EVALUATION OF ANTICONVULSANT ACTIVITY OF CHLOROFORM ROOT EXTRACT OF *Aconitum heterophyllum*

A Dissertation Submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

OCTOBER-2017

DEPARTMENT OF PHARMACOLOGY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD,

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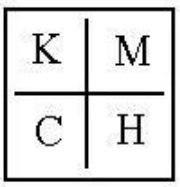

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Reg. No. 261525801

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CERTIFICATE

This is to certify that the dissertation work entitled **"EVALUATION OF ANTICONVULSANT ACTIVITY OF CHLOROFORM ROOT EXTRACT OF *Aconitum heterophyllum*"** was carried out by **Reg. No. 261525801**. The work mentioned in the dissertation was carried out at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, for the partial fulfillment for the degree of Master of Pharmacy during the academic year 2016-2017 and is forwarded to the Tamil Nadu Dr. M. G. R. Medical University, Chennai.

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CERTIFICATE

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This research work either in part or full does not constitute any of any thesis / dissertation.

Date: **Signature of the guide**

Place: Coimbatore

DECLARATION

I do here by declare that to the best of my knowledge and belief ,the dissertation work entitled **"EVALUATION OF ANTICONVULSANT ACTIVITY OF CHLOROFORM ROOT EXTRACT OF *Aconitum heterophyllum*"** submitted to the Tamil Nadu Dr. M.G.R. Medical university , Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology,** was carried out at Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, during the academic year 2016-2017.

Date:

Place: Coimbatore **Reg. No: 261525801**

EVALUATION CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"EVALUATION OF ANTICONVULSANT ACTIVITY OF CHLOROFORM ROOT EXTRACT OF *Aconitum heterophyllum*"** submitted by **Reg No: 261525801** to the Tamil Nadu Dr. M.G.R. Medical university, Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work carried out by the candidate during the academic year 2016-2017 at KMCH College of Pharmacy, Coimbatore, Tamil Nadu and the same was evaluated by us.

Examination Center: KMCH College of Pharmacy, Coimbatore

Date:

Place: Coimbatore

Internal Examiner **External Examiner**

Convener of Examination

Dedicated to Almighty God and My Beloved Parents , Brothers and friends

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Reg No: 261525801

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LIST OF ABBREVIATIONS

SL. NO	ABBREVIATIONS	FULL FORM
18	5-HT	Serotonin
3	AED	Antiepileptic drug
14	BSA	Bovine serum albumine
6	CEAH	Chloroform extract of Aconitum heterophyllum
2	CNS	Central nervous system
17	DA	Dopamine
22	DTNB	5,5'-Dithios (2-nitrobenzoic acid)
10	EEG	electroencephalography
12	FDA	Food and drug administration
7	GABA	Gamma amino butyric acid
19	GLU	Glutamate
1	GNP	Gross national product
8	GSH	Reduced glutathione
20	HPTLC	High performance liquid chromatography
25	HTLE	Hind limb extension
9	LPO	Lipid peroxidation
15	MDA	Malondialdehyde
4	MES	Maximal electroshock
16	NA	Nor adrenaline
11	NMDA	N-methyl-D-aspartate
24	OPT	O-phthaldialdehyde
5	PTZ	Pentylenetetrazole
23	TBARS	Thiobarbituric acid reactive substances
21	TCA	Trichloro acetic acid
13	TMS	Transcranial magnetic stimulation

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ABSTRACT

The present investigation has been undertaken to study the anticonvulsant activity of chloroform root extract of *Aconitum heterophyllum.* The plant *Aconitum heterophyllum* of family *Ranunculaceae* is an Ayurvedic herb which is known for its significant medical properties. Experiments were conducted following standard procedures. The chloroform extract of *Aconitum heterophyllum* were evaluated for their *invivo* antioxidant and anticonvulsant properties and neurotransmitters level. The anticonvulsant activity of CEAH was evaluated using maximal electroshock induced convulsion and pentylenetetrazole induced convulsion models. Diphenylhydantoin was used as standard for MES and Sodium Valproate was used as standard for PTZ. Extracts treated groups showed higher *in vivo* antioxidant, and anticonvulsant activities. They also showed higher activity in neurotransmitters level. CEAH exhibited similar anticonvulsant activity that of the standard but with lesser magnitude. The result may be attributed to the chemical constituents such as diterpene alkaloids present in it which may be due to their individual or cumulative effect that enhanced anticonvulsant activity and provided scientific evidence to the ethnomedicinal features of *Aconitum heterophyllum*. These findings could justify the inclusion of this plant in the management of epilepsy.

Keywords: CEAH, anticonvulsant, chemical constituents.

1. INTRODUCTION

This chapter presents; the background of the study, statement of problem, definition of terms, theoretical basis, purpose of study, hypothesis, specific aims and plan of work.

1.1 **BACKGROUND OF THE STUDY**

Epilepsy is a chronic disorder of the brain that affects people worldwide. As per WHO, epilepsy is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized), and are sometimes accompanied by loss of consciousness and control of bowel or bladder function.[1]

Epilepsy was one of the first brain disorders to be described. It was mentioned in ancient Babylon more than 3,000 years ago. The strange behaviour caused by some seizures has contributed through the ages to many superstitions and prejudices. From greek word attack, the word epilepsy is derived. In earlier times, People once thought that those with epilepsy were being visited by demons or gods. However, in 400 B.C., the early physician Hippocrates suggested that epilepsy was a disorder of the brain, and we now know that he was right.[2]

Epilepsy is a major neurological disorder and upto 5% of the world population develops epilepsy in their lifetime. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity as well as teratogenic effects and approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy.

Traditional systems of medicines are popular in developing countries and upto 80% of the population relies on traditional medicines/ folk remedies for their primary health care need. Hence, there is a need to discover an alternative agent from natural sources.[3]

Aconitum heterophyllum used as a herbal medicine and is well known for its traditional uses such as expectorants, diuretics, laxative etc. Various studies shows that the active principle diterpene alkaloids having a crucial role in treatment of epilepsy.

Aconitum heterophyllum is rich in diterpene alkaloids. Since *Aconitum heterophyllum* have not been studied for its antiepileptic activity, the present study was aimed to evaluate the antiepileptic activity of chloroform extract of *Aconitum heterophyllum*.

1.2 **STATEMENT OF THE PROBLEM**

More than 2 million people in the United States have experienced an unprovoked seizure or been diagnosed with epilepsy. For about 80 percent of those diagnosed with epilepsy, seizures can be controlled with modern medicines and surgical techniques. However, about 25 to 30 % of people with epilepsy will continue to suffer from seizures with the current available treatment. Doctors call this situation intractable epilepsy. Having a seizure does not necessarily mean that a person has epilepsy. Only when a person has had two or more seizures is he or she considered to have epilepsy.[2]

Approximately 50 million people currently live with epilepsy worldwide. An estimate shows that people suffering from epilepsy(i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people. However, some studies shows that the proportion is much higher in low- and middle-income countries, between 7 and 14 per 1000 people.

Globally, each year epilepsy was diagnosed on estimating 2.4 million people. In high-income countries, annual new cases are between 30 and 50 per 100 000 people in the general population. This figure can be up to two times higher in low- and middle-income countries.

Various factors such as higher incidence of road traffic injuries, birth-related injuries, variations in medical infrastructure, availability of preventative health programmes and awareness among people can be the reason for these. Close to 80% of people with epilepsy live in low- and middle-income countries.[1]

It is estimated that there are more than 10 million persons with epilepsy in India. Its prevalence is about 1% in our population. The prevalence is higher in the rural (1.9%) compared to urban population (0.6%). In the Bangalore Urban Rural Neuro-epidemiological Survey (BURNS), estimated that a prevalence rate of 8.8/1000 population was observed, with the rate in rural communities (11.9) being twice that of urban areas (5.7).[4]

Epilepsy accounts for 0.6%, of the global burden of disease, a time-based measure that combines years of life lost due to premature mortality and time lived in less than full health. In terms of health care needs, premature death and lost work productivity, epilepsy has significant economic implications.

An Indian study conducted in 1998 calculated that the cost per patient of epilepsy treatment was as high as 88.2% of the country's per capita Gross National Product (GNP), and epilepsy-related costs, which included medical costs, travel, and lost work time, exceeded $2.6 billion/year (2013 USD).[1]

Estimates suggest that available medication controls the seizures in only 50% of patients or decreases the incidence in only 75% of patients. The search for agents with anticonvulsant activity with more selectivity and lower toxicity continues to be an area of investigation in future.[4]

1.3 **DEFINITIONS**

- **Epilepsy**

These are a group of CNS disorders characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes(seizures) of loss or disturbance of consciousness, with or without characteristic body movements(convulsions), sensory or psychiatric phenomena.[5]

- **Seizures**

A seizure is a sudden surge of electrical activity in the brain.

- **Convulsion**

A convulsion is a condition in which body muscles contract and relax rapidly and repeatedly, results in an uncontrolled shaking of the body.[6]

1.4 **THEORETICAL BASIS**

Despite the successful development of various new antiepileptic drugs (AEDs) in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal. The discovery and development of a new AED relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans. This approach has been very successful and crucially contributed to the

development of numerous clinically effective AEDs. In the discovery and development of new AEDs, animal models of seizures or epilepsy serve a variety of purposes . First, they are used for identifying novel AEDs. Second, animal models are used to evaluate the possible specific efficacies of the compound against different types of seizures or epilepsy if the antiepileptic activity of a novel compound was detected,. Third, specific models of AED-resistant seizures are used to investigate whether the novel drug has advantages towards clinically established AEDs for therapy of difficult-to-treat types of seizures or epilepsies. Fourth, animal models are used to characterize the preclinical efficacy of novel compounds during chronic administration. Such chronic studies can serve different objectives, for instance evaluation of whether drug efficacy changes during prolonged treatment, e.g. because of development of tolerance. Fifth, in view of the possibility that chronic brain dysfunctions, such as epilepsy, might lead to altered sensitivity to drug adverse effects, models with epileptic animals are useful to study whether epileptogenesis alters the adverse effect potential of a given drug. Sixth, animal models can be used to estimate effective plasma concentrations of new AEDs for first clinical trials. And finally, seventh, animal models are crucial in discovering therapies that may prevent or modify the development of epilepsy after brain insults

Not all animal models of seizures and/or epilepsy can be used for all of the above described purposes. Furthermore, the intention of the experiment is essential for selection of a suitable animal model. For instance, simple seizure models such as the maximal electroshock seizure (MES) test, allowing to test high numbers of compounds for anticonvulsant activity in relatively short time, will be preferred above more complex models in screening approaches of anticonvulsant drug development.

For AED discovery, which necessitates screening of large numbers of compounds, animal models should be easy-to-perform, time- and cost-efficient, and predictive of clinical activity. This explains that two simple seizure models in mice and rats, the MES and pentylenetetrazole (PTZ) tests, which have been developed >60 years ago, are still the most widely used animal seizure models employed in the search for new AEDs.[7]

Experimental models

These models for testing antiepileptic drugs have also shed light on the etiopathogenesis of epilepsy.

1. Maximal electroshock seizures: Brief high intensity shock is applied to the head of a rodent produces tonic flexion- tonic extension-clonic convulsions. The tonic phase (especially extensor) is selectively abolished by drugs effective in generalized tonic clonic seizure. Activity in this model represents action on spread of seizure discharge.
2. Pentylenetetrazol clonic seizures(PTZ) : Injection of PTZ in rats or mice produces clonic convulsions which are prevented by drugs effective in absence seizures. Activity in this model represents action on seizure focus itself.
3. Chronic focal seizures: Produced by application of alumina cream on the motor cortex of monkey.
4. Kindled seizures: Brief bursts of weak electrical impulses are applied to the brain (especially amygdala) intermittently over days. After- discharges increase progressively and tonic-clonic seizures are produced after 10-15 shocks; with time spontaneous seizures have a self perpetuating and reinforcing effect: more neuronal circuits are facilitated and recruited in the seizure process. Kindling is probably involved in the genesis of clinical epilepsy.[5]

1.5 **PURPOSE OF THE STUDY**

The current therapy of epilepsy with modern antiepileptic drugs (AEDs) is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy. The discovery of novel antiepileptic drugs relies upon the preclinical employment of animal models to establish efficacy and safety prior to the introduction of the AEDs in human volunteers. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles. For the detection of antiepileptic activity, several plants are used for the treatment of epilepsy in different systems of traditional medicine and these plants have shown activity when tested in modern bioassays and many such plants are yet to be scientifically investigated. Medicinal plants used for the therapy of epilepsy in traditional medicine have been

shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening.[8]Various studies shows that the active principle alkaloids having a crucial role in treatment of epilepsy. *Aconitum heterophyllum* is rich in diterpene alkaloids(11). The purpose of the study is to evaluate the antiepileptic activity of chloform extract of *Aconitum heterophyllum.*

1.6 HYPOTHESIS

I hypothesize that the presence of active constituents like diterpene alkaloids in this plant *Aconitum heterophyllum*after isolation and extraction may produce anti convulsant activity. To test whether the plant producing the anti convulsant activity, MES and PTZ induced convulsion models are selected. The result of these studies will have a translational value to anti convulsant activity.

1.7 SPECIFIC AIMS

1. To determine anticonvulsant activity of CEAH by MES and PTZ induced convulsion models
2. To determine *in vivo* antioxidant activity of CEAH
3. To determine the effect of CEAH on neurotransmitters level in MES and PTZ induced convulsion models.

1.8 PLAN OF WORK

1. Review of Literatures
2. Selection, Collection And Authentication of Plant Material
3. Extraction of plant materials with chloroform
4. Acute toxicity study
5. Pharmacological study

   A. Screening of anti epileptic activity using various models

- Maximal electroshock induced convulsion
- Pentylenetetrazole induced convulsion

B. Estimation of neurotransmitter

a. GABA

b. Serotonin

c. Nor adrenaline

e. Dopamine

7.Invivo antioxidants

- Reduced Glutathione (GSH)
- Lipid peroxidation (LPO)

8. Total protein content

9. Histopathological study

10. Statistical analysis

2.REVIEW OF LITERATURE

2.1. EPILEPSY

Epilepsy is a chronic CNS disorder characterized by brief episodesof seizures and excessive EEG discharge. It is usually associated with loss of consciousness, violent spasmodic contractions of skeletal muscles (convulsions) and autonomic hyperactivity.[9]

Epilepsy is one of the most common neurological disorders. Worldwide, the prevalence is estimated to be 0.5- 1%, and there is a life time incidence of 1- 3%. It has important medical, social and psychological consequences .Epilepsy is a heterogeneous symptom complex, a chronic disorder characterized by recurrent seizures. Seizures resulting from abnormal discharge of cerebral neurons and are finite episodes of brain dysfunction . It is estimated that in India (with population more than 1 billion), there will be 6- 10 million people with epilepsy, accounting for nearly 1/5 of global burden. The current treatment of epilepsy with modern antiepileptic agents is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy . Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.[10]

2.2 NATURE OF EPILEPSY

The term epilepsy is used to define a group of neurological disorders all of which exhibit periodic seizures. Not all seizures involve convulsions. Seizures are associated with episodic high-frequency discharge of impulses by a group of neurons (sometimes referred to as focus) in the brain. What starts as a local abnormal discharge may then spread to other areas of the brain. The site of the primary discharge and the extent of its spread determine the symptoms that are produced, which range from a brief lapse of attention to a full convulsive fit lasting for several minutes, as well as odd sensations or behaviours.

The particular symptoms produced depend on the function of the region of the brain that is affected. Thus, involvement of the motor cortex causes convulsions, involvement of the hypothalamus causes peripheral autonomic discharge, and involvement of the reticular formation in the upper brain stem lead to loss of consciousness.[11]

2.3TYPES

A. **Generalised seizures**

1. **Generalized tonic-clonic seizures(major epilepsy, grand mal)**: commonest, lasts 1-2 min. Prolonged sleep and depression of all CNS functions after the usual sequence that is aura-cry-unconsciousness-tonic spasm of all body muscles-clonic jerking followed by

2. **Absence seizures (minor epilepsy, petit mal)**: prevalent in children, lasts about ½ min. Sudden loss of consciousness, no muscular component or little bilateral jerking, patient apparently freezes and stares in one direction,.

3. **Atonic seizures (Akinetic epilepsy**): unconsciousness with relaxation of all muscles due to excessive inhibitory discharges. Patient may fall.

4. **Myoclonic seizures**: shock-like momentary contractions of muscles of a limb or the whole body.

5. **Infantile spasms (Hypsarrhythmia**): these type of epilepsy seen in infants and probably not a form of epilepsy.Intermittent muscle spasm and progressive mental deterioration.

B. **Partial seizures**

1. **Simple partial seizures (cortical focal epilepsy):** lasts ½-1 min. Often secondary. Depending on the area of cortex involved, convulsions are confined to a group of muscles or localized sensory disturbance, without loss of consciousness.

2. **Complex partial seizures (temporal lobe epilepsy):** attacks of bizarre and confused behaviour and purposeless movements, emotional changes lasting 1-2 min along with impairment of consciousness. An aura often precedes. The seizure focus is located in the temporal lobe.

3. **Simple partial or complex partial seizures secondarily generalized**: The partial seizure occurs first and evolves into generalized tonic clonic seizures with loss of consciousness.[5]

2.4 CAUSES

All forms of epilepsy have their origin in the brain. The different types of epilepsies are not based on a single underlying mechanism, but are multifactorial in origin. Epilepsy results when many neurons in union, under a high excited stage, deliver

massive discharges abolishing a finely organized pattern of the integrative activity of the brain.

John Jackson proposed that these seizures are caused by occasional, sudden, excessive, rapid and local discharges of grey matter and once initiate by the abnormal focus, the seizures attack the neighboring normal brain resulting into generalized convulsions. This abnormal focus may originate as a result of local biochemical changes, ischemia or the loss of vulnerable cell inhibitory systems. However, certain physiological changes may trigger the focus and thus facilitate the spread of abnormal electrical activity to normal tissue. Such factors include

- **Changes in blood glucose concentration**
- **Plasma pH**
- **Total osmotic pressure and electrolytes composition of extra cellular fluids**
- **Fatigue**
- **Emotional stress**
- **Nutritional deficiency.**[4]
- **Genetic Factors**

Several types of epilepsy have now been linked to defective genes for ion channels, the "gates" that control the flow of ions in and out of cells and regulate neuron signaling. Another gene, which is missing in people with progressive myoclonus epilepsy, codes for a protein called cystatin B. This protein regulates enzymes that break down other proteins. Another gene, which is altered in a severe form of epilepsy called LaFora's disease, has been linked to a gene that helps to break down carbohydrates.

- **Other Disorders**

In some cases, epilepsy may develops as a result of brain damage from other diseases. For example, brain tumors, alcoholism, and Alzheimer's disease frequently lead to epilepsy because they alter the normal workings of the brain. Strokes, heart attacks, and other conditions that diminishes the supply of oxygen towards brain, also can cause epilepsy in some cases. About 32 percent of all cases of newly developed epilepsy in elderly people appears to be due to cerebrovascular disease, which reduces the supply of oxygen to brain cells. Meningitis, AIDS, viral encephalitis, and other infectious diseases and also ahydrocephalus -- a condition in which excess fluid builds up in the brain can

lead to epilepsy. Epilepsy also can result from intolerance to wheat gluten (also known as celiac disease), or from a parasitic infection of the brain known as neurocysticercosis.

Epilepsy is having connection with a variety of metabolic diseases such as cerebral palsy, pyruvate dependency, tuberous sclerosis, Landau-Kleffner syndrome, and autism. Epilepsy is just one of a set of symptoms commonly found in people with these disorders.

- **Head Injury**
- **Prenatal Injury and Developmental Problems**

The developing brain is susceptible to many kinds of injury. Some conditions like Maternal infections, poor nutrition, and oxygen deficiencies that may affect the brain of a developing baby. These conditions may lead to cerebral palsy, which often is associated with epilepsy, or they may cause epilepsy that is unrelated to any other disorders.

- **Poisoning**

Exposure to lead, carbon monoxide, and many other poisons may cause seizures. They also can result from exposure to street drugs and from overdoses of antidepressants and other medications.

Seizures are often triggered by factors such as lack of sleep, alcohol consumption, stress, or hormonal changes associated with the menstrual cycle. For some people, a seizure can also be triggered by light flashing at a certain speed or the flicker of a computer monitor and this problemthis type of epilepsy is known as photosensitive epilepsy. Smoking cigarettes also can trigger seizures. The nicotine in cigarettes acts on receptors for the excitatory neurotransmitter acetylcholine in the brain, which increases neuronal firing. Seizures are not triggered by sexual activity except in very rare instances.[2]

Aetiologically, the epilepsies are classified into four groups: idiopathic, symptomatic, cryptogenic and progressive . The idiopathic epilepsies are thought to be genetically determined and are usually associated with particular clinical characteristic and specific electroencephalography (EEG) findings . Structural abnormality of the brain can result in symptomatic epilepsies and are acquired condition . Epilepsy is classified as cryptogenic when no clear abnormality or putative risk factor is identified for what is

presumed to be a symptomatic or acquired epileptic condition . The term progressive epilepsy is used when epilepsy is associated with an evolving neurological condition.[12]

2.5 SYMPTOMS

Repeated seizure is the major cause of epilepsy. The individual should see a doctor If one or more of the following symptoms are present, especially if the symptoms recur:

- a convulsion with no temperature (no fever)
- Confused memory or short spells of blackout
- intermittent fainting spells, during which loss of bowel or bladder control, followed by extreme tiredness
- for a short period, the person is unresponsive to instructions or questions
- the person becomes stiff, suddenly, for no apparent reason
- the person suddenly falls
- the person shows sudden bouts of blinking without apparent stimuli
- sudden bouts of chewing, without any apparent reason
- for a short time the person seems dazed and unable to communicate
- repetitive movements that seem inappropriate
- the person becomes fearful for no apparent reason; they may even panic or become angry
- peculiar changes in senses, such as smell, touch, and sound
- the arms, legs, or body jerk, in babies these will appear as a cluster of rapid jerking movements

The following conditions need to be eliminated as they may appear as similar symptoms and are sometimes misdiagnosed as epilepsy:

- high fever with epilepsy-like symptoms
- fainting
- narcolepsy - recurring episodes of sleep during the day
- cataplexy - periods of extreme weakness
- sleep disorders
- nightmares

- panic attacks
- fugue states - rare psychiatric disorder
- psychogenic seizures[13]

2.6SYNDROMES

Cases of epilepsy may be arranged into epilepsy syndromes on the basis of specific features that are present. These features include, the seizure types, EEG findings, the age that seizure begins. Identifying an epilepsy syndrome is useful as it helps determine the underlying causes as well as what anti-seizure medication should be tried.

Since the onset of seizures is commonly early, the ability to categorize a case of epilepsy into a specific syndrome occurs more often with children. Less serious examples are benign rolandic epilepsy (2.8 per 100,000), childhood absence epilepsy (0.8 per 100,000) and juvenile myoclonic epilepsy (0.7 per 100,000). Severe syndromes with diffuse brain dysfunction caused, at least partly, by some aspect of epilepsy, are also commonly known as epileptic encephalopathies. These are associated with frequent seizures that are resistant to treatment and severe cognitive dysfunction, for instance Lennox–Gastaut syndrome and West syndrome. Genetics is believed to play an important role in epilepsies by a number of mechanisms. Simple and complex modes of inheritance have been identified for some of them. However, extensive screening have failed to identify many single gene variants of large effect.

Syndromes in which causes are not clearly identified are difficult to match with categories of the current classification of epilepsy. Categorization for these cases was made somewhat arbitrarily. In case of 2011 classification (idiopathic category) includes syndromes in which the general clinical features and/or age specificity strongly point towards a genetic cause. Some childhood epilepsy syndromes are included in the unknown cause category in which the cause is presumed genetic, for instance benign rolandic epilepsy. Others are included in symptomatic in some cases despite a presumed genetic cause, for example Lennox-Gastaut syndrome. Clinical syndromes in which epilepsy is not the main feature (e.g. Angelman syndrome) were categorized symptomatic but it was argued to include these within the category idiopathic. Classification of epilepsies and particularly of epilepsy syndromes will change with advances in research.[14]

2.7 PATHOPHYSIOLOGY

Action potential is the the basic mechanism of neuronal excitability . Action potential is a hyperexcitable state can result from increased excitatory synaptic neurotransmission, decreased inhibitory neurotransmission, an alteration in voltage-gated ion channels, or an alteration of intra- or extra-cellular ion concentrations in favor of membrane depolarization. Membrane potential can varies with activation of ligand-gated channels, whose conductance is affected by binding to neurotransmitters; or with activation of voltage-gated channels, whose conductance is affected by changes in transmembrane potential; or with changes in intracellular ion compartmentalization.

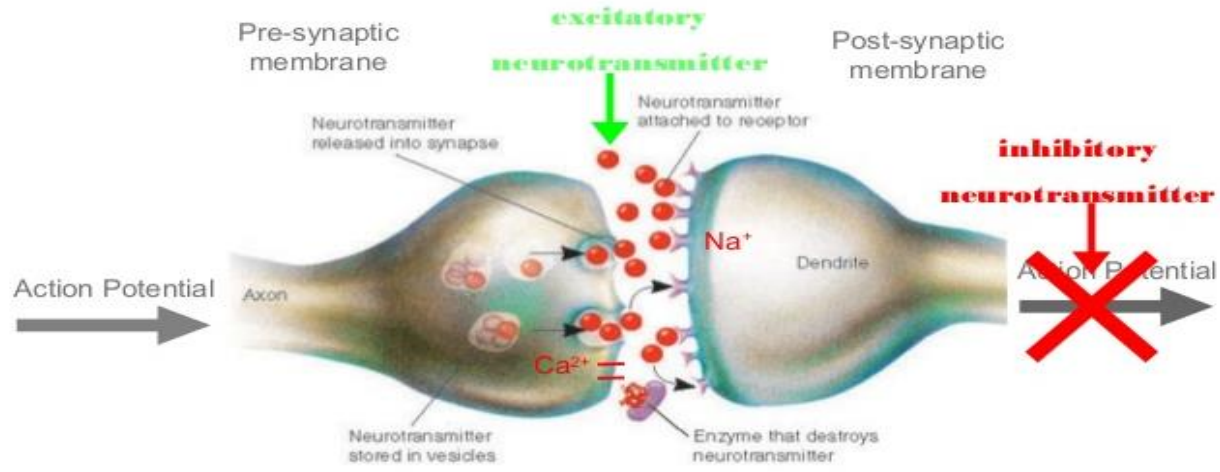
The major neurotransmitters in the brain are glutamate, gamma-amino-butyric acid (GABA), acetylcholine (ACh), norepinephrine, dopamine, serotonin, and histamine. The major excitatory neurotransmitter is the amino acid glutamate. All ionotropic glutamate receptors are permeable to Na+ and K+, and it is the influx of Na+ and outflow of K+ through these channels that contribute to membrane depolarization and generation of the action potential. The NMDA receptor also has a Calcium channel and in resting state, calcium channel is blocked by Magnesium ions, but under conditions of local membrane depolarization, Mg++ is displaced and the channel becomes permeable to Ca++. Influx of Ca++ tends to further depolarize the cell, and is thought also to contribute to Ca++ mediated neuronal injury under conditions of excessive neuronal activation (such as status epilepticus and ischemia), potentially leading to cell death, a process termed excitotoxicity.

The major inhibitory neurotransmitter, GABA, interacts with 2 major subtypes of receptor:

$GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors are found postsynaptically, while $GABA_B$ receptors are found presynaptically, and can thereby modulate synaptic release. $GABA_A$ receptors are permeable to Cl- ions in adult brain and action potential is inhibited by upon activation Cl- influx hyperpolarizes the membrane. Therefore, substances which are $GABA_A$ receptor agonists, such as barbiturates and benzodiazepines, are well known to suppress seizure activity. Rather than Cl- channels, $GABA_B$ receptors are associated with second messenger systems, and due to their presynaptic location, attenuation of transmitter release occurs. The second messenger systems often result in opening of K+ channels, leading to a hyperpolarizing current.

Certain $GABA_B$ agonists, such as baclofen, have been reported to exacerbate hyperexcitability and seizures.[15]

Figure-1: Pathophysiology of epilepsy



2.8 DIAGNOSIS

Abnormal electrical activity during and following a seizure can be detected by electroencephalography (EEG) recording from electrodes distributed over the surface of the scalp. Various types of seizure can be recognized on the basis of the nature and distribution of the abnormal discharge. Modern brain imaging techniques, such as magnetic resonance imaging and positron emission tomography, are now routinely used in the diagnosis of epilepsy to identify structural abnormalities(eg. Lesions, tumors) that cause certain epilepsies.[11]

2.9**TREATMENT**

Once epilepsy is diagnosed, it is important to begin treatment as soon as possible. Once seizures and their consequences become established, research suggests that current available medication and other treatments may be less successful in treating epilepsy.

- **Medications**

By far the most common approach to treating epilepsy is to prescribe antiepileptic drugs. Doctors diagonosing a patient with newly developed epilepsy often

prescribe antiepileptic agents like carbamazepine, valproate, lamotrigine, oxcarbazepine, or phenytoin first, unless the developed epilepsy is a type that is known to require a different kind of treatment. For absence seizures, ethosuximide is often the primary treatment. Other commonly prescribed drugs include clonazepam, phenobarbital, and primidone. Some relatively new epilepsy drugs include tiagabine, gabapentin, topiramate, levetiracetam, and felbamate.[2]

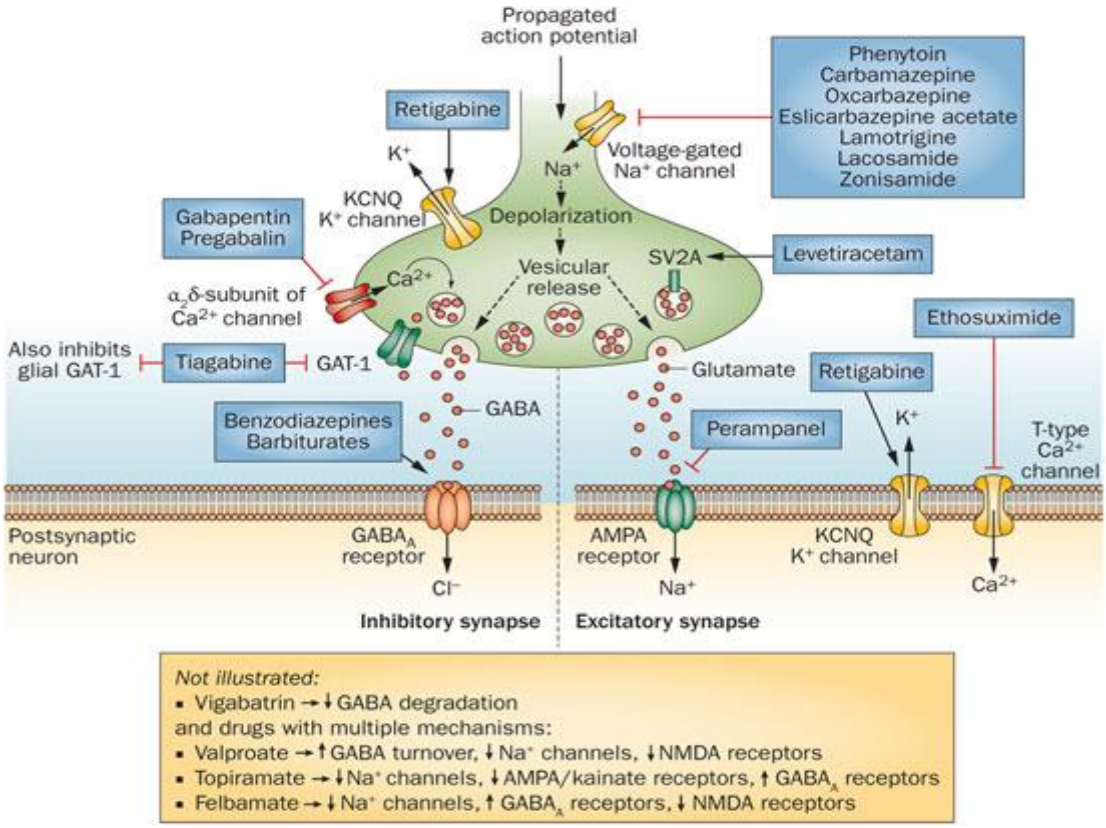
Classification

1. Barbiturate: Phenobarbitone
2. Deoxybarbiturate: Primidone
3. Hydantoin: Phenytoin, Fosphenytoin
4. Iminostilbene: Carbamazepine, Oxcarbazepine
5. Succinimide: Ethosuximide
6. Aliphatic carboxylic acid: Valproic acid, Divalproex
7. Benzodiazepines: Clonazepam, Diazepam, Lorazepam, Clobazam
8. Phenyltriazine: Lamotrigine
9. Cyclic GABA analogues: Gabapentin, Pregabalin
10. Newer drugs: Topiramate, Zonisamide, Tiagabine[5]

2.10 MECHANISM OF ACTION

- The currently available anticonvulsant agents are thought to act by three main mechanisms:
    1. Reducing electrical excitability of cell membranes, mainly through use-dependent block of sodium channels
    2. Enhancing GABA-mediated synaptic inhibition; this may be achieved by an enhanced postsynaptic actionof GABA, by inhibiting GABA transaminase or by inhibiting GABA uptake into neurons and glial cells
    3. Inhibiting T-type calcium channels (important in controlling absence seizures).
- Newer drugs act by other mechanisms, largely yet to be elucidated.
- Drugs that block ionotropic glutamate receptors are effective in animal models but are unsuitable for clinical use.[11]

Figure-2: Mechanism of action of Antiepileptic drugs



- **Surgery to treat underlying conditions**

When seizures are caused by a brain tumor, hydrocephalus, or other conditions that can be treated with surgery, doctors may operate to treat these underlying conditions. In many cases, once the underlying condition is successfully treated, a person's seizures will disappear as well.

- **Surgery to remove a seizure focu**

Removal of a seizure focus, or small area of the brain where seizures originate is the most common type of surgery for epilepsy. This type of surgery, which doctors may refer to as a lobectomy or lesionectomy, is appropriate only for focal seizures that originate in just one area of the brain.

Indications of surgery

1. Medically intractable seizures
2. Seizures significantly affect the quality of life
3. Localized seizure focus
4. Presence of signs predictable of seizure persistence

Contraindications of surgery

1. Benign, self limited epilepsy syndrome
2. Neurodegenerative and metabolic disorders
3. Non compliance with drugs
4. Severe family disfunctions
5. Associated psychosis

- **Multiple subpial transection**

When seizures originate in part of the brain that cannot be removed, surgeons may perform a procedure called a multiple subpial transection

- **Corpus callosotomy**

In children with severe seizures that start in one half of the brain and spread to the other side, Corpus callosotomy, or severing the network of neural connections between the right and left halves, or hemispheres, of the brain, is done.

- **Hemispherectomy and hemispherotomy**

These procedures remove half of the brain's cortex, or outer layer. These are used mainly in children who are suffering from seizures that do not show response to medication because of damage that involves only half the brain, as occurs with conditions such as Rasmussen's encephalitis, Sturge-Weber syndrome, and hemimegencephaly

- **Devices**

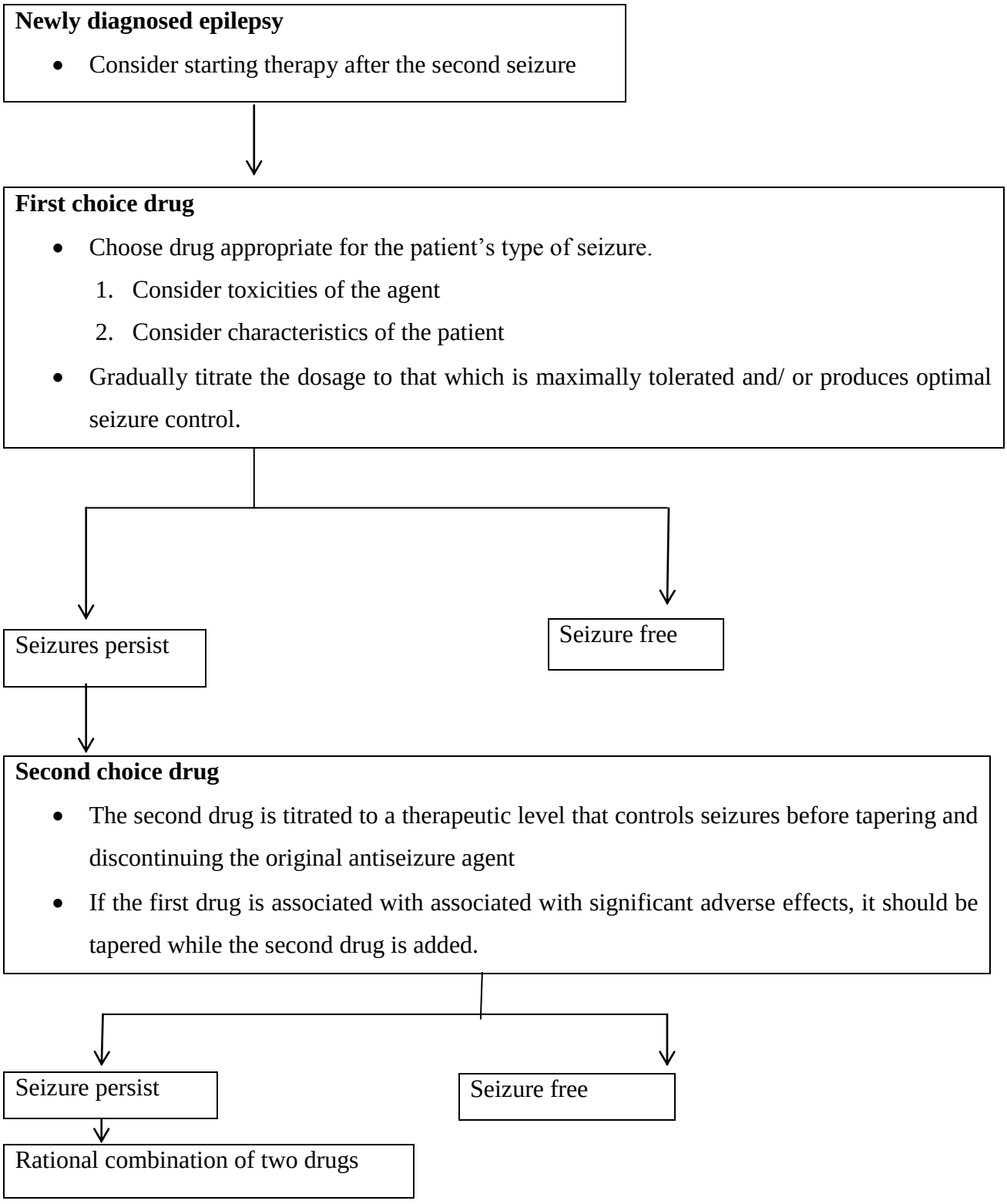
The vagus nerve stimulator was approved by the U.S. Food and Drug Administration (FDA) in 1997 for use in people with seizures that are not well-controlled by medication. The vagus nerve stimulator is a battery-powered device that is surgically implanted under the skin of the chest, much like a pacemaker, and is attached to the vagus nerve in the lower neck. This device delivers short bursts of electrical energy to the brain via the vagus nerve. Researchers are studying whether transcranial magnetic stimulation (TMS), a procedure which uses a strong magnet held outside the head to influence brain activity, may reduce seizures. They also hope to develop implantable devices that can deliver drugs to specific parts of the brain.[2]

- **Diet**

Ketogenic diet is one of the oldest methods of treating childhood epilepsy. In children with refractory seizures who have failed drug therapy and are not candidate for epilepsy surgery, this therapy is as or more effective than the addition of new anti-epileptic drug. For example, it remains a reasonable alternative for children with Lennox-Gastaut syndrome refractory to standard drug therapy.

The ketogenicdiet consist of a higher proportion of fats and small amounts of carbohydrates and protein. The basis of the therapeutic effectiveness of the ketogenic diet is because of the ketosis that develops when the brain is relatively deprived of glucose as an energy source and must shift to utilization of ketone bodies as the primary fuel.

Figure-3: Therapeutic strategies for managing newly diagnosed epilepsy[16]



2.11ANTIEPILEPSY ACTIVITY

- **Avanthi E *et al*., (2016)** evaluated the antiepileptic activity of clove oil by MES model in mice. A total of thirty mice were taken, they were given an electroconvulsive shock. Thirty mice were divided into five groups of six animals each, the control group received distilled water 5ml/kg i.p, standard received Inj. Sodium valproate 200 mg/kg i.p. another group received Sesame oil – 10ml/kg i.p(control), test groups received Clove oil- 0.075 ml/kg i.p., Clove oil-0.1ml/kg i.p respectively. All the injections were given 30 minutes before the test. The results showed Clove oil produced significant antiepileptic effect at all the doses.**[17]**

- **K Sandeep Kumar *et al*., (2015)** evaluated the antiepileptic activity of ethanolic extract of *Biophytum sensitivum* in animal models. The anticonvulsant activity was assessed using MES and PTZ using albino mice. The extract reduced the duration of tonic hind limb extension and delayed the onset of tonic clonic convulsion. The result showed that the ethanolic extract of the plant beneficial in both tonic clonic and absence seizures.[10]

- **Nirmala D (2015)** performed a study which involves in detecting anticonvulsant activity from *Annacyclus pyrethrum* roots by using maximal electroshock seizure (MES) in a dose-dependent way. MES-induced tonic seizures can be prevented either by drugs that inhibit voltage dependent Na+ channels, such as phenytoin, valproate and lamotrigine or by drugs that block glutamatergic excitation mediated by the NMDA receptor such as felbamate. The study showed that ethanolic extract from roots of A. pyrethrum can inhibit voltage dependent Na+ channels as phenytoin in MES induced tonic seizures.[18]

- **Gummalla Pitchaiah*et al*., (2015)** evaluated the anticonvulsant activity of methanolic extract of *Allium cepa* (Onion) bulbs in Swiss albino mice. The anticonvulsant effect was assessed using maximal electroshock (MES) and

Isoniazid(INH) induced seizure models. Methanolic extract (200 and 400 mg/kg) showed significant reduction in the duration of hind limb extensor phase in electroshock convulsions; protected the mice against the Isoniazid induced convulsions. The results showed significant improvement in brain GABA levels after Allium cepa treatment.[19]

- **Santilna K S *et al*., (2014)** studied the anticonvulsant activity study of *Artemisia nilagirica*. The leaves part of the plant was dried, powdered and subjected to maceration using diethyl ether, chloroform and ethanol. The results showed that the alkaloids, flavonoids and terpenoids were identified to be present in all three solvents extracts. The result obtained suggests that the ethanolic and chloroform extracts of Artemisia nilagirica may be beneficial in the treatment of epilepsy.[20]

- **Ravindra C Sutar *et al*., (2014)** evaluated the anticonvulsant activity of leaf extract of *Holoptelea integrifolia.* The petroleum ether and methanolic extract of the leaves was evaluated using Pentylenetetrazole (PTZ) induced convulsions in mice and maximal electro shock (MES) induced Convulsions and lithium-pilocarpine induced status epilepticus in rats. The petroleum ether extract and methanolic extract delayed onset of PTZ- induced convulsions and also prolonged the onset of tonic convulsions in mice. Both the extracts failed to protect the rats from MES induced convulsions. The extracts also protected rats against seizures induced by lithium-pilocarpine. The results indicate that petroleum ether and methanol extracts contained such phytochemial compounds which are active in case of Pentylenetetrazole (PTZ) and lithium pilocarpine induced status epilepticus, which support the ethnomedicinal application of the plant as an anticonvulsant agent.[21]

- **DilnawazPathan*et al*., (2014)** evaluated the anticonvulsant effect of ethanolic extract of roots of *Picrorhiza kurroa* on electrically and chemically induced seizures. The extract was studied for its anticonvulsant effect on maximal electroshock-induced seizures and pentylenetetrazole, picrotoxin induced seizures

in mice. It has been observed in the present study that extract(100 mg/kg) showed significant increase in latency to clonic convulsions and reduced mortality. The results shows that Picrorhizakurroa possess anticonvulsant activity against Pentylenetetrazole , Maximal electroshock and Picrotoxin induced convulsions in mice.[22]

- **Ganapathi G. Varma*et al.*, (2014)** performed the evaluation of antiepileptic activity of methanolic leaves extract of *Tragia involucrata* in mice. In vivo screening models like maximal electroshock-induced convulsion (MES), pentylenetetrazole (PTZ) and picrotoxin (PTX) induced models are used to evaluate the antiepileptic effects of the extracts. In the MES induced convulsion, methanolic extract (800 mg/kg), showed high significant inhibition on tonic hind limb extension and decrease in duration of stupor period . In PTZ and PTX induced model extract(400 mg/kg and 800 mg/kg) showed delay on the onset of convulsions, decreased duration of convulsion and reduced mortality significantly. The results showed that Tragiainvolucrata possesses dose dependent antiepileptic activity.[23]

- **MehrdadModaresi *et al.*,** (2014) studied the antiepileptic activity of hydroalcoholic extract of *Ocimum basilicum* in mice. The experimental groups comprised control, sham, and four treatment groups receiving the extract at 100, 250, 300, and 350 mg/kg doses 65 minutes before PTZ injection. The obtained results of using different doses of the extract indicated that the mice receiving the extract at 100 and 250 mg/kg doses exhibited the highest and lowest frequency of myoclonic twitches, respectively.[24]

- **Chinchawade A B *et al.*, (2013)** observed the anticonvulsant activity of chloroform extract of bark & root of *Erythrina variegate*. The pentyleneterazole (PTZ) and the maximal electroshock seizure (MES) models were used for assessing the anticonvulsant effects of the chloroform extract in mice and rats. The extract produced significant protection against PTZ-induced and MES-induced convulsions in rat. The results obtained from this study indicate that the

chloroform root and bark extract of Erythrinavariegata may be beneficial in both absence and tonic clonic seizures.[25]

- **Abubakar K *et al*., (2013)** evaluated the anticonvulsant effect of methanolic extract of *Evolvulus alsinoides* in mice using pentyleneterazole (PTZ) and the maximal electroshock seizure (MES) model. The extract significantly increased the latency of PTZ induced seizure. In the MES test a dose dependent decrease in the duration of seizure was also observed. These findings suggest that the methanol extract of the plant contains bioactive principles that may be beneficial in the treatment of epilepsy.[26]

- **Ashish P Anovadiya *et al*., (2013)** performed the antiepileptic and memory retention activity of *Curcumin perse* and in combination with antiepileptic drugs. In this study, antiepileptic activity of curcumin and its combination with phenytoin and sodium valproate were studied in chronic model (14 days) of Maximal Electroshock Seizure (MES) and Pentylenetetrazole (PTZ) induced seizure respectively. Curcumin (100 mg/kg) reduced clonic phase and significantly inhibited PTZ induced seizure. Addition of curcumin to sub therapeutic dose of sodium valproate showed synergistic effect..Curcumin found to be effective in absence seizure alone and as add on with sodium valproate.[27]

- **Prabhat Singh *et al*., (2012)** studied antiepileptic activity of aqueous extract of fruits of *Tricosanthes dioica*. The antiepileptic efficacy of aqueous extract was evaluated by hand limb extension induced by MES and PTZ induced seizures in mice models. The aqueous extract was showed significant antiepileptic activity in both models and it was found to be due to activity against generalized tonic-clonic and cortical focal seizures.[28]

- **Vikas Saroch *et al*., (2012)** evaluated the anticonvulsant Activity of *Apasmarari rasa*. *Apasmarari rasa* was subjected to assess the LD 50 and Anti convulsant activity on Male Albino rats was by means of MES (Maximal Electro convulsing Shock) Method. A supramaximal strength was 150mA in rats for 0.2 seconds and

stimulus was applied via ear clip electrodes. The animal dose of Phenytoin (7.2mg/kg), *Smritisagar rasa* (18mg/kg) and *Apasmarari rasa* (5.4mg/ kg) was given orally to different groups. The animals were observed for a period of 180 minutes after being subjected to electro convulsions. Both standard drugs also shown good results when it comes to HLE (hind limb extension), butother factors such as time duration of flexion, tonus, clonus, recovery time amongst others in test drug group *(Apasmarari rasa*) showed significantly better results.[29]

- **Vipin K Garg*et al.*, (2011)** evaluated the anticonvulsant activity of ethanolic extract of *Cynodon dactylon.* The anticonvulsant activity was studied using maximal electroshock (MES) and Pentylenetetrazol (PTZ) induced convulsions in mice. The extract suppressed hind limb tonic extensions (HLTE) induced by MES and also exhibited protector effect in PTZ-induced seizures. The results showed that the ethanolic extract of *Cynodon dactylon* has anticonvulsant effect in the both models suggesting their possible depressant action in the central nervous system.[30]

- **Shyamjith Manikkoth *et al.*, (2011)** performed *Phyllanthus amarus* on maximal electroshock-induced seizures (MES) and pentylenetetrazole (PTZ) induced seizures. The aqueous and ethanolic extracts of the leaves and stems of P. amarus significantly abolished the hind limb extension induced by MES. The same dose also significantly protected the animals from PTZ induced tonic convulsions.[31]

- **Harish Babu B *et al.*, (2010)** performed anticonvulsant activity of the methanolic extract of *Martynia annua* on Maximal Electroshock(MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. These studies showed, the mean duration of extensor phase of test group reduced to significant level as compared to control group. In Pentylenetetrazol induced seizure test, onset of myoclonic spasm and clonic convulsion was delayed in the test group. The study concluded *Martynia annua* possesses an anticonvulsant effect which results from the potentiation of the activity of GABA.[32]

- **N. S. Vyawahare*et al.*, (2009)** evaluated the anticonvulsant activity of roots of *Argyreia speciosa* in mice. The mice were pretreated with different doses of *Argyreia speciosa* extract for 10 days and then, they were subjected to either pentylenetetrazole or maximal electroshock seizures treatment. The hydroalcoholic extract of *Argyreia speciosa* at the dose of 200 and 400 mg/kg significantly delayed the latency to the onset of first clonus and significantly reduced the duration of hind limb extension. This study shows that the hydroalcoholic extract possesses anticonvulsant activity against pentylenetetrazole and maximal electroshock seizures.[33]

- **KarunakarHegde *et al.*, (2009)** studied the anticonvulsant activity of *Carissa carandas* root extract in experimental mice. The ethanolicextract was studied for its anticonvulsant effect on maximal electroshock-induced seizures and pentylenetetrazole-, picrotoxin-, bicuculline- and N-methyl-dl-aspartic acid-induced seizures in mice. The data suggest that the ethanolic root extract reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazole and picrotoxin.[34]

2.12 *Aconitumheterophyllum*

- **S. G. Budhadev *et al.*, (2017)** studied a complete review on Ativisha- *Aconitum heterophyllum.* The phytochemical constituents of *Aconitum heterophyllum* were isolated and characterized with the help of chromatographic separation technique and their structures were explained by using nuclear magnetic resonance techniques. The plant possess ant-inflammatory activity which was evaluated by using cotton pellet granuloma method.[35]

- **DebashishParamanick *et al.*, (2017)** studied the phytochemistry and pharmacognosy as well as the medicinal properties of *Aconitum heterophyllum*. *Aconitum heterophyllum* has been used in some formulations in the traditional healing system of India (Ayurveda). It was reported to have use in treating patients with urinary infection, diarrhea and inflammation. The plant has been also used as an expectorent and for the promotion of hepatoprotective activity.

The chemical studies of plant have revealed that it contain alkaloids, saponins, glycosides, flavonoids etc.[36]

- **Neeraj Sharma *et al*., (2017)** performed an attempt to assemble all the information on *Aconitum heterophyllum* such as botanical, photochemical, pharmacological and toxicological. It possess many pharmacological property such as antioxidant, anti-inflammatory, anti-periodic, expectorant etc. but most species of Aconitum are highly toxic in nature. *Aconitum heterophyllum* is the intoxicating source of phytochemical constituents that are responsible for its pharmacological activities.[37]

- **Rajakrishnan R *et al*., (2016**) performed the standardization of the root tubers of *Aconitum heterophyllum* as per pharmacopoeial testing protocol which include powder microscopy, physic-chemical screening, HPTLC fingerprinting and GC-MS analysis. Preliminary phytochemical test showed the presence of alkaloids, sugars, flavonoids, steroids, quinones and tannins. The GC-MS analysis of the diethyl ether fraction showed the presence of 39 compounds of which 21 were identified.[38]

- **M. Nagarajan*et al*., (2015**) evaluated the pharmacology of *Aconitum heterophyllum* and three other species. The biological properties of Ativisha and Musta are similar according to ayurvedic classification of dravyaguna. This is supported by modern pharmacological studies, which show, both *A. heterophyllum* and *C. rotundus* have antidiarrheal, antipyretic, anti-inflammatory, antihyperlipidemic and hypoglycemic activities. The dravyaguna method of classifying materials (pharmaco-taxonamy), offer a unique way of classifying plant materials.[39]

- **Sadia Khurshid *et al*., (2015)** studied clinical and therapeutic potential of *Aconitum heterophyllum*. The constituents of *Aconitum heterophyllum* such as alkaloids, flavonoids, diterpenoid and nonditerpenoid compounds were isolated and characterized by using chromatographic separation techniques. The study of the structure of these compounds were done by the technique of nuclear magnetic resonance. The anti-inflammatory activity of ethanolic root extract of *Aconitum*

heterophyllum was determined by cotton pellet induced granuloma in rats. The results revealed the activity.[40]

- **Satyendra K Prasad *et al*., (2014)** evaluated anti diarrheal activity of ethanol extract of *Aconitum heterophyllum* at 50, 100 and 200 mg/kg using fecal excretion and castor oil induced diarrheal models. The results depicted a significant reduction in normal fecal output. The study concluded antisecretory and antimotility effect of *Aconitum heterophyllum*, which mediates through nitric oxide pathway.[41]

- **Neelma Munir *et al*., (2014)** studied the antifungal and antioxidant activity of *Aconitum heterophyllum.* The invivo antifungal activity of *Aconitum heterophyllum* were determined by measuring diameters of inhibitory zones of the extract against *Aspergillusniger* and *Alternaliasolani.* The methanolic extract of *Aconitum heterophyllum* showed significant antifungal activity against both the tested organisms. The extract also showed antioxidant activity, measured using a radical scavenging method.[42]

- **Yoirentimameetei *et al*., (2014)** performed the antibacterial activity of the root alkaloid extract of *Aconitum heterophyllum*. This alkaloid extract showed antibacterial activity against S. aureus, B. bronchiseptica, B. subtilis, P. putida and X. campestris. The present study revealed the antibacterial activity of all alkalids from root was due to synergistic effect of different alkaloids.[43]

- **S John Adams *et al*., (2013)** performed a study including the establishment of pharmacognostic and phytochemical characters of *Aconitum heterophyllum* and to compare with its substitutes. They performed histological, phytochemical tests using standard protocols. Based on histochemical analyses, it was revealed the presence of alkaloid, terpenoid-alkaloid complex, lipids and calcium majorly**.**[44]

- **Venu GopalaRao Konda *et al*., (2013)** evaluated the hepatoprotective activity of ethanolic extract of *Aconitum heterophyllum* root in Parecetamol induced hepatic damage in wistar albino rats. The hepatoprotective activity of ethanolic extract of *Aconitum heterophyllum* root was evaluated by the assessment of biochemical

parameters such as SGOT, SGPT, ALP, total bilirubin, serum protein and histopathological studies of the liver. Ethanolic extract of the *Aconitum heterophyllum* root significantly reduced the liver damage and all biochemical parameters.[45]

- **Arunkoorapilly *et al*., (2012)** evaluated the hypolipidemic effect of methanol fraction of *Aconitum heterophyllum* wall. The administration of *Aconitum heterophyllum* was able to reduce serum TG, LDL-C levels. Furthermore, *Aconitum heterophyllum* help to improve lipid HDL-C level. The results shows that the change in lipid profile by *Aconitum heterophyllum* is due to the inhibition of HMGR and the activation of LCAT enzymes. The extract also able to block intestinal fat absorption which helps to reduce cholesterol level. Hence, *Aconitum heterophyllum* methanol fraction exhibits potential hypolipidemic activity.[46]

- **Satyendra K Prasad *et al* (2012)** performed physicochemical standardization and evaluation of in-vitro antioxidant activity of *Aconitum heterophyllum*. The quantitative estimations shows that the root to be highly rich in alkaloids while phenols, tannins, flavonoids and saponins were found in less quantity. The in-vitro antioxidant study showed a moderate to low activity in all models which may be due to low phenolic and flavonoid content.[47]

- **Santhosh Varma etal., (2010)** evaluated the anti- inflammatory activity of *Aconitum heterophyllum* on cotton pellet induced granuloma in rats. The anti-inflammatory activity of ethanolic root extract of *Aconitum heterophyllum* (225, 450 and 900 mg/kg p.o) has reduced inflammation as evidenced by decreased weight of cotton pellet granuloma in rats.[48]

- **M D Ukani*et al*., (1996)** studied pharmacology of *Aconitum heterophyllum* (ativisha) of family Ranunculacea, the plant is an ayurvedic herb which is known for its medicinal properties. The roots of the plant found use in one form/ the other in various ayurvedic preparations. It found to possesss activities like antimalarial, anti-inflammatory, antidiabetic, diuretic etc. The constituents of

Aconitum heterophyllum include atisine, atidine, heteratisine, heterophylline etc.[49]

2.13 PLANT PROFILE

Plant name : *Aconitum heterophyllum*

Family **:** Ranunculaceae

Synonyms **:** Aruna, Ardra, Upavisa, KasayaKrsna, Ghuna Vallabha, Pita vallabha, Prati visa, Bhangura

Vernacular names

- English : Atis root
- Hindi : Atis
- Kannada : Ativisa
- Malayalam : Ativitayam
- Sanskrit : Ativisa
- Tamil : Ativadayam
- Telungu : Ativasa

Natural habitat and distribution

Common in the alpine and sub alpine belts of Himalayan altitudes between 1800 and 4500 km

Morphology

Roots: biennial, paired, tuberous; whitish or grey.

Stem: erect, simple or branched, from 15-20 cm high. glabrous below, finely crispo-pubescent in the upper part.

Leaves: heteromorphous, glabrous: lowest on long petioles (13cm); blade orbicular- cordate or ovate-cordate in outline with a usually narrow sinus (1-1.5 cm deep); usually 5- lobed to the middle, amplexicaul.

Inflorescence: slender raceme or a lax, leafy panicle, crispo-pubescent; Sepals: bluish or violet (rarely whitish); navicular obliquely erect, shortly or obscurely

beaked, 18-20 mm high, 8-9 mm wide. Carpels: 5, elliptic-oblong. Follicles: contagious, linear-oblong, straight, 16-18 mm long.
Seeds: pyramidal, 3-4 mm long, blackish brown.[60]

Chemical constituents

Aconitum heterophyllum contains atidine, atisine, hetisine, heteratisine, Diterpene alkaloids like – heterophylline, heterophyllidine, heterophyllisine, hetidine.

Tuber contains aconitic acid, tannic acid, pectin, ample starch, flat, oleic, palmitic and stearic glycerin mixture, vegetable mucilaginous matter, sucrose and ash 2 percent.

Root – The roots yield 0.79 per cent of total alkoloids. The following alkoloids are reported to have been isolated; Atisenol, Atsine, Heteratisine, Histisine, heterophyllisine, heterophylline, heterophyllidine, – atidine, Hetidine, Banzolheteratisine, F-dihydroatisine and Hetisinone.

Traditional uses

- Atees has been used from centuries to cure various diseases externally and internally as well.
- Externally the crushed leaves and seeds are used to be applied on the throat to treat tonsillitis.
- For Internal uses the juice of Atees roots along with milk is considered as an expectorant. The root powder of this plant is taken orally to cure cervical lymphadenitis.
- The seeds and roots of Atees help in making digestive system strong.
- Seeds are also thought to have diuretic properties which help in alleviating the burning sensation in urinary tract and increase the intensity of urine.(12)

Figure 4: ***Plant Aconitum heterophyllum***

Figure 5: ***Root Aconitum heterophyllum***

3. METHODOLOGY

3.1 PLANT COLLECTION AND AUTHENTICATION

The root powder of the *Aconitum heterophyllum* was collected from Andhra Pradhesh and authenticated from Sri Venkateswara University, Tirupati. The authentication certificate number is No.

3.2 EXTRACTION OF THE PLANT MATERIAL[50]

The extraction is done by using Soxhlet apparatus. The coarse powder of the roots were first extracted with petroleum ether. Obtained defatted material is again extracted with chloroform. After extraction, the chloroform extract were evaporated or concentrated by using rotary evaporator and dried at room temperature to give a viscous mass. The obtained crude extracts were weighed and stored at 4^0C for the further analysis.

3.3 PHARMACOLOGICAL STUDY

3.3.1 ANIMALS AND MANAGEMENT[51]

Healthy adult Wistar albino rats of either sex weighing 180-250g will be selected. The animals will be housed in large, spacious, hygienic cages during the course of experimental period. The animal house will be well maintained and the animals will have 12 ± 1 hour day and night schedule with a temperature [64-79°F] maintained at standard experimental condition. The animals will be fed with standard rodent pellet feed and water *ad libitum*. The animals will be fasted 12 hours prior to the experiment with free access to only water.. The experimental procedure was approved by IAEC (Institutional animal ethical committee of KMCH, governed by CPCSEA, Government of India.

3.3.2 ACUTE TOXICITY STUDY[52]

Rats were kept overnight fasting prior to drug administration. A total of five animals were used which received a single oral dose (2000mg/kg) of chloroform extract of the root of *Aconitum heterophyllum*. After administration of the test extract, food was withheld further 3–4hr. Animals were observed individually at least once during the first

30min after dosing, periodically during the first 24hr (with special attention during the first 4hr) and daily thereafter for a period of 14days. Once daily, cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks. LD50 was done as per OECD guidelines for fixing the dose for biological evaluation .

3.3.3 EVALUATION OF ANTIEPILEPTIC ACTIVITY OF CEAH

A. Maximal electroshock seizure [MES] model[28]

Experimental design

Wistar albino rats weighed around 150-250g were used for the study. Rats were divided into four groups of 5 animals each.

Table-1: Maximal electroshock seizure model

Model I: Maximal electroshock seizure [MES] Model	
Group 1:	*Vehicle control* [Equivalent normal saline i.p]
Group 2:	*Standard* [Diphenylhydantoin 25 mg/Kg BW i.p]
Group 3:	*Aconitum heterophyllum* low dose (75 mg/kg) orally
Group 4:	*Aconitum heterophyllum* high dose (150 mg/kg) orally

Procedure

Animals in the control group [Group 1] will be administered equivalent volume of normal saline by i.p route. Animals in Group 2 will be administered standard drug Diphenylhydantoin. In Groups 3 and 4 *Aconitum heterophyllum* low dose and high dose will be administered by oral route in 1% Sodium lauryl sulphate solution respectively.

After 30 minutes of administration of above drugs, all the rats will be given electroshock with electro convulsiometer through ear electrodes [after moistening the ear of animals with drop of normal saline] at intensity of 150 mA, 60Hz for 0.2 seconds. There after various parameters will be recorded.

B. **Pentylenetetrazol [PTZ] model**[28]

Experimental design

Wistar albino rats weighed around 150-250g were used for the study. Rats were divided into four groups of 5 animals each.

Table-2: Pentylenetetrazole model

Model II: **Pentylenetetrazole Model**	
Group 1:	*Vehicle control* [Equivalent normal saline i.p]
Group 2:	*Standard* Sodium valproate (150 mg/Kg BW i.p)
Group 3:	*Aconitum heterophyllum* low dose (75 mg/kg) orally
Group 4:	*Aconitum heterophyllum* high dose (150 mg/kg) orally

Procedure

Animals in the control group [Group 1] will be administered equivalent volume of normal saline by i.p route . Animals in Group 2 will be administered standard drug Sodium Valproate. . In Groups 3 and 4 *Aconitum heterophyllum* low dose and high dose will be administered by oral route in 1% Sodium lauryl sulphate solution respectively. After 30 minutes of administration of above drugs, all the animals will be given Pentylenetetrazol [PTZ] and the various parameters will be recorded.

3.4 INVIVO ANTIOXIDANT STUDY

Preparation of tissue homogenate:

The animals were sacrificed after treatment by euthanasia. Brain was isolated and washed with normal saline and stored for in-vivo antioxidant studies. The separated brain weighing 0.5 gm were homogenized with motor driven Teflon coated homogenizer with 5 mL of ice-cold 0.1 M Tris-HCl buffer pH 7.4 to get 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min at 5˚C. The supernatant was collected and used for the estimation and in-vivo antioxidant activity.

3.4.1 ESTIMATION OF PROTEINS[53]

Reagents

- Alkaline copper reagent
- Solution A: 2% sodium carbonate in 0.1 N NaOH
- Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate
- Solution C: 50 mL of solution A was mixed with 1 mL of solution B
- Folin's phenol reagent (commercial reagent, 1:2 dilution)
- Bovine serum albumin (BSA)

Principle

This method is a combination of both Folin-ciocalteau and Biuret reaction which involves two steps.

Step: 1

Protein binds with copper in alkaline medium and reduces it to Cu++.

Step: 2

The Cu++ formed catalyses the oxidation reaction of aromatic amino acid by

reducing phosphor molybdotungstate to heteropolymolybdanum, which leads to the formation of blue colour and its absorbance was measured at 640 nm.

Procedure

0.1 mL homogenate was made up to 1 mL with distilled water and to this; 5 mL of alkaline solution was added, mixed well and allowed to stand for 10 min. Then a volume of 0.5 mL Folin's reagent was added, mixed well and incubated at room temperature for another 10 min. The blue color developed was measured at 660 nm against reagent blank. Bovine serum albumin (1 mg/mL) served as the standard and from the standard graph obtained; the amount of protein in the sample was calculated and expressed as mg/100 mg tissue.

3.4.2 ENZYMATIC ANTIOXIDANT ACTIVITY

Antioxidant Enzymes:

An antioxidant is any substance that when present in low concentration compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. The term 'oxidizable substrate' includes every type of molecule found in-vivo. The various antioxidant systems are Superoxide dismutases, Catalases, Glutathione peroxidase family and other peroxidases.

3.4.2.1 ESTIMATION OF REDUCED GLUTATHIONE (GSH) [54]

Principle:

DTNB (5, 5'-dithiobis (2-nitrobenzoic acid)), known as Ellman's Reagent, was developed for the detection of thiol compounds. DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG). Since 2-nitro-5-thiobenzoic acid is a yellow colored product, GSH concentration in a sample solution can be determined by the measurement at 412 nm.

Reagents

- 5% TCA
- 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB)

- 0.2 M Phosphate buffer, pH 8.0

Procedure

To 250 μL of tissue homogenate taken in 2 ml eppendroff tube, 1 mL of 5% TCA was added and the above solution was centrifuged at 3000 g for 10 min at room temperature. To 250 μL of the above supernatant, 1.5 ml of 0.2 M phosphate buffer was added and mixed well. 250 μL of 0.6 mM of Ellman's reagent (DTNB solution) was added to the above mixture and the absorbance was measured at 412 nm within 10 min. A standard graph was plotted using glutathione reduced solution (1 mg/mL) and GSH content present in the tissue homogenates was calculated by interpolation. Amount of glutathione expressed as μg/mg protein.

3.4.2.2 LIPID PEROXIDATION ASSAY[55]

Principle

Thiobarbituric Acid Reactive Substances (TBARS) is a well established assay for screening and monitoring lipid peroxidation. Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as malondialdehyde (MDA) which forms a 1:2 adduct with thiobarbituric acid.

Procedure:

To 100 μL of the tissue homogenate, 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (TBA 0.37%, 0.25 N HCl and 15% TCA) was added and mixed. The above content was incubated in a boiling water bath for 15 min, cooled and centrifuged at 3500 rpm for 10 min at room temperature. The pink colour developed was estimated at 535 nm against a reagent blank, in a spectrophotometer. LPO was expressed as nmol of MDA/mg protein.

3.5 ESTIMATION OF BRAIN NEUROTRANSMITTER

Preparation of brain tissue extracts[56]

All groups were sacrificed, whole brain was dissected and separated the forebrain. Weighed quantity of tissue was homogenized in 5ml HCL- butanol for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1ml) was removed and added to centrifuge tube containing 2.5 ml heptane and 0.31 ml HCL of 0.1 M. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above inorder to separate the 2 phases, and the overlaying organic phase was discarded. The aqueous phase (0.2 ml) was taken for Gama-Aminobutyric acid (GABA), Glutamate (GLU), Serotonin (5-HT), Nor adrenaline (NA) and Dopamine (DA) assay. All steps were carried out at 0ºC.

3.5.1 NOR ADRENALINE AND DOPAMINE ASSAY[57]

To the 0.2 ml of aqueous phase, 0.05 ml 0.4 M HCL and 0.1 ml of sodium acetate buffer (pH 6.9) were added, followed by 0.1 ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.1 ml Na_2SO_3 solution. 0.1 ml Acetic acid is added after 1.5 min. The solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectroflourimeter. The readings were taken at 330-375 nm for dopamine and 395- 485 nm for nor adrenaline.

3.5.2 SEROTONIN AND GLUTAMATE ASSAY[58]

To 0.2 ml aqueous phase, 0.25 ml of OPT reagent was added. The fluorophore was developed by heating to 100ºC for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at for serotonin 360- 470 nm and Glutamate 515 nm in the spectrofluorimeter.

3.5.3 GABA ASSAY[59]

A sample (0.1 ml) of tissue extract was placed in 0.2 ml of 0.14 M Ninhydrin solution in 0.5 M carbonate- bicarbonate buffer (pH 9.95) kept in a waterbath at 60ºC for 30 min, then cooled and treated with 5 ml of copper tartarate reagent (0.16% disodium

carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10 min fluorescence at 377/455 nm in a spectroflourimeter was recorded.

3.6 HISTOLOGICAL ASSESSMENT[61]

Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

Collection of materials

Thin pieces of 3 to 5 mm, thickness were collected from tissues showing gross morbid changes along with normal tissue.

Fixation:

Kept the tissue in fixative for 24-48 hours at room temperature

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein,
b) Prevents autolysis,
c) Preserves the structure of the tissue, and
d) Prevents shrinkage

Common Fixatives: 10% Formalin

Haematoxylin and eosin method of staining:

Deparaffine the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

3.7 STATISTICAL ANALYSIS

The datas of all the parameters were analyzed using the software Graph pad Prism 5. Analysis of variance (ANOVA); one way ANOVA followed by Dunnet's test was performed. The values were expressed as Mean ± SEM.

4. RESULTS

4.1 EXTRACTIVE YIELD OF EXTRACTS OF *Aconitum heterophyllum*

Percentage Yield

The root powder of *Aconitum heterophyllum* were extracted with chloroform using soxhlet apparatus after deffating with petroleum ether. The percentage yield of extract was found to be 10 % w/w.

4.2 ACUTE TOXICITY STUDY

The acute toxicity test was performed by using the chloroform extract at concentrations 2000 mg/kg, 1000 mg/kg, 900 mg/kg, 850 mg/kg, 800 mg/kg and 750 mg/kg. As it is a natural substance and is not expected to be particularly toxic. Hence 2000 mg/kg of the test animal was administered orally. And 3 animals were died. As mortality was observed after administration of 2gm/kg body weight, then a lower dose of 1000 mg/kg and 900 mg/kg was given. And mortality was observed for all animals for both doses. Hence a lower dose of 850 mg/kg was given. Two animals died and one animal survived after administration of 850 mg/kg. Hence a lower dose of 800 mg/kg was given. Two animals survived and one animal died for 800 mg/kg. Then a dose of 750 mg/kg was given. All animals survived for 750 mg/kg. and no signs of toxicity was observed following administration of 750 mg/kg. Hence *Aconitum heterophyllum* was found to be safe at 750 mg/kg.

Table-3: Acute toxicity study of *Aconitum heterophyllum*

No. of animals used	Dose (mg/kg)	No.of animals survived
3	2000	0
3	1000	0
3	900	0
3	850	1
3	800	2
3	750	3

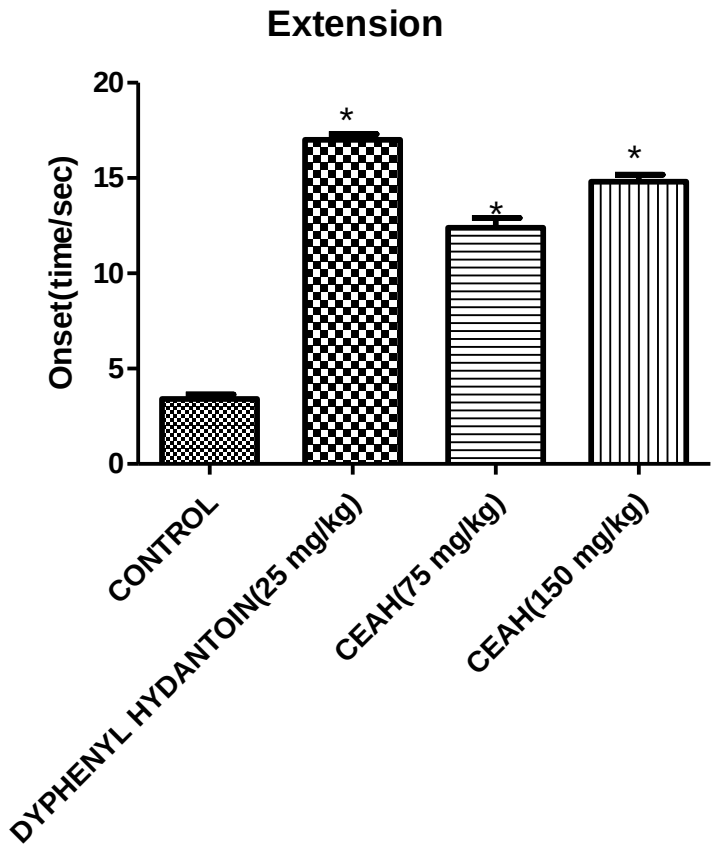
4.3 EVALUATION OF ANTIEPILEPTIC ACTIVITY

Table-4: Effect of Chloroform extract of *Aconitum heterophyllum* on onset of hind limb extension in MES induced seizures models

Groups	Onset time (sec)		Recovery/ Mortality
	Extension	Clonus	
Control	3.40± 0.24	33.20± 1.15	Recovery
Diphenylhydantoin (25mg/kg)	17.00±0.31 *	0***	Recovery
CEAH(75 mg/kg)	12.40± 0.50*	0***	Recovery
CEAH(150mg/kg)	14.80± 0.37*	0***	Recovery

Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (High dose) are compared with control ($p<0.05$-* , $p<0.01^{**}$, $p<0.001^{***}$)

Figure-6: Effect of Chloroform extract of *Aconitum heterophyllum* on onset of hind limb extension in MES induced seizures models



Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (High dose) are compared with control ($p<0.05$-* , $p<0.01^{**}$, $p<0.001^{***}$)

Table-5: Effect of Chloroform extract of *Aconitum heterophyllum* on MES induced seizures models

Groups	Flexion	Extension	Clonus	Stupor	Recovery/ Mortality
Control	5.60±0.4	18.6±0.5	24±1.68	50.40±1.77	Recovery
Diphenylhydantoin (25 mg/kg)	2.20±0.20***	4±1.04***	0***	11.00±0.44***	Recovery
CEAH (75 mg/kg)	3.60±0.24***	6±0.66***	0***	19.40±0.60***	Recovery
CEAH (150 mg/kg)	2.80±0.20***	5.4±1.50***	0***	14.40±0.67***	Recovery

Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (HIGH DOSE) are compared with control ($p<0.05$-*, $p<0.001$**, $p<0.0001$***)

Figure-7: Effect of CEAH on duration of flexion after MES

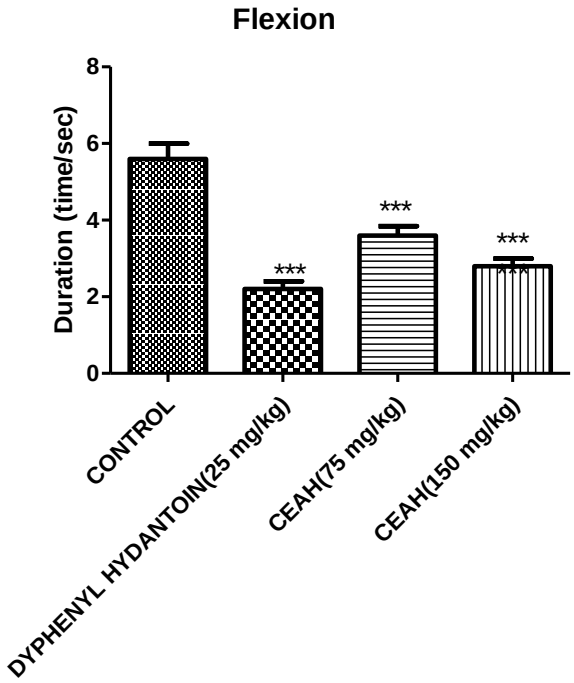


Figure-8: Effect of CEAH on duration of extension after MES

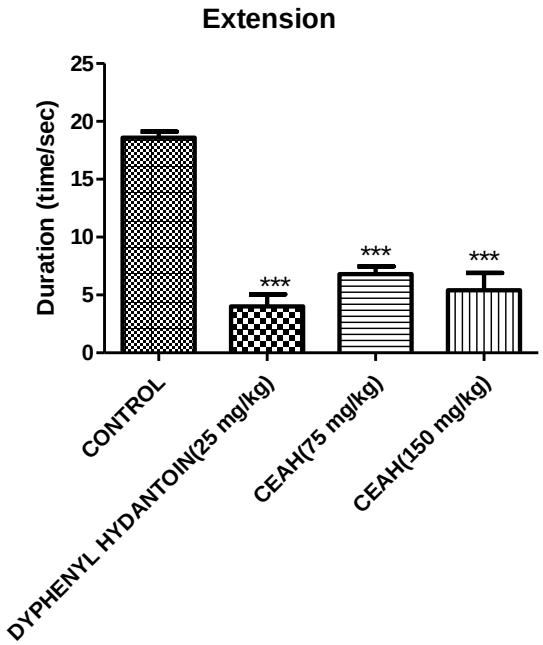
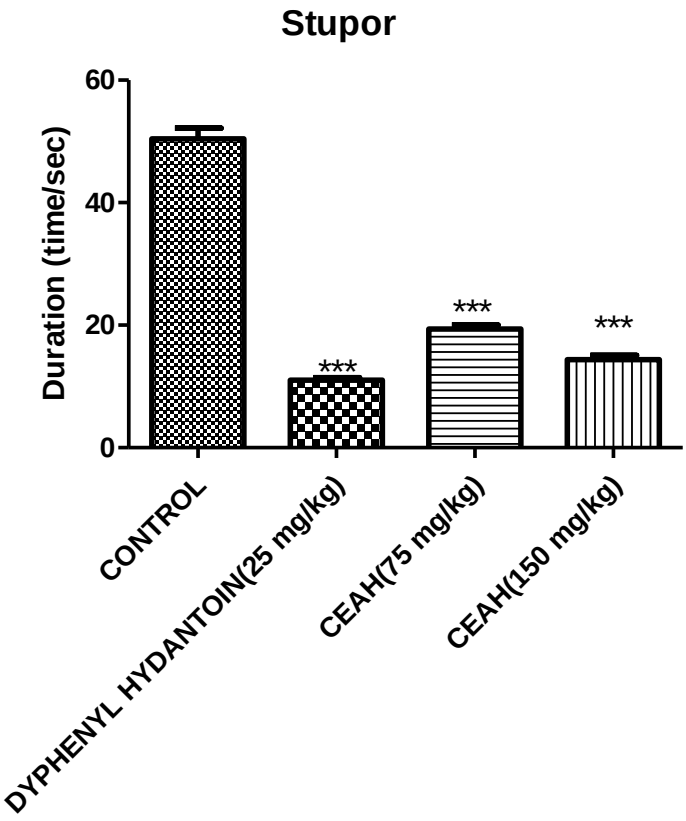


Figure-9: Effect of CEAH on duration of stupor after MES



Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (HIGH DOSE) are compared with control ($p<0.05$-* , $p<0.001^{**}$, $p<0.0001^{***}$)

Table-6: Effect of Chloroform extract of *Aconitum heterophyllum* on PTZ induced seizures models

Groups	**Onset of convulsion (sec)**	**Duration of convulsion (sec)**	**Recover/ Mortality**
Control	107.40 ±1.32	74.00 ±1.41	Mortality
Sodium valproate(150mg/kg)	680.60 ±1.28***	11.20 ±0.37***	Recovery
CEAH (75 mg/kg)	384.40 ±2.29***	27.40 ±0.67***	Mortatility
CEAH (150 mg/kg)	478.80 ±1.35***	45.80 ±0.73***	Recovery

Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (HIGH DOSE) are compared with control ($p<0.05$-*, $p<0.001$**, $p<0.0001$***)

Figure-10: Effect of Chloroform extract of *Aconitum heterophyllum* on onset of convulsion in PTZ induced seizure models

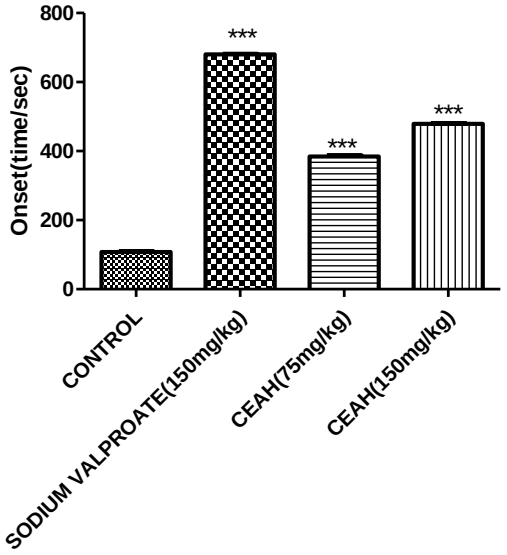
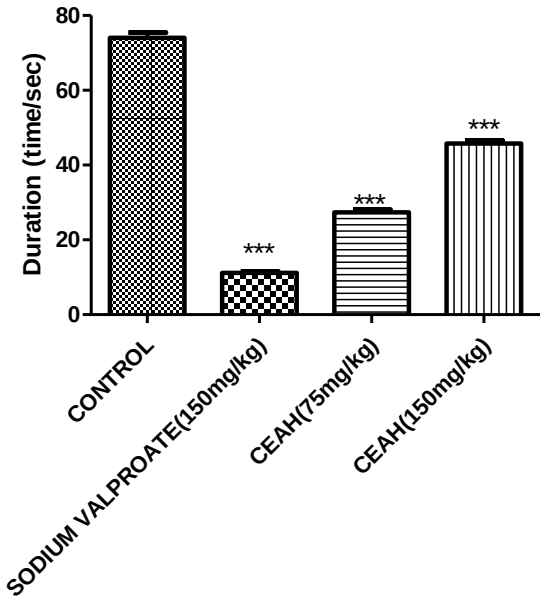


Figure-10: Effect of Chloroform extract of *Aconitum heterophyllum* on duration of convulsion in PTZ induced seizure models



4.4 INVIVO ANTIOXIDANT ACTIVITY

Table-7: Effect of CEAH on brain antioxidant GSH, Total protein, LPO in MES induced seizure models

Groups	Total protein (mg/dl)	GSH(mM/mg of tissue extract)	LPO (nMoles of MDA released/ mg protein)
Control	61.8± 0.80	35.2± 0.37	57.8± 0.42
Diphenylhydantoin (25mg/kg)	13.4 ±0.40***	92.8± 0.33***	14.2 ±0.37***
CEAH (75 mg/kg)	32.80 ±0.86***	73.8± 0.37***	28.8± 0.33***
CEAH (150 mg/kg)	24.0± 0.44***	80.2± 0.80***	23.8± 0.40***

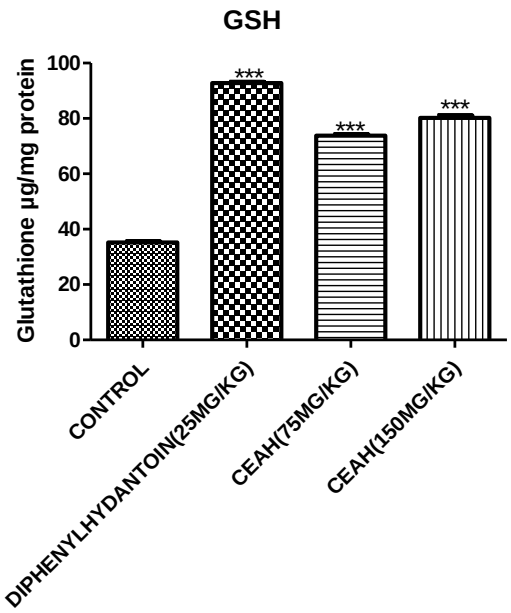
Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (high dose) are compared with control ($p<0.05$-*, $p<0.001$**, $p<0.0001$***)

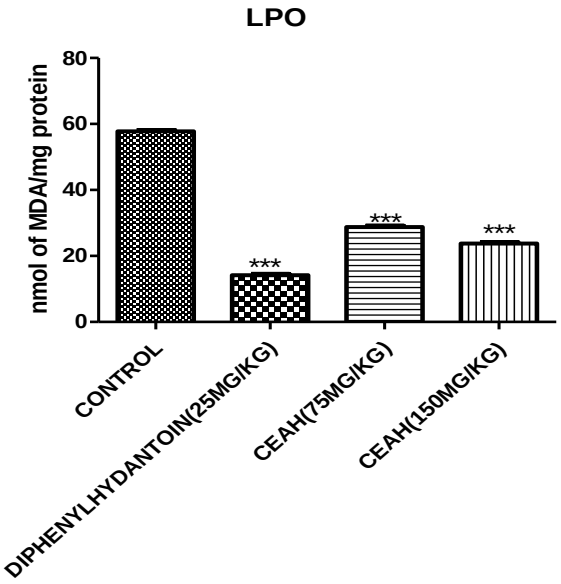
Table-8: Effect of CEAH on brain antioxidant GSH, Total protein, LPO in PTZ induced seizure model

Groups	Total protein (mg/dl)	GSH (mM/ mg of tissue protein)	LPO (nMoles of MDA released/ mg protein)
Control	21.8± 0.33	98.0± 0.40	96.6± 0.40
Sodium Valproate (150 mg/kg)	99.2± 0.37***	42.4± 0.24***	58.8± 0.58***
CEAH (75 mg/kg)	76.0± 0.44***	57.0± 0.31***	53.8± 0.37***
CEAH (150 mg/kg)	86.2± 0.37***	52.0± 0.54***	55.0± 0.31***

Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (HIGH DOSE) are compared with control ($p<0.05$-* , $p<0.001^{**}$, $p<0.0001^{***}$)

Figure-12: Effect of CEAH on brain antioxidant GSH, Total protein, LPO in MES induced seizure models





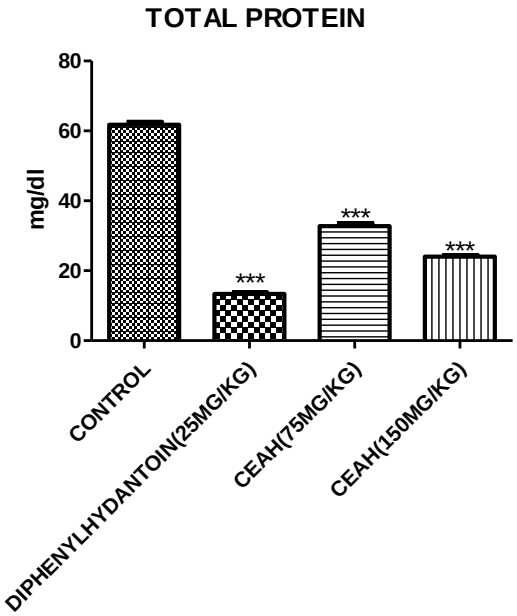
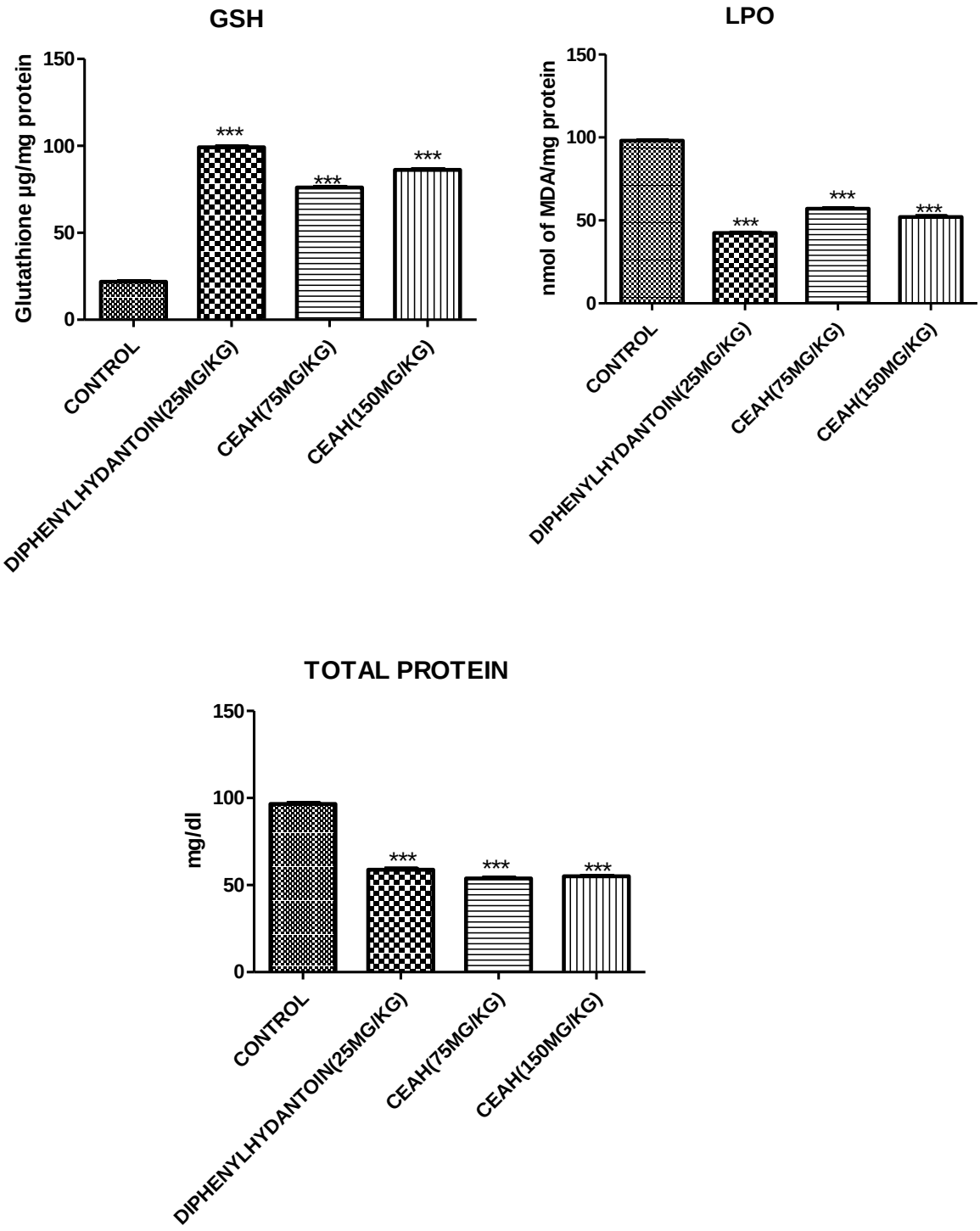


Figure-12: Effect of CEAH on brain antioxidant GSH, Total protein, LPO in PTZ induced seizure model



4.5 EFFECT OF CEAH ON BRAIN NEUROTRANSMITTERS

Table-9: Effect of CEAH on neurotransmitters levels in rat brain after MES induced epilepsy

Groups	Nor adrenaline (µg/g tissue)	Dopamine (µg/g tissue)	Serotonin (µg/g tissue)	GABA (µg/g tissue)
Control	431.60±1.86	444.00±2.21	73.60±1.72	218.00±3.04
Diphenylhydantoin (25mg/kg)	584.20±1.35***	741.20±2.55***	136.80±1.65***	292.80±2.22***
CEAH(75mg/kg)	530.80±2.08***	575.40±2.27***	95.80±1.28***	251.60±2.33***
CEAH(150mg/kg)	545.60±2.58***	645.60±2.06***	114.60±1.77***	271.20±1.88***

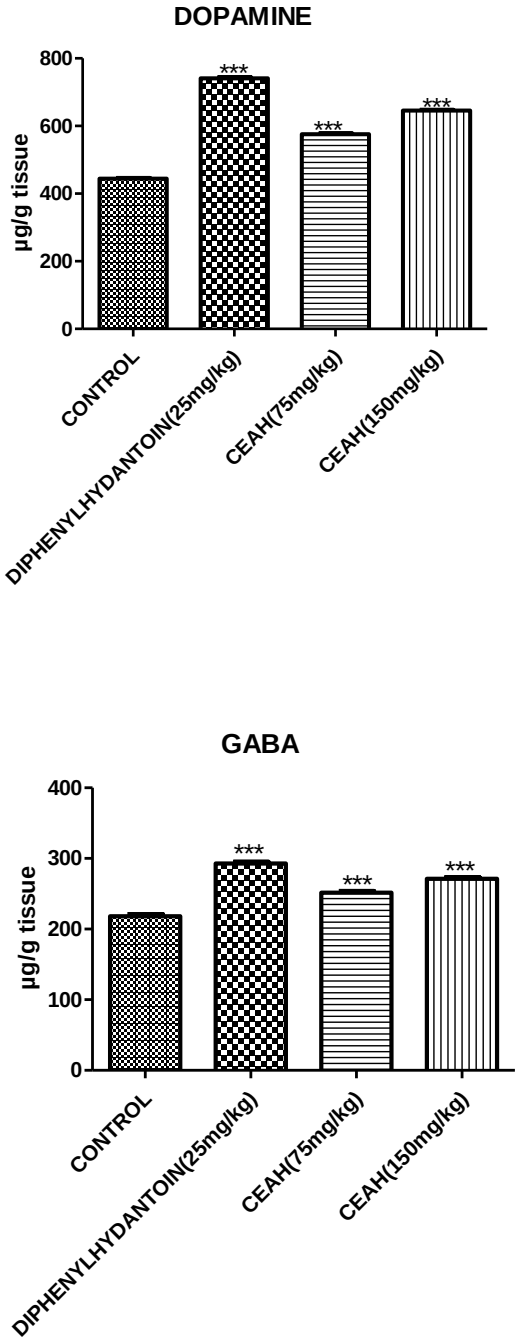
Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (HIGH DOSE) are compared with control ($p<0.05$-*, $p<0.001$**, $p<0.0001$***)

Table-10: Effect of CEAH on neurotransmitters level in rat brain after PTZ induced epilepsy

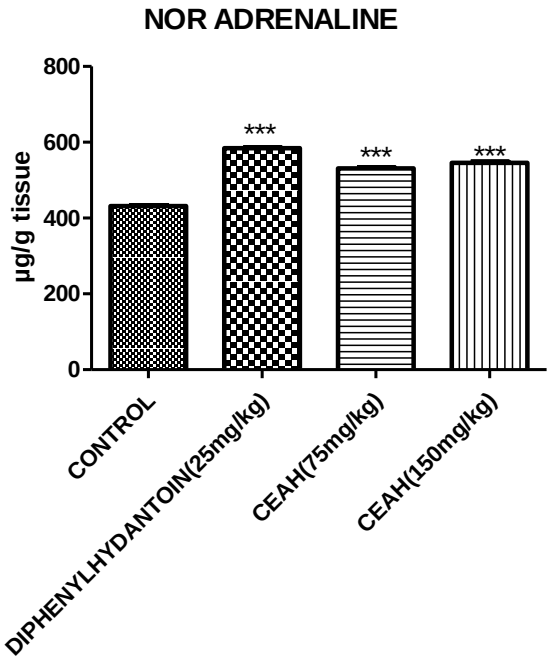
Groups	Nor adrenaline (µg/g tissue)	Dopamine (µg/g tissue)	Serotonin (µg/g tissue)	GABA (µg/g tissue)
Control	524.00±1.51	212.80±2.08	93.00±1.78	205.40±1.07
Diphenylhydantoin (25mg/kg)	790.40±1.63***	292.20±1.85***	134.80±1.98***	293.20±1.82***
CEAH(75mg/kg)	682.80±1.93***	252.80±2.51***	112.20±1.98***	254.00±1.41***
CEAH(150mg/kg)	749.40±1.56***	273.20±1.71***	123.20±1.01***	273.20±1.85***

Statistical comparison: One way ANOVA, followed by Dunett's multiple comparison test. Standard, CEAH (low dose) and CEAH (HIGH DOSE) are compared with control ($p<0.05$-*, $p<0.001$**, $p<0.0001$***)

Figure-14: Effect of CEAH on neurotransmitters levels in rat brain after MES induced epilepsy



Effect of CEAH on neurotransmitters levels in rat brain after MES induced epilepsy



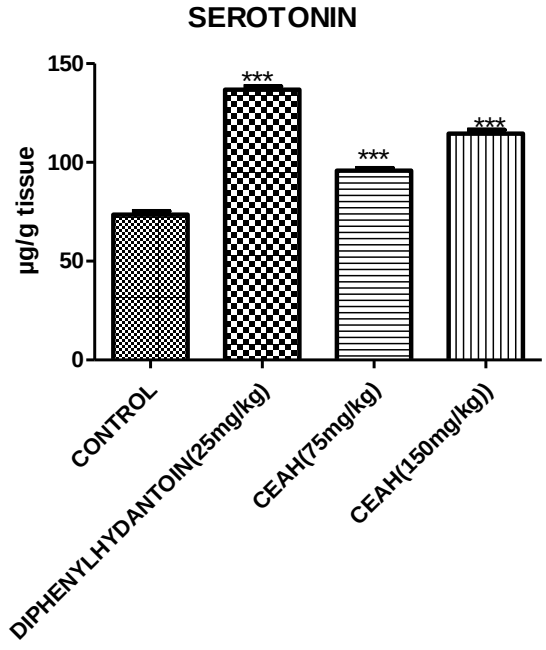
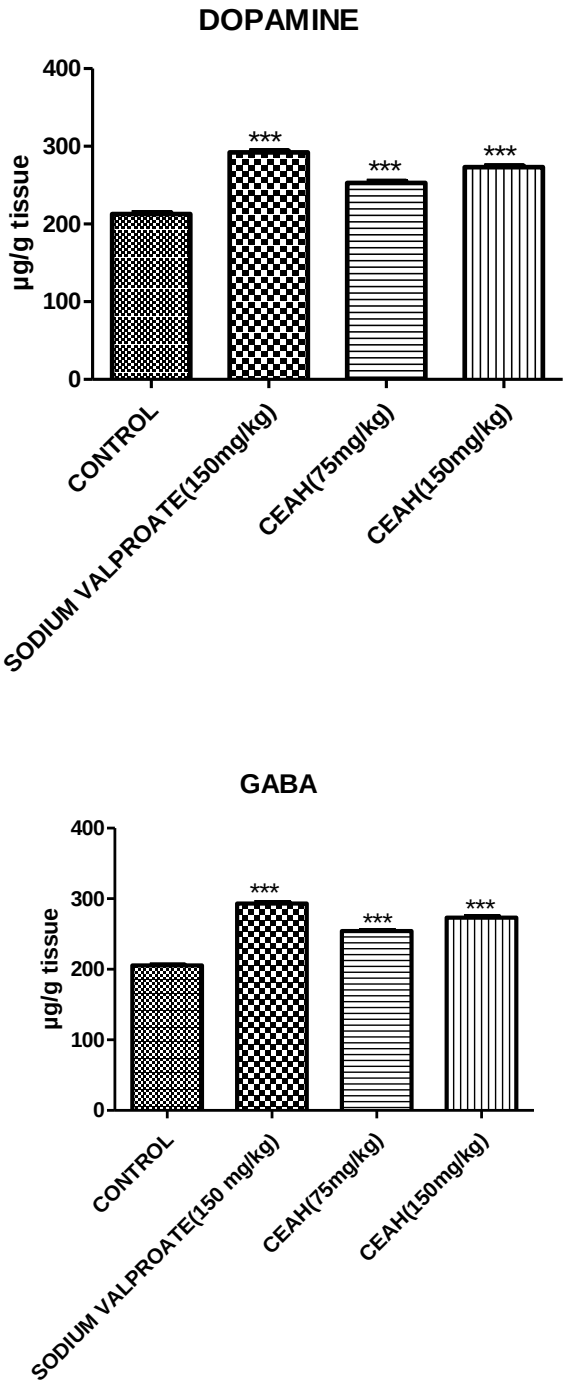
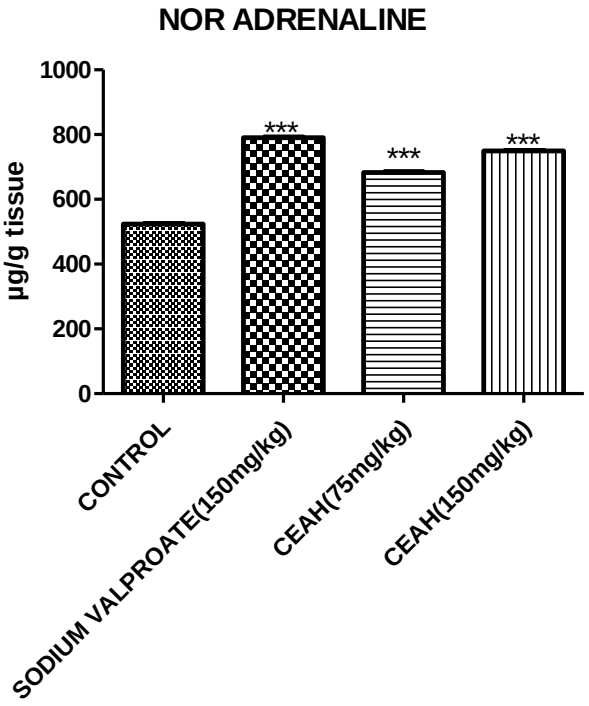
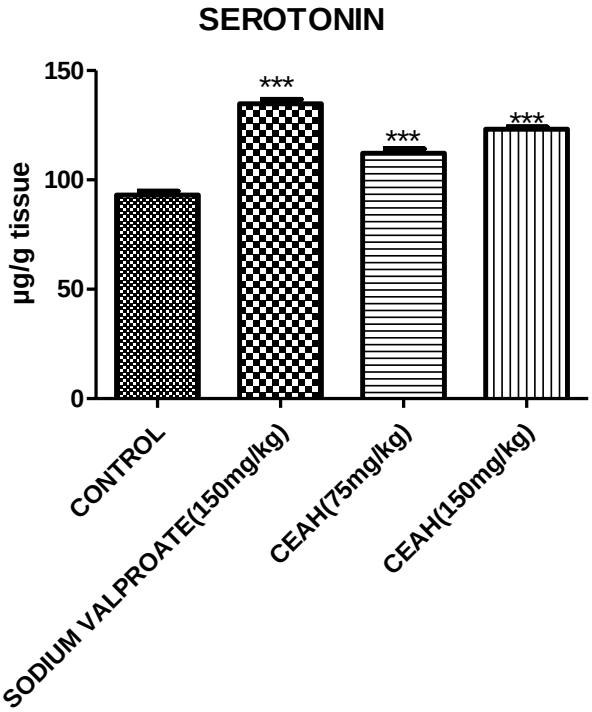


Figure-15: Effect of CEAH on neurotransmitters level in rat brain after PTZ induced epilepsy



Effect of CEAH on neurotransmitters level in rat brain after PTZ induced epilepsy

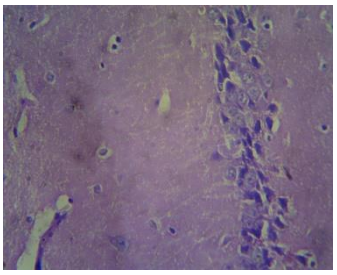




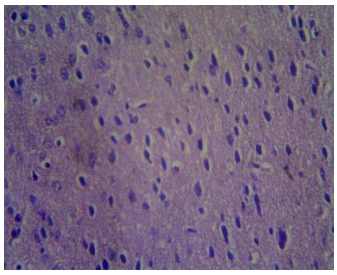
4.6 HISTOPATHOLOGICAL EVALUATION

4.6.1 Histopathological evaluation of MES model

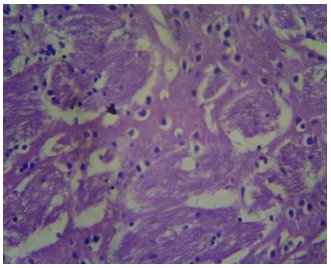
Figure 16: Group 1: ONLY MES TREATED GROUP



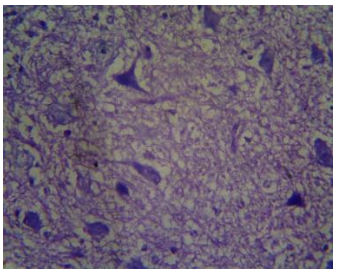
Normal hippocampus



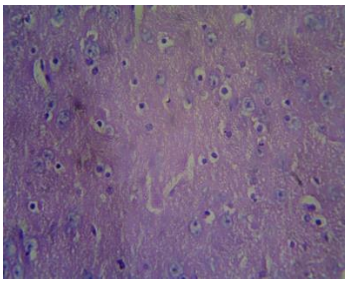
Normal thalamus



Normal corpus striatum



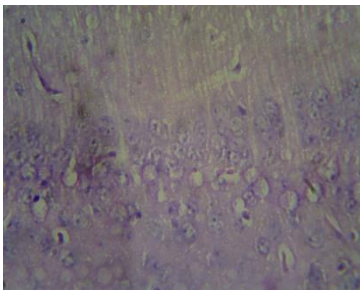
Normal substantia nigra



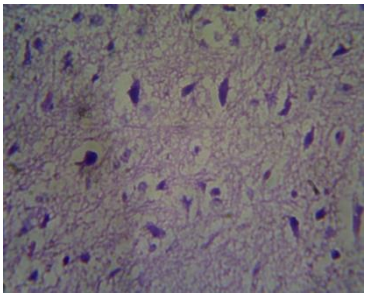
cerebral cortex with neuronal loss

From the results the rat brain shows normal hippocampus (dentate gyrus), thalamus, corpus striatum and substantia nigra and globus pallidus. Cerebral cortex shows neuronal loss.

Figure 17: Group 2: MES + Standard PHENYTOIN TREATED GROUP



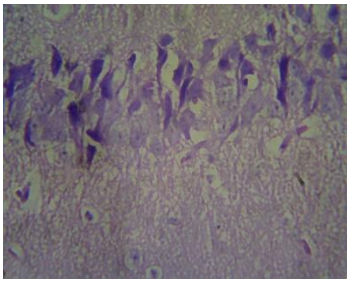
Degenerated hippocampus



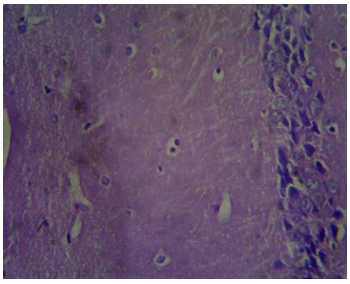
Substantia nigra with edema

From the result rat brain shows hippocampus with degeneration, Substantia nigra shows with edema.

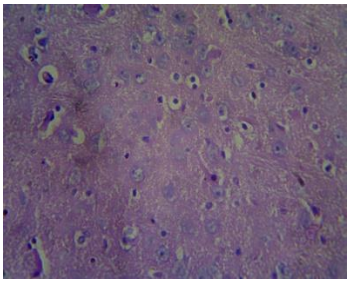
Figure 18: Group 3: MES + CEAH (75 mg/kg) TREATED GROUPS



Hippocampus degeneration



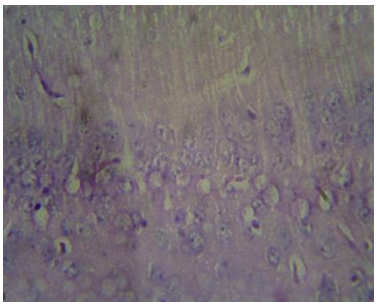
Hippocampus sclerosis



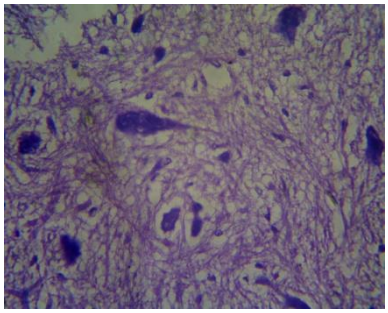
cerebral cortex with edema

From the results the rat brain shows hippocampus showing sclerosis and degeneration, cerebral cortex with edema.

Figure 19: Group 4: MES + CEAH (150 mg/kg) TREATED GROUP



Hippocampus degeneration

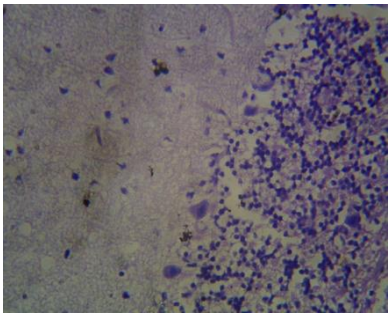


Substantia nigra with edema

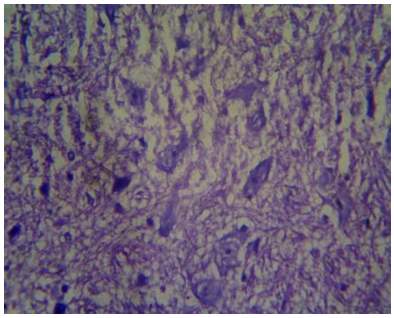
From the result rat brain shows hippocampus showing degeneration. Substantia nigra, shows neuronal loss with sclerosis.

4.6.2. Histopathological evaluation of PTZ model

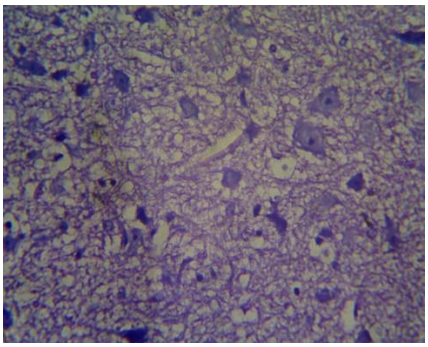
Figure 20: Group 1: ONLY PTZ TREATED GROUP



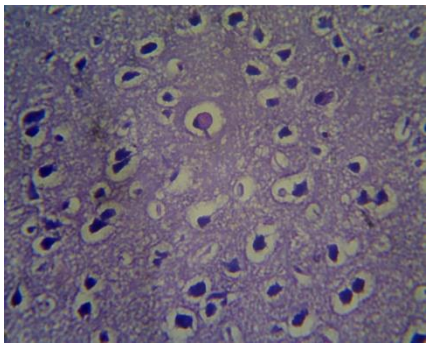
Normal cerebellum with molecular purkinji cell layer



Cerebrum shows neuronal loss



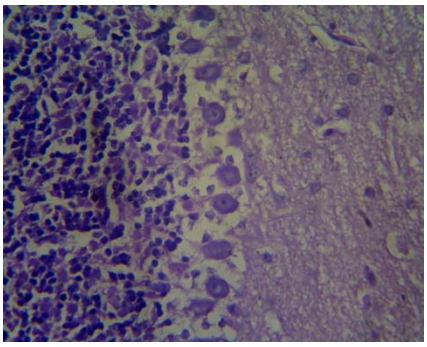
Cerebral cortex shows edema



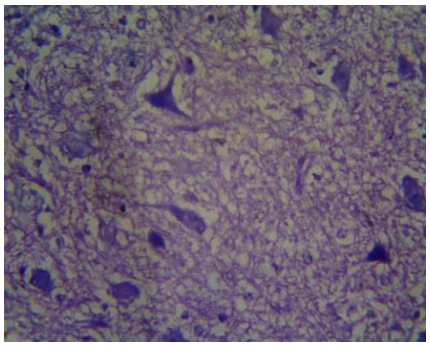
Cerebral cortex shows piknosis

From the result rat brain shows normal cerebellum with molecular purkinji layer. The cerebrum shows neuronal loss. Cerebral cortex shows edema and piknosis.

Figure 21: Group 2: PTZ + SODIUM VALPROATE TREATED GROUP



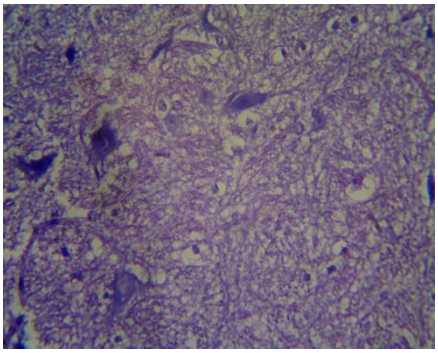
Cerebellum shows normal purkinji cells with molecular layer



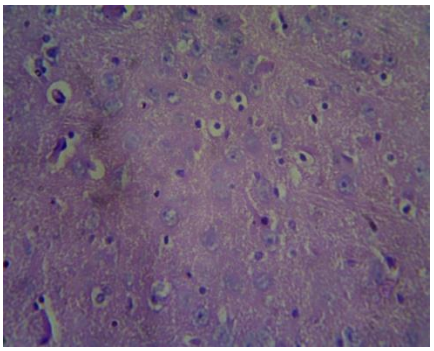
Substantia nigra with edema

From the result rat brain shows normal cerebellum shows normal purkinjiec cells with molecular layer and white matter. The substantia nigra shows neuronal loss and edema.

Figure 22: Group 3: PTZ + CEAH (75 mg/kg) TREATED GROUP



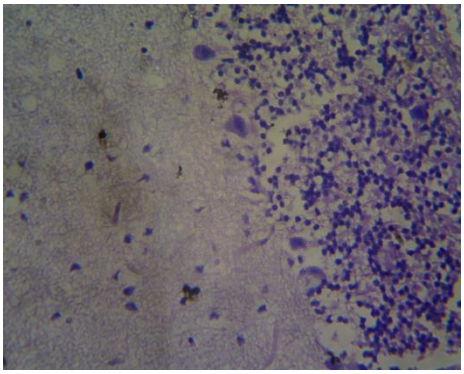
Cerebellum with normal purkinji cells and molecular layer



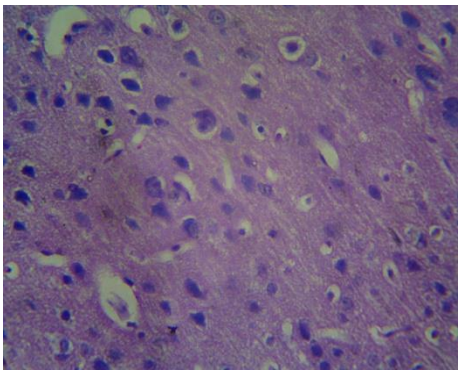
Normal cerebral cortex

From the result rat brain shows cerebellum with normal purkinjiec cells and molecular layer and white matter shows atrophy and gliosis. The cerebral cortex shows normal.

Figure 23: PTZ + CEAH (150 mg/kg) TREATED GROUPS



Normal cerebellum shows purkinji cells with molecular layer



Cerebral cortex shows scelorosis

From the result rat brain shows normal cerebellum shows purkinji cells with molecular layer and white matter shows sclerosis and gliosis. The cerebral cortex shows sclerosis.

5. DISCUSSION

Epilepsy is a chronic disorder of the brain that affect people worldwide. Nearly about 50-80% of the patients with epilepsy are controlled with currently available antiepileptic drugs. But these drugs cannot able to control seizures effectively in about 10-20% of the patients. The treatment of epilepsy still remains inadequate even though new anticonvulsants are being developed. Furthermore the current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose related and chronic toxicity as well as teratogenic effects.

Traditional systems of medicines are popular in developing countries and upto 80% of the population relies on traditional medicines/ folk remedies for their primary health care need. Hence, there is a need to discover an alternative agent from natural sources.[3]

Aconitum heterophyllum used as a herbal medicine and is well known for its traditional uses such as expectorants, diuretics, laxative etc. Various studies shows that the active principle diterpene alkaloids having a crucial role in treatment of epilepsy. *Aconitum heterophyllum* is rich in diterpene alkaloids.[60] Since *Aconitum heterophyllum* have not been studied for its antiepileptic activity, the present study was aimed to evaluate the antiepileptic activity of chloroform extract of *Aconitum heterophyllum*

The maximal electroshock induced convulsion in animals represents grand mal type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure.[19] The result of the present study shows that the chloroform extract of *Aconitum heterophyllum* at doses 75 and 150 mg/kg significantly delayed the onset of HTLE and reduced the duration of HTLE. And also both doses completely abolished the phase of convulsion in MES induced convulsion models.

In case of PTZ induced convulsion, the result of the present study shows that the chloroform extract of *Aconitum heterophyllum* , at doses 75 and 150 mg/kg significantly reduced the duration and also delayed the onset of convulsion when compared to control group. PTZ may be exerting convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors. The results revealed that the CEAH possess anticonvulsant activity.

Oxidative stress was described as an imbalance between generation and elimination of reactive oxygen species and reactive nitrogen species. The brain is

particularly susceptible to oxidative stress because it utilizes the highest amount of oxygen than other body organs. It has been postulated that lipid peroxidation may be casually associated with certain types of epilepsy. A decrease in free radical scavenging activity may lead to an increased risk of seizure recurrence.[61] The effect of CEAH on oxidative stress in MES and PTZ induced convulsion was evaluated. CEAH at doses 75 and 150 mg/kg dose showed significant decrease in LPO level.

Glutathione reductase is an important free radical scavenging compound that prevents membrane lipid peroxidation. The decreased level of reduced glutathione in control group seen in the present study indicates that there was an increased generation of free radicals and that the reduced glutathione was depleted during process of combating oxidative stress.[61] CEAH at doses 75 and 150 mg/kg dose showed significant increase in the GSH levels in brain tissue. The decrease in lipid peroxidation level and increase in the glutathione level in PTZ and MES induced convulsion models indicates that CEAH exhibit good antioxidant activity.

Epilepsy may develop because of an imbalance of nerve signaling chemicals called neurotransmitters. In case of epilepsy, there may be abnormally high level of excitatory neurotransmitters(glutamate) that increase neuronal activity, while abnormally low level of inhibitory neurotransmitters(GABA) that increase neuronal activity in the brain. Hence, GABA hypoactivity and glutamate hyperactivity can enhance an epileptic seizure. In epileptic foci, GABA hypoactivity, which reduces the activity of dopaminergic neurons through a presynaptic effect through $GABA_A$ receptors. At low doses, NA can enhance an epileptic seizures, whereas at high doses, it has a protective effect on seizures. Glutamate hyperactivity is exerted through presynaptic N- methyl- D-aspartate receptors, which strongly inhibit serotoninergic neurons and through post synaptic ionotropic glutaminergic receptors, which can induce epileptic seizures.[15] The result of the present study shows that CEAH significantly increased the level of inhibitory neurotransmitter GABA and also showed significant increase in the levels of DA, NA and 5-HT when compared to control group. The histopathological study shows alteration in neuronal activity in only MES and PTZ treated groups compared to standard, CEAH low dose and high dose. Hence, the result indicates that CEAH have good anticonvulsant activity.

6. CONCLUSION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. Approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of antiepileptic drugs with novel structures and better safety and efficacy profiles.[34]

The chloroform extract of *Aconitum heterophyllum* delayed the onset and reduced the duration of convulsion in MES and PTZ induced convulsion models and can be used as an adjuvant therapy against cognitive deficit in convulsions. The extract also shows significant decrease in lipid peroxidation level and increase in reduced glutathione level, indicates that CEAH possess good antioxidant activity. Also CEAH significantly increased the level of inhibitory neurotransmitter GABA and also showed increase in DA, NA and 5-HT levels. Hence it can be concluded that the CEAH possesses good anticonvulsant activity. Further studies are needed to explore the mechanism as well as the active principle responsible for the anticonvulsant activity of *Aconitum heterophyllum.*

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