVERDİYEV sorumluluğunda yürütülmesi planlanan ve yukarıda başvuru bilgileri verilen doktora projesi, gerekçe, amaç ve yöntemler dikkate alınarak ilgi başvuru belgeleri incelendi. Çalışmanın etik açıdan uygun olduğuna, projenin aşağıdaki hususlar dikkate alınarak yürütülmesine ve proje yürütücüsüne iletilmesine oy birliği / oy çokluğu ile karar verildi. 1) Projede herhangi bir değişiklik gerektiğinde kurulumuzdan onay alınması. 2) Projede çalışacağı bildirilen araştırıcılarda değişiklik olduğunda kurulumuzdan onay alınması. 3) Deney hayvanları üzerinde yapılacak girişimin başlangıç ve bitiş tarihlerinin bildirilmesi. 4) Çalışma süresinde tamamlanamaz ise ek süre talebinde bulunulması. 5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.	

	BAŞKAN/CHAIR Prof. Dr. Semiha DEDE	
ÜYE Prof. Dr. N. Tuğba BİNGÖL	**ÜYE** Prof. Dr. Süddik KESKİN	**ÜYE** Prof. Dr. Sephi DENİZ
ÜYE Prof. Dr. Nalan ÖZDAL	**ÜYE** Doç. Dr. Atilla DURMUŞ	**ÜYE** Doç. Dr. Yıldıray BAŞBUĞAN
ÜYE Doç. Dr. Ferda KARAKUŞ	**ÜYE** Dr. Öğr. Üyesi Oruc ALLAHVERDİYEV	**ÜYE** Dr. Öğr. Üyesi Cansel Yılmaz DEMİR
ÜYE Dr. Öğr. Üyesi Hacer ŞAHİN AYDINYURT	**ÜYE** Dr. Öğr. Üyesi Şükrü ÖNALAN	**ÜYE** Vet. Hek. Kerem OĞRAK
ÜYE Vet. Hek. İsmail Hakkı BEHÇET	**ÜYE** Zir. Müh. Kenan YILDIRIMOĞLU	

*Bu form VAN YÜHADYEK tarafından doldurulacaktır.

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

VAN YÜHADYEK
VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

ARAŞTIRMA KESİN SONUÇ ONAY BELGESİ

VAN YUZUNCUYILUNIVERSITY (TURKEY)
ANIMAL RESEARCHES LOCAL ETHIC COMMITTEE
RESEARCH FINAL REPORT APPROVAL CERTIFICATE

Araştırmanın Adı *Research Title*	Aç hayvanlarda atropin uygulanması ve yem verilmesi ile oluşan konvülsiyonlara, glibenklamid, minoksidil, kafein, ve üsnik asidin etkisinin araştırılması. Application of atropine in fasted animals and convulsions caused by feeding, investigation of the effect of glibenclamide minoxidil, caffeine, and usnic acid
Araştırıcı(lar) *Investigator(s)*	**Yürütücü** / *Chief investigator* : Dr. Öğr. Ü. Oruç YUNUSOĞLU
	Yardımcı Araştırıcı(lar) / *Co-investigator(s):* Taha Safauldeen Taha ALHAJJAR

Araştırmanın Başlama Tarihi / *Research Starting Date: 28.07.2019*

Araştırmanın Bitiş Tarihi / *Research Completion Date:27.09.2019*

Proje Süresi / *Total Time of Project:12 ay*

Proje No / *Project Number:* TYL-2019-7794

Araştırmayı Destekleyen Kuruluş (varsa)/ *Funding institution(s) (if available): Van YYU. BAP*

Destek Şekli ve Miktarı / *Type and amount of funding: 6500 tl*

Karar:
Yukarıda bilgileri verilen araştırma projesinin kesin sonuç raporu Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 03/10/2019 tarih ve 2019/09 sayılı kararı ile kabul edilmiştir.
Decision:
Final report of the research project detailed above was approved by Van Yuzuncu Yil University Animal Researches Local Ethic Committee in the session held on 03/10/2019 (decision number 2019/09)

	BAŞKAN/CHAIR Prof. Dr. Semiha DEDE	
ÜYE/Member Prof. Dr. N. Tuğba BİNGÖL	**ÜYE/Member** Prof. Dr. Sıddık KESKİN	**ÜYE/Member** Prof. Dr. Nalan ÖZDAL
ÜYE Prof. Dr. Atilla DURMUŞ	**ÜYE/Member** Doç. Dr. Ferda KARAKUŞ	**ÜYE/Member** Doç. Dr. Yıldıray BAŞBUĞAN
ÜYE/Member Doç. Dr. Canser Yılmaz DEMİR	**ÜYE/Member** Doç. Dr. Hacer ŞAHİN AYDINYURT	**ÜYE/Member** Dr. Öğr. Üyesi Oruç YUNUSOĞLU
ÜYE/Member Dr. Öğr. Üyesi Şükrü ÖNALAN	**ÜYE/Member** Vet. Hek. Kerem OĞRAK	**ÜYE/Member** Vet. Hek. İsmail Hakkı BEHÇET
	ÜYE/Member Zir. Müh. Kenan YILDIRIMOĞLU	

ATTACHMENT 3. Master Thesis Orıgınality Report

T.C.
VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
Sağlık Bilimleri Enstitüsü

YÜKSEK LİSANS TEZİ ORİJİNALLİK RAPORU

Tarih: 09/12/2019

Tez Başlığı / Konusu: Aç hayvanlarda atropin uygulanması ve yem verilmesi ile oluşan konvülsiyonlara, glibenklamid, minoksidil, kafein, ve üsnik asidin etkisinin araştırılması

Yukarıda başlığı/konusu belirlenen tez çalışmamın Kapak sayfası, Giriş, Ana bölümler ve Sonuç bölümlerinden oluşan toplam 86 sayfalık kısmına ilişkin, 09/12/2019 tarihinde şahsım/tez danışmanım tarafından Turnitin intihal tespit programından aşağıda belirtilen filtreleme uygulanarak alınmış olan orijinallik raporuna göre, tezimin benzerlik oranı %16 (on altı) dır.

Uygulanan filtreler aşağıda verilmiştir:
- Kabul ve onay sayfası hariç,
- Teşekkür hariç,
- İçindekiler hariç,
- Simge ve kısaltmalar hariç,
- Gereç ve yöntemler hariç,
- Kaynakça hariç,
- Alıntılar hariç,
-Tezden çıkan yayınlar hariç,
- 7 kelimeden daha az örtüşme içeren metin kısımları hariç (Limit match size to 7 words)

Van Yüzüncü Yıl Üniversitesi Lisansüstü Tez Orijinallik Raporu Alınması ve Kullanılmasına İlişkin Yönergeyi inceledim ve bu yönergede belirtilen azami benzerlik oranlarına göre tez çalışmamın herhangi bir intihal içermediğini; aksinin tespit edileceği muhtemel durumda doğabilecek her türlü hukuki sorumluluğu kabul ettiğimi ve yukarıda vermiş olduğum bilgilerin doğru olduğunu beyan ederim.

Gereğini bilgilerinize arz ederim.

Taha Safauldeen Taha Alhajjar
İmza

Öğrencinin Adı Soyadı		Taha Safauldeen Taha Alhajjar
Anabilim Dalı	:	Enstitünüz Eczacılık Meslek Bilimleri Anabilim Dalı
Öğrenci No		169306009
Programı	:	☒ Yüksek Lisans ☐ Doktora

DANIŞMAN ONAYI
UYGUNDUR
(Dr. Öğr. Üyesi Onur YUNUSOĞLU)

ENSTİTÜ ONAYI
UYGUNDUR
(Doç. Dr. Hamit Hakan ALP)