A STUDY ON ANTICONVULSANT EFFECT OF AQUEOUS EXTRACT OF CISSUS QUADRANGULARIS

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CERTIFICATE

This is to certify that this dissertation entitled ***"A STUDY ON ANTICONVULSANT EFFECT OF AQUEOUS EXTRACT OF CISSUS QUADRANGULARIS"*** is a bonafide record of work done by **Dr.K.GEETHA,** under my guidance and supervision in the Institute of Pharmacology, Madurai Medical College, Madurai, during the period of her post graduate study for M.D. Pharmacology from 2005 -2008.

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DECLARATION

I **Dr. K. GEETHA** solemnly declare that the dissertation entitled ***"A STUDY ON ANTICONVULSANT EFFECT OF AQUEOUS EXTRACT OF CISSUS QUADRANGULARIS"*** has been prepared by me under the able guidance and supervision of my guide **Dr. R. MEHER ALI, M.D.,** Former Director and Professor of Pharmacology, Institute of Pharmacology, Madurai Medical College, Madurai, in partial fulfillment of the regulation for the award of M.D. (PHARMACOLOGY) degree examination of The Tamilnadu Dr.M.G.R. Medical University, Chennai to be held in March 2008.

This work has not formed the basis for the award of any other degree or diploma to me previously from any other university.

Place : Madurai

Date :

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CONTENTS

ABBREVIATIONS

CNS	-	Central Nervous System
GABA	-	Gaba Amino Butyric Acid
$ABCB_1$	-	ATP Binding Casette B_1
NMDA	-	N Methyl D Aspartate
ILAE	-	International League Against Epilepsy
EEG	-	Electro Encephalogram
AED	-	Anti Epileptic Drugs
VDRL	-	Venereal disease Research Laboratory
ELISA	-	Enzyme Linked Immunosorbant assay
GTCS	-	Generalised tonic Clonic Seizures.
CYP	-	Cytochrome P 450
PTZ	-	Pentylenetetrazole
MES	-	Maximal Electro Shock
SE	-	Status Epilepticus
CHS	-	Continuous Hippocampal Stimulation

INTRODUCTION

Software can never replace greyware

- Anonymous

A Seizure (Latin – to take possession of) is a paroxysmal event due to abnormal, excessive, high frequency, hypersynchronous action potential discharges from an aggregate central nervous system neurons while epilepsy is the tendency to have recurrent seizures[1]. 5-10% of the population will have at least one seizure with the highest incidence occurring at early childhood and late adult hood.

Many people with epilepsy feel stigmatized by the society and become isolated unnecessarily[2]. The causes of seizure and epilepsy could be a normal brain capable of having a seizure under the appropriate circumstances and there are differences between individuals in the susceptibility or threshold for seizures.

The conditions that may commonly result in chronic seizure disorder are

- Severe penetrating head trauma
- Stroke
- Infections
- Tumours

- Abnormalities in CNS development

Seizures are made episodic by precipitating or provocating factors, some of them being intrinsic physiological processes like psychological or physical stress, sleep deprivation, hormonal changes, extrinsic factors like exposure to toxic substances and certain medication. These emphasize that many causes of seizures and epilepsy results from a dynamic interplay between endogenous, epileptogenic and precipitating factors.

Epileptogenesis refers to the transformation of a normal neuronal network into one that is chronically hyperexcitable[1]. In many genetic and idiopathic forms of epilepsy, epileptogenesis is presumably determined by developmentally regulated events. The mechanism of initiation and propagation of most generalized seizures remain rudimentary and reflects the limited understanding of the connectivity of the brain at a system level.

The treatment of epilepsy can be considered in four parts

- Use of antiepileptic drugs
- Removal of causative / precipitating factors
- Surgical excision of epileptic foci and other surgical measures
- Regulation of physical / mental activity.

Drug treatment should be considered after more than one seizure has occurred. Of patients whose epilepsy in controllable only a single drug is necessary in 80%.

Antiepileptic drugs appear to act primarily by blocking the initiation and spread of seizures. This occurs through a variety of mechanisms that modify the activity of ion channels or neurotransmitters and in most cases the drugs have pleiotropic effects. The mechanism includes inhibition of Na^+ dependent action potentials in a frequency dependent manner.

Compounds are sought, so that they act by one of the three mechanisms.

- Enhancement of GABAergic inhibitory transmission
- Diminution of excitatory usually glutamatergic transmission
- Modification of ionic conductance[3].

The most important recent progress in epilepsy research has been the identification of genetic mutation. It appears that many of the inherited, idiopathic epilepsies are due to mutations affecting ion channel function. These syndromes are therefore part of the large group of channelopathies causing paroxysmal disorders such as cardiac arrhythmias, episodic ataxia, periodic weakness and familial hemiplegic migraine[4].

Significance of Medicinal Plants:

Somewhere in the plant kingdom there is a remedy for everything

- Norman Fransworth

Even with the advent of so many antiepileptic drugs because of the commonly appearing adverse effects, the hunt for a safer antiepileptic agent is

going on. Existing antiseizure drugs provide adequate seizure control in about two thirds of patients[3]. A fraction of the epileptic population is resistant to all available drugs and this may be due to increased expression of multidrug transporter P glycoprotein. 170, a product of the $ABCB_1$ gene.

And even when epilepsy is under control special issues like adverse effects, management of epilepsy during pregnancy because of the teratogenecity, a continuous search for a relatively safe remedy is always there.

The medicinal plants continue to play a significant role in modern medicine due to their inherent, distinct chemical and biological properties. In nature a plant is able to synthesize complex molecules from simple ones through highly specific reaction mechanisms.

Herbal medicines are widely used in epilepsy in many parts of the world. About 150 plants are said to be recorded in traditional medicine and 10 warrant further investigation. The Chinese mixture Saiko-Keishi-To which is made up of 9 plants has produced reduction in seizure frequency, severity or even freedom form seizure[5].

"Let your food be your medicine" said Hippocrates the Father of medicine who compiled a list of 400 herbs and their uses[6]. While modern or allopathic medicine is barely a century old, the practice of natural medicine

using nature as a pharmacy can be traced back through all of the ancient civilization. And with the advent of biotechnology, the unfolding of human genome and the various tools of bio-informatics new frontiers in the treatment of diseases like personalized medicine will open up. So one such attempt has been made here to evaluate the antiepileptic effect of aqueous extract of Cissus quadrangularis and the efficacy has been compared with the standard anti epileptic drug – diphenyl hydantoin.

AIM & OBJECTIVE

To evaluate the anticonvulsant effect of aqueous extract of Cissus quadrangularis in MES induced seizures in adult male albino rats in comparison with diphenyl hydantoin.

REVIEW OF LITERATURE

The word “Epilepsy” derived from Greek verb epilamvanein which means “to be seized”. It is a broad category of symptom complexes arising from a number of disordered brain function that themselves may be secondary to a variety of pathological processes[7].

A generalized convulsion is the most readily diagnosed epileptic phenomenon. Classically there is a cry, stiffening of body and limbs, followed by rhythmic jerking of all four limbs, associated with loss of awareness, eyes staring blankly, tongue biting and urinary incontinence. The generalized convulsive movements usually last for a minute or so and as the attack proceeds, the jerking slows in frequency and increases in amplitude. This is followed by a postictal stage or depression, from which the patient recovers in a few minutes.

History

Epilepsy is as old as mankind, described about 3000 years ago in Mesopotamia and was attributed to the God of moon. Hippocrates (460B.C) was the first person who attributed epilepsy to brain having an abnormal consistency caused by superfluity of phlegm, contradicting the then belief-epilepsy was a sacred disease caused by Greek gods. About 1000 yrs later, Paracelsus a swiss physician cum alchemist treated epilepsy with a mixture of

piety, alchemy, magic, astrology and accelerated the use of chemicals into treatment[5].

At the turn of 17th century William Gilbert a contemporary of Shakespeare and Queen Elizabeth and one of the Queen's physician accelerated a change in approach from mystical and supernatural to scientific, particularly of magnetic and electrical phenomena.

In 1791 Luigi Galvani found the field of electrophysiology, which opened the gate towards understanding epilepsy. In 1872, Richard Caton hypothesized local motor responses followed electrical stimulation of various cortical areas as well as in a reverse direction i.e peripheral stimulation might evoke local electrical responses in the brain.

In the period between 1849 & at the turn of the century, with close interaction among physician – scientists and clinicians, following the lead of Email Du Bois-Reymond that "No other force than the common physical-chemical ones, are active within the organism", there was a melding of ideas and discoveries at an unusual rate. The clinical giants in the study during that era were the neurologists J. Hughlings Jackson and William Gowers and the neurosurgeon David Ferrier. Jackson localized the seizures to areas of brain by their clinical manifestations with confirmation by autopsy.

Epidemiology[5]

Epilepsy is a common condition with an incidence of 80 cases per 1,00,000 persons per year. The prevalence is higher in underdeveloped countries perhaps due to poor perinatal care, standards of nutrition, public hygiene and the greater risk of brain injury, cerebral infection or other symptomatic cerebral condition. It is also slightly high in low social economic classes. The most striking feature than any difference in frequency is the fact that it occurs in all parts of the world and can affect all strata in a population. Males are slightly more affected and the highest incidence occurs in the first year of life and second peak in later life - > 50yrs. Standardized mortality rate is also 2-3 times higher in patients with epilepsy attributable to

- Underlying cause of epilepsy
- Higher rates of accidents
- Sudden unexpected death in epilepsy (SUDEP)
- Suicides

Epilepsy:

Epilepsy is a group of neurological conditions the common and fundamental characteristics of which is recurrent, usually unprovoked epileptic seizures.

Epileptic seizures result from excessive, synchronous abnormal firing patterns of neurons that are located predominantly in the cerebral cortex[7,8].

The human brain is the most complicated biological machine on this planet

- George Chencher

Viewing seizures only as the result of too much excitation or too little inhibition will be major oversimplification. Two essential physiologic elements interact here being,

a) Abnormal cellular excitability neuronal deregulation and
b) Network defect.

Accumulated knowledge from a number of animal models of epilepsy and seizures have evidenced that the fundamental defect in most seizures is the paroxysmal depolarization shift and an associated high frequency burst firing of neurons.

Molecular and cellular factors

A number of factors control neuronal excitability, which include voltage gated ion channels, neurotransmitter activated ion channels, neuromodulators and second messenger systems. Ligand gated ion channels are responsible for communication between cells, while voltage gated channels determine how inhibitory and excitatory influences are integrated in a way that determines the

propagation of impulses to other neurons. The major voltage dependent channels are that of Na^+, Ca^{++} and K^+. The major inhibitory neurotransmitter being GABA. Binding of GABA leads to opening of the chloride ion channel & resultant hyperpolarisation[9].

The major excitatory neurotransmitters are the amino acids L glutamate and L aspartate. Binding of these to AMPA (α Amino 3 hydroxy 5 methyl isoxazole propionic acid) receptors make ion channels permeable to Na^+ & K^+. The other receptors involved are kainate receptors and NMDA (N methyl D aspartate) receptors.

Epileptic activity in Neuronal system

With the help of many acute and chronic animal models of epilepsy, where acute models are used for screening of antiepileptic agents, chronic models are used for availing information about the basic mechanism of epilepsy[9].

Focal

A number of animal models of focal epilepsy are studied with topical application of aluminum hydroxide, systemic use of tetanus toxin, kainic acid, pilocarpine. These models produce acute seizures followed often after a latent period by the development of a chronic epilepsy and behaviour like that of complex partial seizures of human.

Generalised

The animal models used are maximal electroshock, chemical induced convulsions, using- rat and mice. These models produce sudden bilateral synchronous seizures and in case of simple absence and myoclonus, non evolving pattern of electrical activity which clearly suggests a generalized disturbance of neuronal activity in both hemispheres.

In a nutshell epileptogenesis could be due to either one of the following

- Diminution of GABA ergic inhibitory transmission
- Enhancement of excitatory usually glutaminergic transmission
- Modification of ionic conductances.

Aetiology[1]

There could be a number of aetiologies for epilepsy the main being

- Idiopathic
- Genetic
- Congenital malformations
- Epilepsy syndromes of childhood
- Infection
- Trauma
- Vascular
- Neoplasm

- Hippocampal sclerosis
- Degenerative disorders
- Unknown – cryptogenic

Genetics

The identification of a chromosomal locus for some epilepsy syndromes and the elucidation of the responsible genes has recently received considerable attention. The review of biochemical and structural basis of syndromes due to single gene disorder can provide additional understanding of the mechanism of epileptogenesis. An integrated effort by epidemiologists, molecular and population geneticists, clinical and basic neurophysiologists, pathologists is clearly needed to acquire an understanding of specific genetic actions[10].

Though epilepsy may be caused by a number of aetiologies the idiopathic form occupies 10-30%. The other causative factors differ according to age group –

Neonatal & infancy	-	Congenital maldevelopment, birth injury, anoxia, metabolic disorders.
Early Child hood	-	Infantile spasm, febrile convulsion, birth injury, anoxia, accidental drug poisoning.
Childhood	-	Perinatal anoxia, cortical malformations, Lennox – Gastaut syndrome

Adolescence	-	Genetically transmitted types, Juvenile myoclonic epilepsy, trauma, drugs
Early adulthood, and middle age	-	Trauma, neoplasm, withdrawal from alcohol / sedatives, vascular disease
Late life	-	Vascular disease, tumour, trauma, degenerative disease

Drug induced epilepsy could be due to[10]

Antimicrobials	-	β lactam & related compounds
		Quinolones
		Acyclovir
		Isoniazid
Anaesthetics & analgesics	–	Meperidine
		Tramadol
Immuno modulatory drugs	–	Cyclosporine
		Interferons
Psychotropics	-	Lithium
		Anti psychotics

Theophylline
Radiographic contrast agents
Withdrawal of alcohol / sedative – hypnotics
Drug abuse - amphetamine, cocaine
Flumazenil

Classification of seizures[10]

According to International League Against Epilepsy (ILAE) 1981 the criteria used for classification are

a. Seizure phenomenology.

b. EEG findings

I. Partial (Focal, Local) Seizures

A. Simple partial seizures

With motor signs

With somatosensory or special sensory symptoms

With autonomic symptoms and signs

With psychic symptoms

B. Complex partial seizures

Simple partial onset followed by impairment of consciousness

With impairment of conscious at onset

C. Partial Seizures evolving to secondarily generalized

Seizures (Tonic, clonic, Tonic or Clonic)

Simple partial seizures evolving to generalised seizures

Complex partial seizures evolving to generalised seizures

Simple partial evolving to complex partial then to generalized seizures.

II. Generalized seizures (Convulsive and Non convulsive)

A. Absence seizures

Absence seizures

Atypical absence seizures

B. Myoclonic seizures

C. Clonic seizures

D. Tonic seizures

E. Tonic clonic seizures

F. Atonic seizures (Astatic seizures)

III. Unclassified epileptic seizures

The problems that are faced in the above classification are

- Difficulty in dividing simple and complex partial seizures on basis of impaired consciousness.
- Large number of seizures which cross the boundaries of clinical classification especially those modified by treatment
- Difficulty in defining focality

Among the above different classifications, incidence of partial seizures predominates. According to aetiology, idiopathic form is the predominant one. The generalised tonic clonic (grand mal epilepsy) is the main seizure type in 10% of all persons with epilepsy.

Pathogenesis

Partial seizure activity may begin in a very discrete region of cortex and then spread to neighbouring regions i.e., seizure initiation phase and a seizure propagation phase. The bursting activity is caused by a relatively long lasting depolarization of the neuronal membrane due to influx of extracellular calcium which leads to opening of the voltage dependent sodium channels, influx of Na^+ & generation of repetitive action potentials. This is followed by a hyperpolarisation after potential, mediated by GABA receptors or potassium channels depending on the cell type. Many factors control neuronal excitability and there are many potential mechanisms for altering a neuron's propensity to have bursting activity[11].

Intrinsic mechanisms

Changes in

(a) Conductance of ion channels

(b) Response characteristics of membrane receptors

(c) Cytoplasmic buffering,

(d)Second messenger systems,

(e)Proteins expressing - Gene transcription

- Translation

- Post translation modification[1]

Extrinsic mechanism are

a) Changes in amount or type of neurotransmitter present in the synapse

b) Modulation of receptors by extra cellular ions /other molecules

c) Temporal, spatial properties of synaptic and nonsynaptic input.

The mechanism responsible for initiation and propagation of most generalised seizures remain rudimentary due to the limited understanding of the connectivity of the brain at a system level.

Epileptogenesis generally refers to the transformation of a normal neuronal network into one that is clinically hyperexcitable. There is often a delay of months to years between an initial CNS injury such as trauma, stroke or infection and the first seizure. The injury appears to initiate a process that gradually lowers the seizure threshold in the affected region until a spontaneous seizure occurs. In many genetic and idiopathic forms of epilepsy, epileptogenesis is presumably determined by developmentally regulated events.

Clinical manifestation[10,12]

Simple partial seizures

Most simple partial seizures are short lived, lasting a few seconds. Prolonged simple partial motor seizures tend to be confined to a limited anatomical region & take the form of epilepsia partials continua.

The common features are

- No alteration of consciousness
- No amnesia
- Focal symptoms or sign
- Sensory and special sensory
- Psychic – dysphasia, affective, hallucinative
- Sudden onset and cessation
- Due to focal cortical pathology

Focal symptoms reflect anatomical origin of the seizure and are thus useful in localizing the underlying pathology. Most commonly affected being the frontal and temporal lobes.

The EEG during localized simple partial seizure is often normal due to small, circumscribed discharges. On temporal lobe epilepsy typical rhythmic theta activity is seen

Complex partial seizures

This type has three components – Aura

Altered consciousness

Automatism

Features

- Aura
- Absence (altered consciousness)

- Amnesia
- Automatism - Oroalimentary, mimicry, gestural ambulatory, verbal, responsive
- Sudden onset and gradual recovery
- Due to focal cortical pathology
- Localization - temporal lobe 60%; frontal cortex 30%

The routine interictal EEG in patients with complex partial seizures is often normal or may show brief discharges termed epileptiform spikes or sharp waves. Since complex partial seizures can arise from the medial temporal lobe or inferior frontal lobe i.e, regions distant from the scalp, the EEG recorded during the seizure may be non localizing, and this can be detected by using sphenoidal or surgically placed intracranial electrodes.

Generalised Seizures

Generalised Seizures are attacks in which epileptic disturbance involves wide areas of both cerebral hemispheres simultaneously from onset of attack with no evidence of an anatomic or functional focus.

- Consciousness is almost invariably impaired from the onset of the attack,
- Motor changes are bilateral and more or less symmetrical and

- EEG patterns are bilateral, grossly synchronous and symmetrical over both the hemispheres.

Typical absence Seizures:

- Blank stare
- Consciousness lost (With total amnesia)
- Other signs – usually slight, including minor tone changes, blinking, eye rolling, jerking
- Sudden onset and cessation usually short lived
- Occur as part of the syndrome of idiopathic generalized epilepsy.

Atypical absence seizures

- Blank stare
- Consciousness only partially impaired
- Focal signs more prominent
  - Tone changes,
  - Clonic jerking,
  - Motor spasm,
  - Automatism
- Onset cessation – gradual
- Seizure may be prolonged
- Occurs in patients with diffuse cerebral damage usually with mental retardation, other seizure types and other neurological defect.

Myoclonic seizures

- Brief jerk, singly or in series – may be induced by stimulus
- Varying intensity – Tremor to massive jerk
- Varying distribution – Single muscle to generalized jerking
- Consciousness usually not altered
- Rapid onset & cessation
- Associated features depend upon the underlying CNS malformations.

Clonic seizures

- Asymmetrical and irregular rhythmic jerking
- Rapid onset and cessation

Tonic seizures

- Tonic muscle spasm three forms
    - Axial
    - Axorhizomelic
    - Global
- Fall – commonly with injury
- Can be brief or prolonged with fluctuating motor signs
- Postictal phase short
- Sudden onset and cessation

- Usually in patients with cerebral damage and associated with mental retardation, neurologic handicap.

Tonic clonic seizure

- Loss of consciousness
- Fall – often with injury
- Tonic phase
- Clonic phase
- Tongue biting, incontinence, cyanosis, vocalization, automatic features.
- Postictal confusion, drowsiness, sedation, headache, muscle pain
- Sudden onset and gradual recovery
- An aura may proceed an attack
- Diurnal pattern common
- Occurs in cryptogenic and symptomatic epilepsy with a wide variety of underlying pathology and in idiopathic generalised epilepsy.

Atonic seizures

Atonia with fall – a static or drop attack

Atonia may be more limited head nodding, bending

Attack may progress in a stepwise fashion with increasing atonia.

Usually occurs in diffuse cerebral damage associated with mental retardation, other seizure types and neurological handicap.

Unclassifiable Seizures

Upto one third seizures are considered as unclassifiable using the current ILAE classification.

Diagnosis[13]

Get the facts first. You can assort them later

- Mark Twain

The principal objective of investigating new and chronic patients are

- To differentiate epileptic attacks from non epileptic attacks
- To determine the nature of seizure type - In case of partial seizures to identify laterality and localization
- To identify the aetiology
- To identify concomitant problems – general, neurological.
- To monitor the progression of the condition, the consequences of the epilepsy and its treatment.

Hence when a patient presents shortly after a seizure the first priority is

- Attention to vital signs
- Respiratory and cardiovascular support and
- Treatment of seizures.

If not acutely ill, evaluation should focus on the above mentioned factors. If the patient is with a history of prior seizures evaluation is directed towards,

- Identification of underlying cause and precipitating factors.
- Determination of adequacy of current therapy[1].

Laboratory Investigations

Sr. electrolytes	Blood Hb
Blood Glucose	BT, CT
Sr. Calcium	Folate level
Sr. Magnesium	VDRL
	ELISA
Hepatic – Sr. Bilirubin	Western Blot
SGOT	
SGPT	

Screening for toxins in blood, urine

Lumbar puncture – to rule out infection of CNS

Electro encephalography:

EEG measures the electrical activity of the brain by recording from electrodes placed on the scalp. The potential difference between pairs of electrodes is amplified and displayed on a computer monitor, oscilloscope or paper. The characteristics of the normal EEG depend on the patients age and level of arousal. In normal awake adults lying quietly with the eyes closed a

8-13 Hz alpha rhythm is seen posteriorly in the EEG intermixed with a variable amount of generalised faster Beta activity which is attenuated when eyes are opened.

The EEG is best recorded from several, different electrode arrangements in turn and activating procedures are usually performed in an attempt to provoke abnormalities, which commonly include

- Hyperventilation
- Photic stimulation
- Sleep
- Sleep deprivation on the night prior to the recording.

In the evaluation of a patient with suspected epilepsy, the presence of electrographic seizure activity during the clinically evident event – an abnormal repetitive, rhythmic activity having an abrupt onset and termination, clearly establishes the diagnosis. The absence of electrographic seizure activity does not exclude a seizure disorder.

Since seizures are typically frequent and unpredictable, it's not possible to obtain EEG during a clinical event. With the advent of continuous monitoring for prolonged periods in video EEG telemetry units, for hospitalized patients or the use of portable equipment to record EEG continuously on cassettes for ≥ 24 hrs in ambulatory patients, it is now possible to obtain

electrophysiologic activities during a clinical event. EEG can also help in classifying the seizure to assess the prognosis[1].

The other modalities available are[10]

EEG monitoring in intensive care unit

Sphenoidal electrodes

Brain mapping

Computed Axial Tomography Scan (CT Scan)

Magnetic Resonance Imaging (MRI)

Fluid attenuated inversion recovery (FLAIR)

Positron emission tomography (PET)

Single photon emission computed tomography (SPECT) for evaluation of seizures refractory to medical management[1].

Other investigations are[10]

- Cardiac – ECG monitoring
- Biopsy of eccrine sweat glands, liver shows presence of Lafora body – (polysaccharide containing body) seen with PAS stain – which is found in progressive myoclonic epilepsy.
- Brain biopsy is very rarely indicated when less invasive investigation haven't yielded an answer - When there is rapid progression
  - Possibility of a remediable aetiology

- Patient and family wishes to have as precise an explanation as possible
- When diagnosis has implications for genetic counselling[10].

Management

Therapy for a patients with a seizure disorder is almost always multimodal and include

(a) Treatment of underlying conditions that cause or contribute to the seizures

(b) Avoidance of precipitating factors

(c) Suppression of recurrent seizures by prophylaxis with antiepileptic drugs

d) Addressing psychological and social issues.

Pharmacological management

In 1857 after two millennia of innumerable ineffective remedies, Sir. Charles Locock introduced potassium bromide for epilepsy. In 1990 Chloral hydrate, paraldehyde, urethane, sulfonal were introduced. In 1912 Dr. Alfred Hauptmann in Germany reported a decrease in seizures when he used phenobarbital for soporific effect in his clinical practice with no marked side effect[14].

The advent of diphenyl hydantoin began the new area of active searching for antiepileptic drugs. It started as an imaginative, well planned search for a

better tolerated and more effective antiepileptic drug than Phenobarbital, by neurologists Tracy Putnam and M. Houstan Merritt between 1937 and 1941 at Harvard service of Boston city Hospital[5]. The study was based on three hypothesis.

- That effective anticonvulsants needn't be soporific
- Anticonvulsants will protect against experimentally induced seizures and indicate their clinical value
- That components with phenyl groups are good candidates.

The electrical model devised by them was the first to be used as an experimental method to predict the effectiveness of an anticonvulsant.

Trimethadione introduced in 1946 by Richards Everett, is ineffective in electrical screening but highly protective against pentylenetetrazole induced seizures hence useful in petit mal epilepsy. Later ethosuximide was developed which became the drug of choice for classic petitmal epilepsy.

In 1953 Geigy a swiss chemist synthesized carbamazepine and was found to be very effective against seizure production by both types of screening tests and currently occupies the major role in treating epilepsy as first line drug.

In 1881 valproic acid was synthesised and widely used as organic solvent, and is the only first line drug effective against petit mal, partial and generalised motor seizures.

Depending upon the mechanism of action [15,16]

I. Prolongation of Na^+ channel inactivation

Phenytoin

Carbamazepine

Valproate

Lamotrigene

II. Facilitation of GABA mediated Cl^- channel opening

Barbiturates

Benzodiazepines

Vigabatrin

Valproate

Gabapentin.

III. Inhibition of T type Ca^{++} current

Ethosuximide

Trimethadione

Valproate

IV. GABA Facilitator – Gabapentin

V. GABA Transaminase inhibitor –

Vigabatrin

Valproate.

Drug treatment should be considered after more than one seizure has occurred. But even after a single seizure there is a 40% risk of subsequent seizures[17]. The use of antiepileptic drugs after a single seizure reduces the frequency of second seizures by half over 2 years but doesn't alter the long term prognosis. Of patients whose epilepsy is controllable, only a single drug is necessary in (80%) provided the patients receive an appropriate agent at its appropriate dosage. The combination of more than two drugs is seldom necessary. Dose regimens should be kept as simple as possible to promote compliance.

Guidelines for choice of Antiepileptic Drugs [2,18] :

Type	First Line	Second Line	Third Line
Partial and / or secondary Generalised Tonic Clonic Seizures	Carbamazepine	Lamotrigine Sodium valproate Topiramate Tiagabine Gabapentin	Clobazam Phenytoin Primidone Phenobarbital Oxcarbazepine Levetriacetam Vigabatrin Acetazolamide
Primary Generalised Tonic Clonic Seizures	Sodium Valproate	Lamotrigine Topiramate Carbamazepine	Phenytoin Gabapentin Primidone Phenobarbital Tiagabine Acetazolamide

Absence	Ethosuximide	Sodium valproate	Lamotrigine clonazepam Acetazolamide
Myoclonic	Sodium valproate	Clonazepam	Piracetam Lamotrigine Phenobarbital

Duration of therapy

Once initiated Antiepileptic Drugs are typically continued for at least 2 years. If the patient is seizure free after 2 years, consideration should be given to tapering and discontinuing therapy. The risk of recurrent seizures approximates 25-50%. Typically 80% recurrences occur within 4 months of discontinuing therapy. The rate of relapse following 2 years of single antiepileptic drug therapy during which no seizure has occurred is 40% after 2½ years and 50% after 5 years while it is 20% for patients remaining on medication[4,19]. Any tapering of drugs ideally is performed slowly over a period of several months usually 2-3 months.

Monitoring of therapy

Too often a prescription signalize the end to an interview rather than the start of an alliance

- Barry Blackwell

Patients should be carefully educated about the approach to therapy. Most anticonvulsants are introduced relatively slowly to minimize side effects. Monitoring of serum antiepileptic drug levels can be very useful in establishing the initial dosing schedule[20].

If seizures continue despite gradual increases to the maximum tolerated dose and documented compliance then switching to other drug is mandatory, as monotherapy should be the goal whenever possible[1].

OTHER MEASURES:

NON PHARMACOLOGICAL

I. Avoiding precipitating factors

Stress is an ignorant state.
It believes that everything is an emergency

- Natalie Goldberg

Like Sleep deprivation

Alcohol intake

Video game monitoring

Music

Reduction of stress by

Physical exercise

Meditation

Counselling

A person with incompletely controlled epilepsy should not be allowed to

- Drive an automobile
- Operate unguarded machinery
- Climb ladders
- Take bath behind locked doors.

Apart from these, simple advises and reassurance helps the patient to overcome inferiority. Oversolicitude and overprotection should be discouraged. It is important that the patient be allowed to live as normal a life as possible.

II. Diet[5]

Lefevre and Aronson have evidenced a ketogenic diet in patients with failure of antiepileptic drugs to control seizures adequately particularly in children <5yrs of age. The diet will be unpleasant which restricts carbohydrate and protein and supplying 80% of energy intake as fat.

III. Vagal Stimulation[1]

A pacemaker like device is implanted in the anterior chest wall and stimulating electrodes are connected to the vagus at the left carotid bifurcation.

Surgical treatment

20% of patients are resistant to medical therapy despite combination of antiepileptic drugs.

- Temporal lobectomy
- Corpus callosotomy
- Multiple subpial transactions
- Amygdalohippocampectomy
- Lesionectomy
- Multilobar resection or hemispherectomy

Postoperatively patients generally need to remain on antiepileptic drug therapy.

Status epilepticus[21]

Whenever man comes up with a better mousetrap,

nature comes up with a better mouse

- James Carswell

Status epilepticus refers to continuous seizures or repetitive, discrete seizures with impaired consciousness in the interictal period, lasting beyond 5

minutes prompting the acute use of anticonvulsant therapy. Status epilepticus is an emergency and must be treated immediately. The most common causes of status epilepticus are

- Anticonvulsant withdrawal
- Noncompliance
- Metabolic disturbance
- Drug toxicity
- CNS infection, tumor
- Refractory epilepsy
- Head trauma

There may be uninterrupted seizures, paroxysmal episode of tachycardia, hypertension, pupillary dilatations.

Management[22]

- Initial Assessment - Adequate ventilation
  Intravenous line
  Intubation if necessary.
- Immediate suppression of convulsion
  - Lorazepam / diazepam 2 -4 mg/min IV
    with blood pressure monitoring.
  - Initiation or reloading with anticonvulsants
    phenytoin 15-18 mg / kg or fosphenytoin 50-75 mg/kg.

- General anaesthetic doses for persistant status
    - Midazolam 0.2 mg/kg loading dose followed by infusion at 0.1-0.4 mg/kg or propofol 2mg/kg/hr.

Further treatment

May add valproate / Phenobarbital IV or carbamazepine or levetriacetam by nasogastric tube. Consider neuromuscular paralysis with EEG monitoring - pentobarbital 10mg/kg/hr.

Inhalational anaesthetic – Isoflurane

Antiseizure therapy and pregnancy[4]

The main issues considered are,

- Potential teratogenic effect and
- Effects on Vit K metabolism in pregnant women. Infants of epileptic mothers are at two fold increased risk of congenital malformations.

Hence women with epilepsy who wishes to become pregnant be put on monotherapy. Careful monitoring of drug levels could do little harm than polytherapy. Folate supplementation (0.4mg/kg) is recommended.

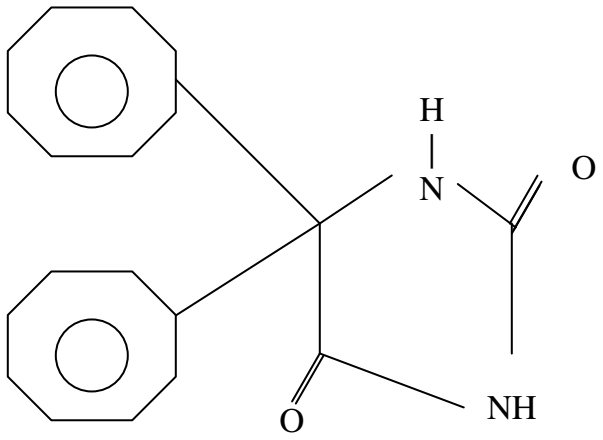
In addition, the newborn of mothers on anti convulsant therapy have Vit K deficiency leading to coagulopathy and intracranial haemorrhage which can be prevented by treatment with vitamin K, 20mg/day orally during last month of gestation as prophylaxis[5,10].

Diphenylhydantoin

Phenytoin is effective against all types of partial and tonic clonic seizures but not absence seizures. Though was synthesized in 1908, the anti convulsant property was discovered in 1938.

Structure – Activity Relationship[23]

Phenytoin has the following structural formula



A 5 phenyl or other aromatic substitution appears essential for activity against generalised tonic clonic seizures. Alkyl substitution in position 5 contribute to sedation, a property absent in phenytoin. The carbon 5 position permits asymmetry but there appears to be little difference in activity between isomers.

The most significant effect of phenytoin is its ability to modify the pattern of maximal electroshock seizures which corresponds to generalised tonic clonic seizures. The characteristic tonic phase can be abolished

completely, but the residual clonic seizure may be exaggerated and prolonged. The mechanism of action is by slowing the rate of recovery of voltage activated Na^+ channels from inactivation, an action that is both voltage and use dependent. At therapeutic drug concentration, the effect on Na^+ channels are selective and no changes in spontaneous activity.

Pharmacokinetic properties

Both rapid release and extended release tablet forms are available. Phenytoin is extensively bound to serum proteins especially albumin 90%, metabolism is mainly by CYP 2C9/10, CYP 2C19 and eliminated under nonlinear kinetics. The principal metabolite parahydrophenyl derivative is inactive. The elimination varies as a function of its concentration. The plasma half life is between 6 - 24 hours.

The low aqueous solubility of phenytoin hindered its intravenous use and led to production of fosphenytoin, a water soluble prodrug. It is converted to its active form in liver and red blood cells. This drug is very useful in adults with partial or generalised seizures when intravenous or intramuscular administration is indicated.

Drug interactions[20]

Being an inducer of CYP 2C, 3A families, the metabolism of drugs metabolized by these CYP are enhanced. Of particular note in this regard is the

metabolism of oral contraceptives leading to unplanned pregnancy. The other antiepileptic drugs carbamazepine, oxcarbazepine, phenobarbital and primidone also induce CYP 3A4 and increase the degradation of oral contraceptives.

Carbamazepine, warfarin, steroids, thyroxine, tricyclic antidepressants which induce CYP 2C9/10 can enhance the metabolism of phenytoin and produce well documented decrease in phenytoin concentration. Drugs that inhibit phenytoin metabolism are cimetidine, cotrimoxazole, chloramphenicol, NSAIDs and disulfiram.

Therapeutic uses

Phenytoin is one of the more widely used antiepileptic drug and it is effective against

- Partial seizures
- Tonic clonic seizures
- Status epilepticus
- Trigeminal and related neuralgias
- Cardiac arrhythmias

Toxicity[24]

At therapeutic level:

- Gum hypertrophy – Commonest, more in young patients
- Hirsutism, coarsening of facial features , acne

- Hypersensitivity reactions – rashes, lymphadenopathy
- Neutropenia – rare but warrants discontinuation
- Megaloblastic anemia
- Osteomalacia – phenytoin desensitizes target tissues to vit D and interferes with calcium metabolism.
- Inhibits insulin release and cause hyperglycemia.
- Used during pregnancy – cause foetal hydantoin syndrome probably caused by areneoxide metabolite[5].

At high plasma levels (dose related toxicity)

Cerebellar, vestibular manifestation – ataxia, vertigo, diplopia, nystagmus.

Epigastric pain, nausea vomiting

Intravenous injection causes local vascular injury, oedema, discolouration of injected limb.

Fall in blood pressure and arrthythmias only on IV injection, probable cause being the solvent.

Pharmacogenetics and drug resistance[10]

This addresses the effect of genetic variation on drug response and adverse effects. Common variation in the gene SCN – 1A affects the maximum dose of phenytoin which act on sodium channel subunit encoded by this gene.

Pharmaco resistance has received fresh attention with two key hypothesis.

- Alteration in drug targets at some stage leading to poor response to drug treatment.
- Certain multidrug transporters expressed in the brain could reduce antiepileptic drug concentration around neurons in the seizure focus by active transport away from the neurons back into capillary lumina.

Newer treatment Prospects

Early prediction of seizures with the use of EEG can have enormous effect on treatment of epilepsy with advances in stem cell science and viral gene expression system, interest has grown in focal approaches to the treatment of epilepsy. At present such approaches remain experimental.

Focal treatments use two approaches[10]

- Focal application of drugs, cells or a virus to the epileptogenic zone. This is dependent on identification of sites of origin of seizure.
- If areas could not be identified, similar methods can be used to express or release antiepileptic drugs into the areas that regulate cortical excitability, seizure threshold and propagation or both.

CISSUS QUADRANGULARIS[25]

Botanical Name	:	Cissus Quadrangularis
Family	:	Vitaceae
Synonyms[25]	:	Cissus adnata
		Cissus latifolia
		Cissus discolor
		Cissus javanica
		Cissus repanda
		Cissus repens
		Cissus setosa
		Cissus trilobata

Vernacular Names[26]

Tamil	-	Pirandai, Vachiravalli
Telugu	-	Nalleeru – teega
Malayalam	-	Changalamparanda
Kannadam	-	Mangarahalli
Sanskrit	-	Asthisandana
Hindi	-	Hadjora
English	-	Edible stemmed vine, Bone setter
		Adamant creeper

Distribution and Habitat[26,27]

A genus of climbing shrubs distributed in the tropical and subtropical regions. Particularly in the hotter parts of India also cultivated in gardens.

General features: [27]

A cactus like jointed tendril climber with stout fleshy jointed quadrangular stem, tendrils simple, long, slender and opposed. In addition to the normal roots, aerial roots develop during rainy season from the jointed nodes grow downwards and strike the soil. Leaves simple, broadly reniform, entire or toothed, rounded, truncated or cuneate at the base. Flowers small, greenish, in shortly peduncle cymes, petals four in number, hooded at the apex, fruits ovoid or globose, red berries, seeds ellipsoid.

Properties and uses:

Local herbal practitioners prescribe for eye diseases, chronic ulcers, digestive disorders, healing of bone fractures and epilepsy in human. The young stems are fed to cattle to induce flow of milk. The ash of the plant is used as a substitute for baking powder. The leaf extract shows antifungal activity. The stem and root yield a strong fibre. The plant is rich in vitamin C. The stem contains two steroidal principles with anabolic steroidal activity which shows marked influence in the rate of fracture healing by influencing early regeneration of all connective tissues involved in the quicker mineralization of the callus. The aqueous extract of the plant given topically or by intramuscular injection hastens fracture healing by reducing the total convalescent period.

ANIMAL MODELS FOR EPILEPSY

A peculiar thing in medicine is that we never believe in anything unless it can be demonstrated in animals

- John A. Schindler

The usual approach to anticonvulsant drug screening in animals is to observe the effect of prior drug administration on seizures produced by some type of electrical stimulation of the brain or the systemic administration of a convulsant drug[28]. Chronic epileptogenic lesion of the cerebral cortex may also be made or animal strains with spontaneous or sensory evoked convulsions be employed for drug study.

The drug effect chosen for study may cause a change in threshold, a qualitative change in pattern of the motor seizure or of the EEG or a change in incidence of seizures. In addition to quantification and comparison of drug potencies, undesired side effects are also observed to obtain the animal equivalent of clinical therapeutic ratio.

Experimental models for seizure studies[29,30]

2.1.1 Invitro methods

Brain slices

Monosynaptic systems

Neuronal cultures for studying specific epileptogenic mechanisms

2.1.2 Invivo methods

These employ animals like mice, rat, guinea pig, gerbils, cats, dogs, rabbits, monkeys etc using different physical, chemical, pharmacological stimuli to induce seizures.

2.2 Experimental Seizure models

2.2.1 Electrical seizures

Threshold model – Quantitates seizure threshold

Maximal Electro Shock Test – supramaximal stimulation

Focal electrical stimulation – kindled seizures

2.2.2 Chemical seizures

Systemic : Pentylenetetrazole

Bicuculine

Strychnine

Kainic acid

Central : Penicillin

Quinolinic acid

Topical : Aluminum cream

Cobalt

Tungstic acid

Premarin

Genetic : Spontaneous model

Semispontaneous

Photic seizures

Audiogenic

Electrically induced seizures

Maximal Electro Shock:.

An electrical stimulus of sufficient intensity to induce maximal seizures– tonic extension of the hind limbs is applied to mice or rat, by means of an external device stimulator / convulsiometer. A supramaximal current strength i.e., a stimulus about 5-10 times higher than the individual electrical seizure threshold of the animals is used 50 mA in mice or 150mA in rats for 0.2 seconds. The stimulus is applied via corneal or ear clip electrodes.

The resultant seizure in normal rat shows a latent phase, a preliminary phase of tonic hind limb flexion, tonic extension, clonic phase and post seizure depression[31].

Drugs like phenytoin sodium abolishes the extensor component entirely but not clonic phase which may even be prolonged. As a single measure of potential anti grand mal potency, the PD50 (Protective dose in 50% of rat) against any tonic seizure activity is found.

Phenacemide abolishes the clonic phase also, bromide abolishes the extensor component leaving a flexor type of seizure.

The maximal electroshock test is a measure of the ability of an anticonvulsant drug to abolish the tonic extensor component of the hind limb in the seizure pattern[32].

Substances are compared in groups of 4-6 animals. The animals are fed with the test compound after over night fasting to avoid regurgitation and aspiration while producing convulsions. After 2 hours the convulsions are induced using Techno convulsiometer by placing ear clips.

In threshold rest, the ability of a drug to alter the seizure threshold for tonic hind limb extension is determined. This test is a better predictor of generalized tonic clonic seizures and is much more sensitive.

In kindling, repeated administration of an initially subconvulsive electrical stimulus results in progressive intensification of seizure activity, culminating in a generalized seizure. The seizures evolve through five stages.

a) Immobility, eye closure, twitching of vibrissae
b) Facial clonus, head nodding
c) Unilateral forelimb clonus
d) Rearing often accompanied by bilateral forelimb clonus
e) Rearing and falling accompanied by generalized clonic seizures stage.

a) and b) correspond to complex partial, while stages c) to e) signify limbic seizures evolving to generalized motor seizures. But kindling is a time consuming procedure, requiring chronic implantation of stimulating and

recording electrodes and regular electrical stimulation. Drug can be screened here aiming at their ability to block establishment of kindling (phenobarbitone, valproate.) or block seizures once kindling has occurred (phenytoin, carbamazepine)

Chemically induced seizure models:

Innumerable chemical and drug induced seizures at toxic doses and many of them can be used to produce epileptiform phenomena in experimental animals. The chemoconvulsants may be administered either systemically or topically.

Pentylenetetrazole as convulsant (PTZ)

The systemic administration of PTZ is one of the most commonly employed method for anti convulsant screening introduced by Richard and Everett. PTZ 60mg/kg has been found to produce generalized clonic seizures in 100% animals with least mortality.

The seizures produced by PTZ are paralled by spike wave complexes – (clonic seizures) or sharp hypersynchronised polyspikes - (tonic seizures) in EEG. The mechanism of convulsant action of PTZ seems to be related to the inhibition of the inhibitory functions of the GABA neurotransmitter. In addition to clonic seizure as endpoints, drug effects in tonic seizure can be studied by using higher dose of PTZ which induces tonic seizures.

Drugs like phenobarbitone and primidone block PTZ seizures more potently suggesting that PTZ is a model for myoclonic rather than petit mal seizures.

Genetic models:

By definition of epilepsy, animals with chronically recurring, spontaneous seizures, represent ideal models for human epilepsy.

Spontaneous seizures[33]

Beagle dogs – high incidence of epilepsy resembling complex partial generalized tonic clonic seizures.

Mice – Homozygous tottering mouse – Spontaneous seizures.

Rat – Spike wave petitmal discharges

Syrian hamsters – Myoclonic seizures, GTC seizures.

These spontaneous models for seizures may be ideal for chronic drug efficacy.

Semi spontaneous seizures

In certain genetically predisposed species seizures donot occur spontaneously but can be induced by specific sensory stimulation such as audiogenic or photic stimuli.

Photic seizure:

In adolescent baboons, domestic fowls myoclonic responses appear for intermittent photic stimulation which is inhibited by drugs like benzodiazepines, barbiturates and valproic acid. The main drawbacks with this model are

- Predictive value against a particular clinical subtype of seizure is questionable.
- High prime and maintenance cost of baboons.

Audiogenic seizure:

The DBA/2J inbred strain of house mouse Mus musculus between the age of 2 and 4 weeks is highly susceptible to sound induced seizures. Audiogenic seizures are prevented by phenytoin, phenobarbital and valproate. Sound induced convulsions also occur in rats – Genetically Epilepsy Prone Rats (GEPR). This GEPRs are valuable alternative to traditional MES test.

The major drawback with such seizures is that

- Sound induced seizures are exceedingly rare in man and
- Questions as to correlation.

Mongolian Gerbil:

In this model seizures can be precipitated by environmental stimuli like onset of bright light, audiogenic stimuli, vigorous shaking of cage, different handling techniques and or air blast.

The drawbacks of this model are

- Non specificity of sensory stimulation for epileptic myoclonic seizures.
- Time consuming experiments
- Skill in handling is mandatory to avoid induction of seizures
- Half life of drugs is shorter than in humans and maintenance of effective levels during chronic treatment difficult.

Consideration for selecting an animal model:

Since different animal models are believed to mimic different types of seizures the major consideration in selecting an animal model depends upon the seizure type, under study.

A. Generalised tonic – clonic

Maximal Electro Shock (MES)

Chemical convulsions – PTZ, picrotoxin

Genetic – photosensitive baboons etc,

Metabolic derangements – hypoxia, uremia

B. Generalised absence

Thalamic stimulation

Systemic penicillin

C. Simple partial

Acute – Topical convulsants – penicillin, strychnine

Chronic – implanted metals – alumina, cobalt

D. Complex partial

Kainic acid

Kindling

E. Status Epilepticus

High dose pilocarpine

Continuous hippocampal stimulation – CHS

F. Post traumatic

Ferric chloride

Haemoglobin

As the seizure under study was generalised tonic-clonic type, the electrically induced seizures was the method selected for inducing seizures. Of the three broad electrically induced testing methods it appears that the supramaximal electroshock method has a high degree of predictivity, for drugs effective against human generalised tonic clonic seizures.[28]

II. Species[29]

Though most convulsive stimuli actively produce seizures in many species there may be a difference in drug response, Screening at initial stages is carried out generally in two species – rat & mice to avoid the possibility of missing out a potential agent.

Male Vs female rats for anticonvulsant studies[29]

A relationship between seizure susceptibility and the estrous cycle is well known. In studies in human as well, a subset of women with epilepsy show changes in seizure frequency in relation to hormonal variation during the menstrual cycle. Thus with the use of female rats, at least there is a theoretical possibility of variation in response with the changing hormonal status of the animal. Furthermore, female rats are known to eliminate several antiepileptic drugs less rapidly than males do. This again is not unambiguous.

MATERIALS AND METHODS

Setting :

This study was carried out in the central animal house, Institute of pharmacology, Madurai Medical College, Madurai.

Design of Study

It was an experimental study

Period of study

The study was conducted for a period of 6 months from September 2006 to February 2007.

Ethical Clearance

Ethical clearance was obtained from the Institutional Animal Ethical Committee, Madurai Medical College, Madurai. Annexure – I

Materials Required for the Study:

Animals

Male albino rats of age 10 months weighing about 250-300 g.

Drugs and chemicals

Aqueous extract of Cissus quadrangularis

Diphenylhydantoin sodium

Gum acacia

Appliances / Equipments

Electroconvulsiometer

Oral feeding tubes

Disposable syringe 2cc

Stirrer

Beakers

Tray

Experimental animals

Selection of laboratory model:

Animals such as mice, rats, guineapigs, gerbils, cats, dogs, monkeys etc have been used in the experimental study of in vivo models of epilepsy. In the present study rats have been used because the major consideration in selecting an animal model remains on the seizure type under study. Here it is GTC seizures. The maximal electroshock model in rats resembles the generalized tonic, clonic seizures and also screening at initial stages is carried out commonly in rats and mice to avoid the possibility of missing out potential agents.

As there are predictable variations during estrous cycle and drug disposing mechanism, male albino rats were selected. The convulsions induced by MES method resembles generalized tonic clonic seizures of human.

Albino rats:

Healthy adult male albino rats, 10 months old, weighing around 250-300 g (mean wt – 286 gms) were used. The animals were selected from the inbred colony maintained in the central animal house, Madurai Medical College. They were fed with commercially available standard pellet diet obtained from AMRUT FEEDS, Pranav agro industries limited and water ad libitum.

Drugs and chemicals

Preparation of extract of Cissus quadrangularis

The whole plant of Cissus quadranguloris were collected and dried in shade for 10 days. It was coarsely powdered. 200g of the powdered plant was soaked in sufficient quantity of distilled water overnight. The contents were transferred to a Soxhlet apparatus and extracted for about two hours using hot water bath. The process was repeated several times with fresh powder to get sufficient quantity of the extract. The semisolid extract obtained was weighed accurately and utilized for experimental studies. The extract was suspended in 1% gum acacia in distilled water to yield the required graded concentrations i.e, 30, 45, 60mg in 0.5ml of the suspension.

Gum acacia

This is the dried gummy exudate obtained from the stem and branches of Acacia Senegal or other African species of acacia and is used here as a suspending agent for the oral administration of the standard drugs and test compound in 1% strength.

Diphenyl Hydantoin

Tablet diphenylhydantoin 100 mg (T. Eptoin – Knoll Pharmaceuticals Ltd) was powdered and suspended in 1% gum acacia. The suspension prepared provided 30mg/ml and it was administered orally in the dose of 50mg/kg body weight[34,35,36]. Diphenylhydantoin is the prototype drug that abolishes the tonic extensor phase of MES seizure and either prolongs or unalters the clonic phase. In addition it shortens the duration of post seizure depression[23].

Appliances / Equipments

Electro Convulsiometer

For electrical stimulation of rat Techno Electro convulsiometer was used. It is provided with various pulse stimulators and an inbuilt timer with which we can select and set the amplitude of electricity to be delivered and its duration. Stimulation is given through a pair of ear clip electrodes made of stainless steel. Saline solution or commercial ECG salt paste is used to moisten the electrodes[28].

Oral feeding tube:

A 16 gauge hypodermic needle of 3 or 4 inch length serves as an useful stomach tube for the rat. The needle is blunted and a small ball of solder is applied around the distal end. A gentle 20-30° bend is made about 2cm proximal to the solder. The tube thus constructed is attached to a 2ml syringe[37].

ANTI EPITEPTIC STUDY

Methodology

15 adult male albino rats were divided into 5 groups with 3 animals in each. All animals were put on overnight fasting. Group I served as control received – 0.5ml of 1% gum acacia, normal feed and water. Group II received the standard drug diphenylhydantoin at the dose of 50mg/kg[38]. The three test groups received the aqueous extract of Cissus quadrangularis at the dose of 100, 150, 200 mg/kg along with normal feed and water[39]. The test was conducted after 2 hours following drug administration. Convulsions were induced using electro convulsiometer which was set to deliver a current strength of 150mA for a period of 0.2 seconds. The control animal evolved though a latent phase, phase of tonic flexion, phase of tonic extension, clonic phase and postictal depression[40]. Convulsions were induced in all three animals in each group at a gap of ten minutes each, so that observations can be made without any difficulty.

Group	Category	Treatment
I	Control	Normal feed + water + 0.5ml of 1% gum acacia
II	Standard	Normal feed + water + Phenytoin sodium 50mg/kg oral
III	T1	Normal feed + water + extract of Cissus quadrangularis 100mg/kg oral
IV	T2	Normal feed + water + Extract of Cissus quadrangularis 150mg/kg oral
V	T3	Normal feed + water + Extract of Cissus quadrangularis 200mg/kg oral

Following recovery from postictal depression, the animals were maintained on normal feed and water after the experiment and observed for a week and also for four weeks thereafter to find out any changes in behavioural, neurological, autonomic profile and mortality.

Results

The results observed were tabulated, data were analyzed statistically using Student's 't' – test. Probability values less than 0.05 were considered significant.

RESULTS

15 adult male albino rats weighing 250-300g of either sex were divided into five groups of three each. Efforts were made to follow animals everyday in the central animal house. Group I which served as control received 0.5 ml of 1% gum acacia; Group II - standard – received diphenylhydantoin; Groups III, IV and V received the test compound aqueous extract of Cissus quadrangularis at a graded dose of 100,150 and 200 mg/kg body weight respectively. All animals were fed orally using oral feeding tube.

On close follow up the animals which received diphenylhydantion (or) the aqueous extract of Cissus quadrangularis (or) placebo, did not show any behavioural abnormalities (or) weight loss. Similarly animals belonging to the Groups III, IV and V didn't show any evidence of bowel disturbances (or) change in eating or drinking habits. These indicated that the extract did not have any systemic toxicity as well.

After 2 hours of oral feeding convulsions were induced 10 minutes apart in each animal and the phases of convulsion along with its duration were observed. The observations were tabulated and compared for their significance in Tables 1 – 5.

None of the rats considered for the present study expired either during the study period or during post study follow up of another 28 days. The guidelines provided by the Ethical Committee for the animals were adhered strictly.

Anticonvulsant effect

Latent Phase:

The mean latent phase in Groups I, II, III, IV and V were 2.67 ± 0.5574, 2.33 ± 0.5774, 2.33 ± 0.5774 2.0 ± 0.5774 and 1.33 ± 0.5774 respectively with no significant change in the duration of latent phase (Table 1).

Phase of tonic flexion

The mean duration of phase of tonic flexion in Groups I, II, III, IV and V were 4.33 ± 0.5774, 3.33± 1.1547, 2.67 ± 0.5774, 3.33 ± 0.5774 and 2.33 ± 0.5774 respectively with no significant change in the duration of phase of tonic flexion (Table 2).

Phase of tonic extension

The mean duration of phase of tonic extension of Groups I, II, III, IV and V were 19.33 ± 3.0557, 1.33 ± 1.1547, 11.67 ± 0.5774, 11.0 ± 1.0 and 6.67 ± 1.1547 respectively with a significant reduction in Group II ($p < 0.001$) and Group V ($p < 0.01$) when compared to the control (Table 3).

Clonic Phase

The mean duration of clonic phase in Groups I, II, III, IV and V were 2.67 ± 0.5774, 28.0 ± 5.2915, 11.67 ± 1.5275, 22.67 ± 9.0185 and 21.67 ± 1.5275 with a significant prolongation of the clonic phase in Group II ($p < 0.01$), IV ($p < 0.05$) and V ($p < 0.001$) when compared to control (Table 4).

Phase of post ictal depression

The mean duration of post ictal depression in Groups I, II, III, IV and V were 4.87 ± 0.3683, 1.18 ± 0.1277, 3.69 ± 0.3630, 3.39 ±0.0503 and 2.40 ± 0.0950 with a significant reduction in the duration in Group II ($p < 0.001$) and Group V ($p < 0.01$) when compared to control (Table 5).

DISCUSSION

Epilepsy is one of the oldest known diseases. 5-10% of the general population suffer at least one seizure during their lifetime. An ideal antiseizure drug should suppress all seizures without any unwanted effects. Unfortunately the available antiseizure drugs provide symptomatic relief in two thirds of patients only. In addition they cause adverse effects ranging from minimal impairment of CNS to death from aplastic anemia and hepatic failure. Hence researchers are making efforts to identify molecules to prevent generation of seizures or to abort the abnormal electrical activity.

The observations emanated in the present study indicated that the duration of tonic extension and post ictal depression were significantly shortened in experimental groups (Groups II and V) compared to control (Group I). The reduced duration of these components are due to the anticonvulsant effect of the drug and the extract. Statistical analysis also revealed that Group V had a significant anticonvulsant effect almost comparable to that of Group II. It is likely that plant extracts are safer and as they are already components of dietary supplements in day to day life, further evaluation is required to understand the exact molecular mechanism. Hopefully the data so obtained will form the nucleus for further research in the treatment of seizures.

SUMMARY & CONCLUSION

Our Institution has been very keenly involved in the review, research and revitalization. The objective of the present work is to evaluate the anticonvulsant effect of aqueous extract of Cissus quadrangularis.

Fifteen adult male albino rats, following overnight fasting were divided into five groups with three animals each. The animals received orally Group I Control – 1% gm acacia, Group II – standard – 50mg / kg of diphenyl hydantoin, Groups III, IV and V aqueous extract of Cissus quadrangularis at a graded dose of 100, 150 and 200 mg / kg respectively. The anticonvulsant effect was assessed by the ability of the extract to produce statistically significant reduction in duration of tonic extensor component and post ictal depression, in MES seizures comparable with that of diphenyl hydantoin.

The results of the present study indicates that the aqueous extract of Cissus quadrangularis possesses significant anticonvulsant effect.

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ETHICAL CLEARANCECERTIFICATE

DR.M.SHANTHI.M.D. (Pharmacology), Dean i/c & Chairman Animal Ethical Committee, Madurai Medical College, Madurai, hereby endorse ethical clearance to the proposal.

" THE ANTI CONVULSANT EFFECT OF CISSUS QUADRANGULARIS IN COMPARISION WITH PHENYTOIN"

Submitted by

Dr.K.Geetha
Post Graduate student
Institute of Pharmacology
Madurai Medical College
Madurai.

The study did not violate the regulations and guidelines prescribed by ICMR and are within the permitted norms of animal experimentation in this country. The outcome of the study may be beneficial to the Human and animals.

Remarks : . 1. Animal ethical Committee permits to use 3 AlbinoRats per group .Totally 15 Albino Rats
If she wantsto use 6 animal per group for examination purpose needs to submit Documentary evidence.

2.. Start with dose of 100 mg per Kg if desired effect is found reduce the dosage.

DEAN I/C & CHAIRMAN

Date : 30.11.06
Place : Madurai
Office seal:

TABLE – 1

LATENT PHASE

Group		Duration in secs	Mean ± SD	Remarks
I	Control	3 2 3	2.67 ± 0.5774	p > 0.05 Not Significant
II	Standard	2 2 3	2.33 ± 0.5774	
III	Test – 1	3 2 2	2.33 ± 0.5774	
IV	Test – 2	2 2 2	2 ± 0.5774	
V	Test – 3	1 1 2	1.33 ± 0.5774	

TABLE – 2

PHASE OF TONIC FLEXION

Group		Duration in secs	Mean ± SD	Remarks
I	Control	5 4 4	4.33 ±0.5774	p > 0.05 Not Significant
II	Standard	2 4 4	3.33 ± 0.5774	
III	Test – 1	2 3 3	2.67 ± 0.5774	
IV	Test – 2	4 3 3	3.33 ± 0.5774	
V	Test – 3	3 2 2	2.33 ± 0.5774	

TABLE – 3

PHASE OF TONIC EXTENSION

Group		Duration in secs	Mean ± SD	Remarks
I	Control	22 16 20	19.33 ± 3.0551	
II	Standard	2 0 2	1.33 ± 1.1547	p< 0.001
III	Test – 1	12 11 12	11.67 ± 0.5774	p< 0.05
IV	Test – 2	10 12 11	11.0 ± 1.0	p< 0.05
V	Test – 3	6 8 6	6.67 ± 1.1547	p< 0.01

TABLE –4

PHASE OF CLONUS

Group		Duration in secs	Mean ± SD	Remarks
I	Control	3 3 2	2.67 ± 0.5774	
II	Standard	30 22 32	28.0 ± 5.2915	p< 0.01
III	Test – 1	12 13 10	11.67 ± 1.5275	p<0.001
IV	Test – 2	14 22 32	22.67 ± 9.0185	p< 0.05
V	Test – 3	20 23 22	21.67 ± 1.5275	p< 0.001

TABLE – 5

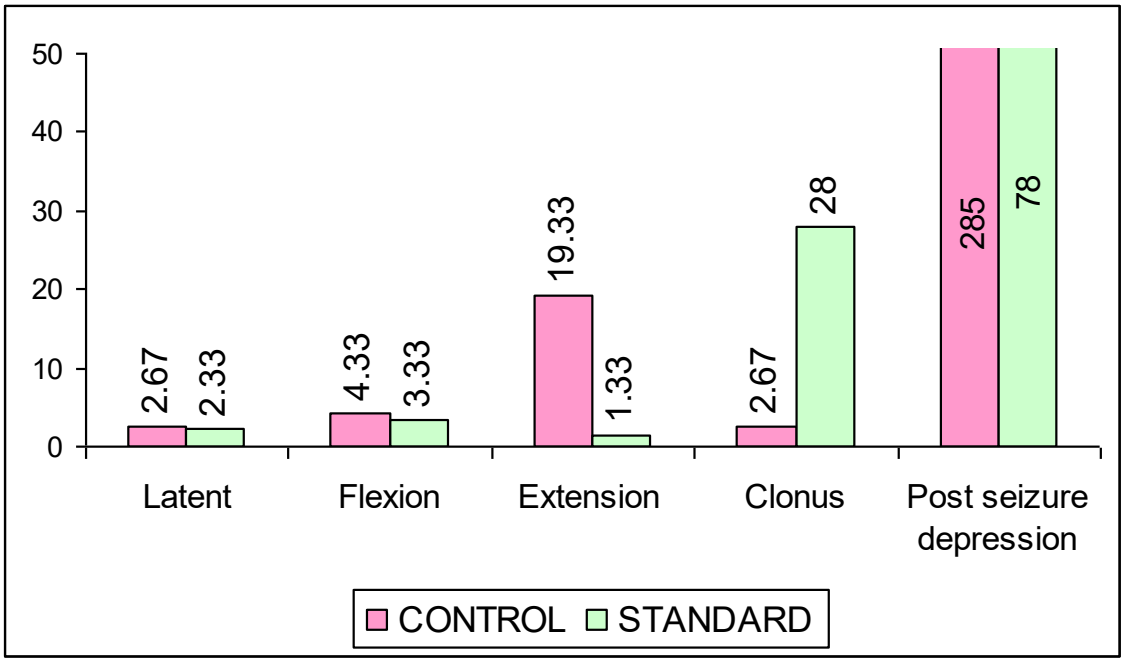
PHASE OF POST ICTAL DEPRESSION

Group		Duration of secs	Mean ± SD	Remarks
I	Control	300 245 312	2.85.67 ± 35.73	
II	Standard	92 67 75	78 ± 12.77	p< 0.001
III	Test – 1	234 222 250	235 ± 14.05	p< 0.1
IV	Test – 2	214 224 220	219.33 ± 5.03	p< 0.05
V	Test – 3	160 150 169	159.67 ± 9.50	p< 0.01

BAR CHART I

PHASES OF CONVULSION – CONTROL Vs STANDARD

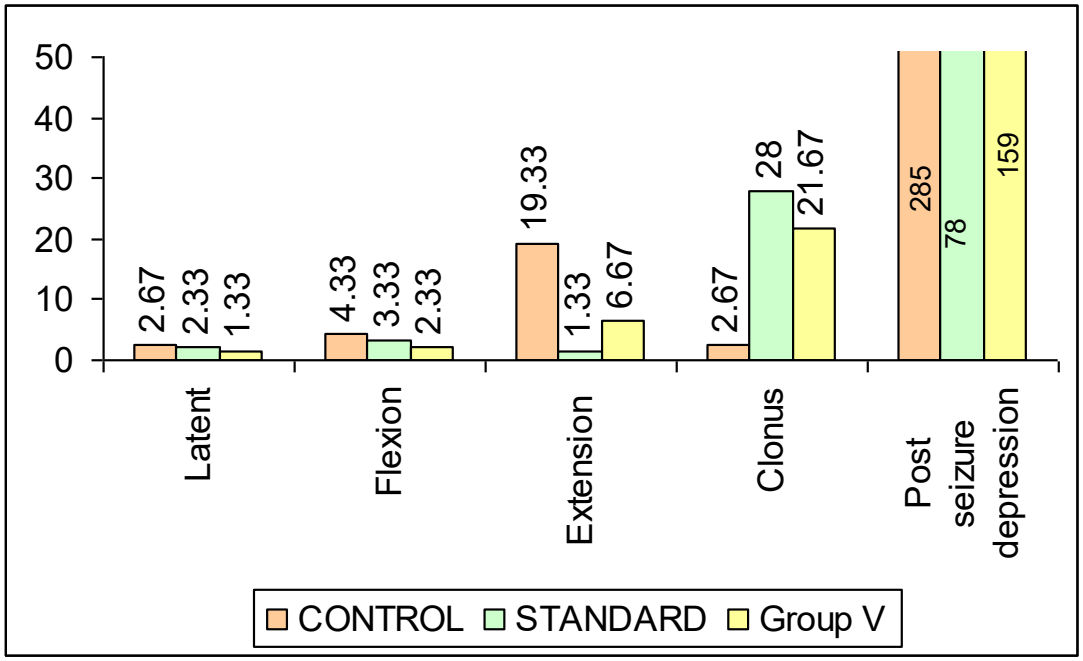
DURATION IN SECONDS



BAR CHART 4

PHASES OF CONVULSION – CONTROL, STANDARD Vs GROUP V

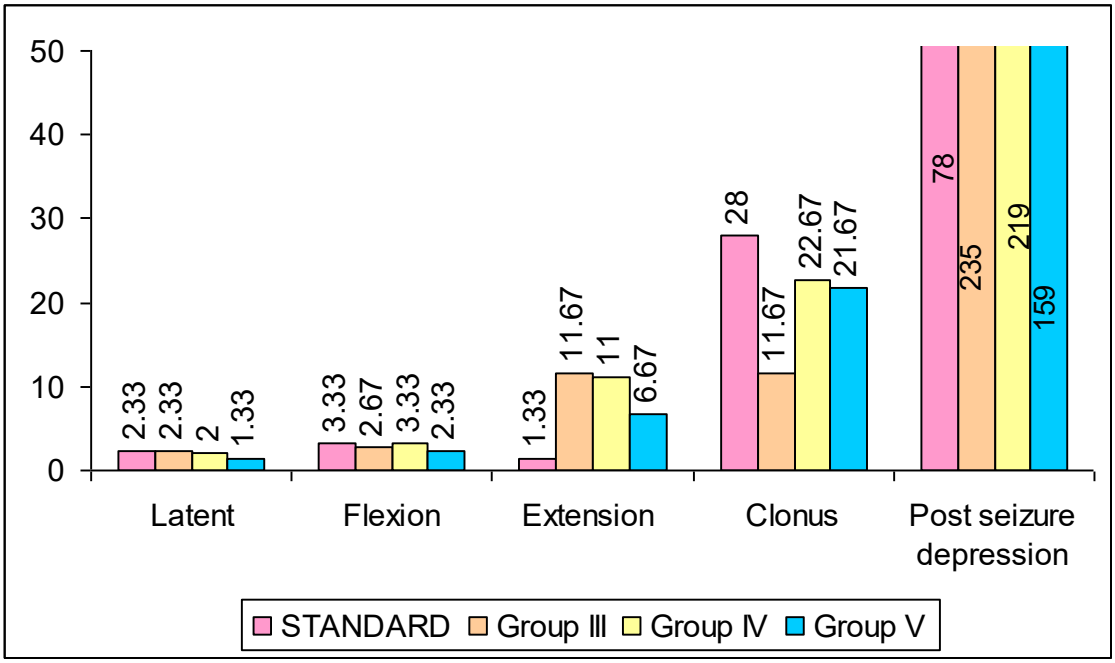
DURATION IN SECONDS



BAR CHART 2

PHASES OF CONVULSION – STANDARD Vs GROUPS III, IV, V

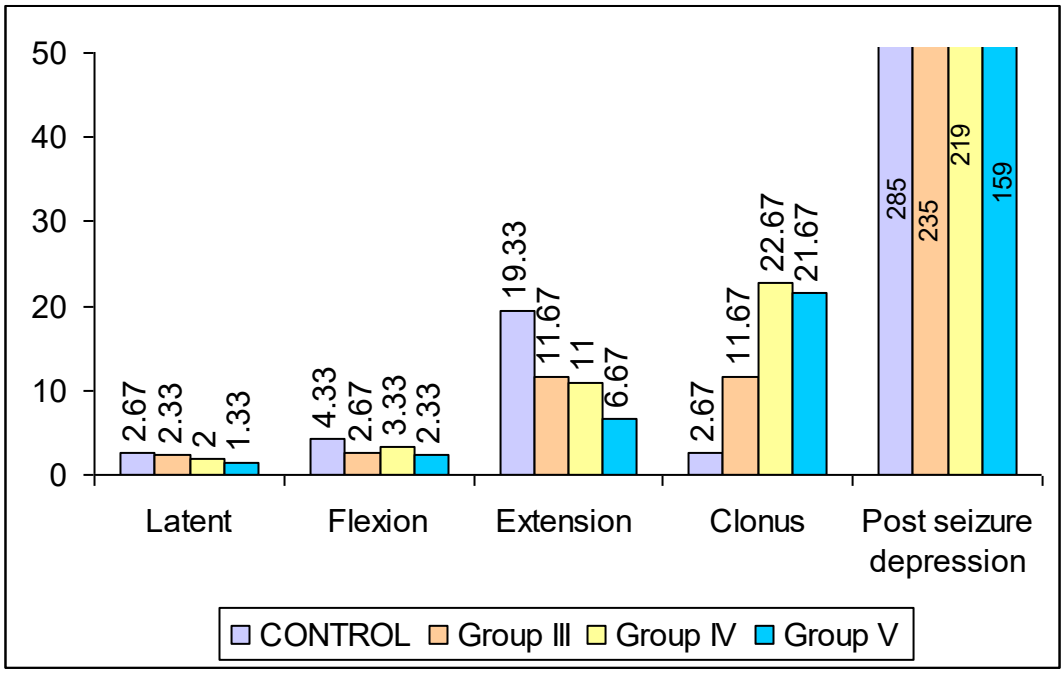
DURATION IN SECONDS



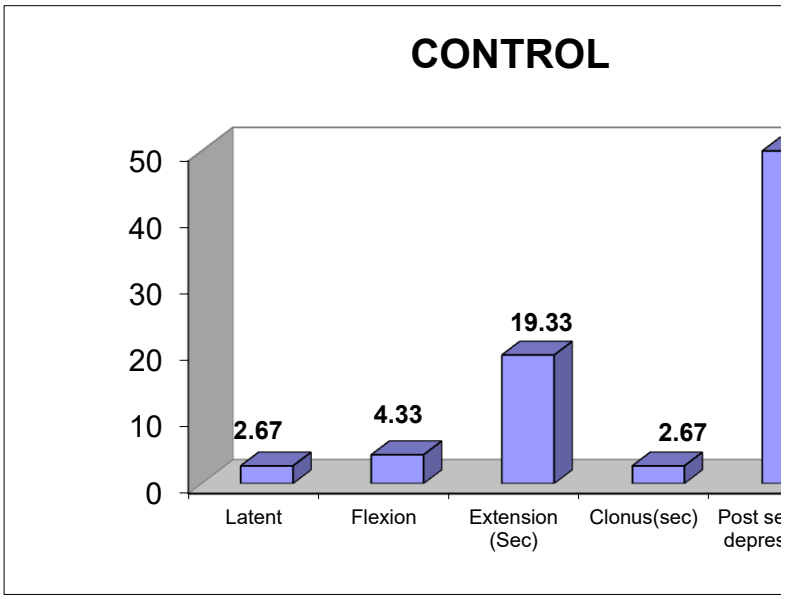
BAR CHART 3

PHASES OF CONVULSION – CONTROL Vs GROUPS III, IV, V

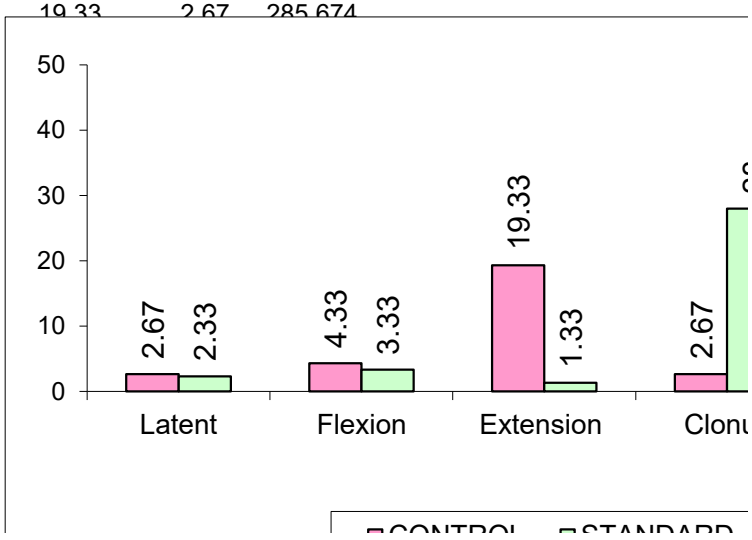
DURATION IN SECONDS



	CONTROL				
Latent	Flexion	Extension	Clonus(sec	Post seizure depression	
2.67	4.33	19.33	2.67	285.674	



	Latent	Flexion	Extension	Clonus	Post seizure depression
CONTROL	2.67	4.33	19.33	2.67	285.674
STANDAR	2.33	3.33			

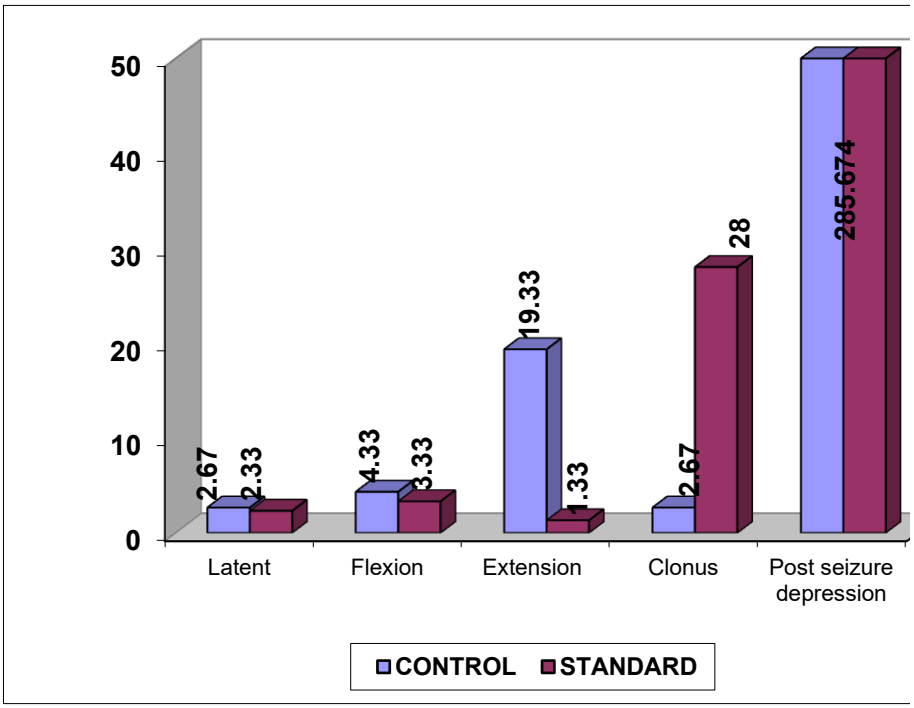



CONTROL
STANDARD

285.674
eizure
ssion

28
285
78
us
Post seizure
depression

	Latent	Flexion	Extension	Clonus	Post seizure depression
CONTROL	2.67	4.33	19.33	2.67	285.674
STANDAR	2.33	3.33	1.33	28	78

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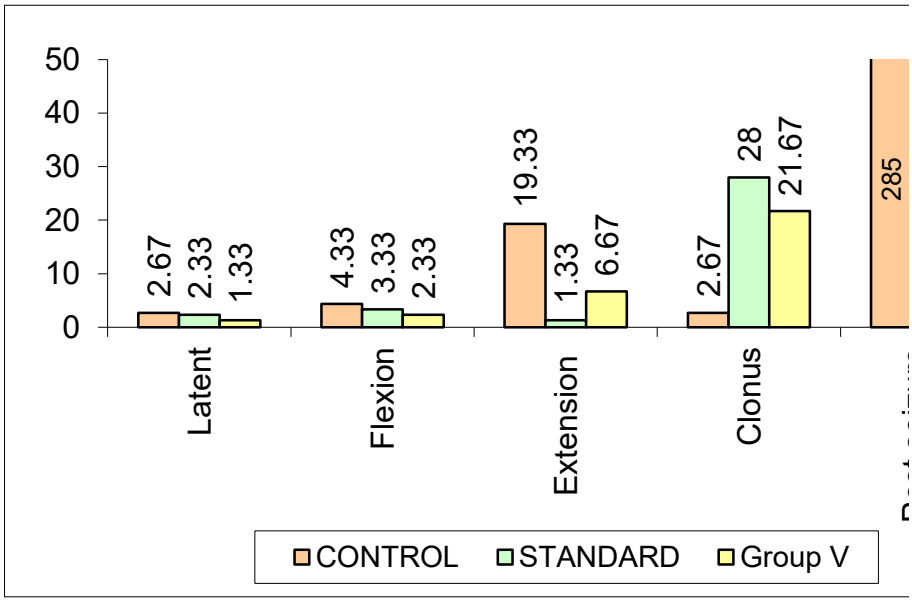
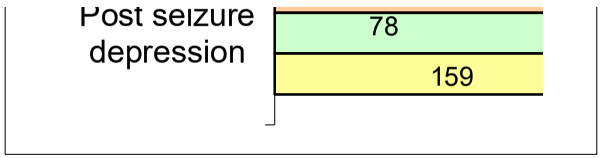
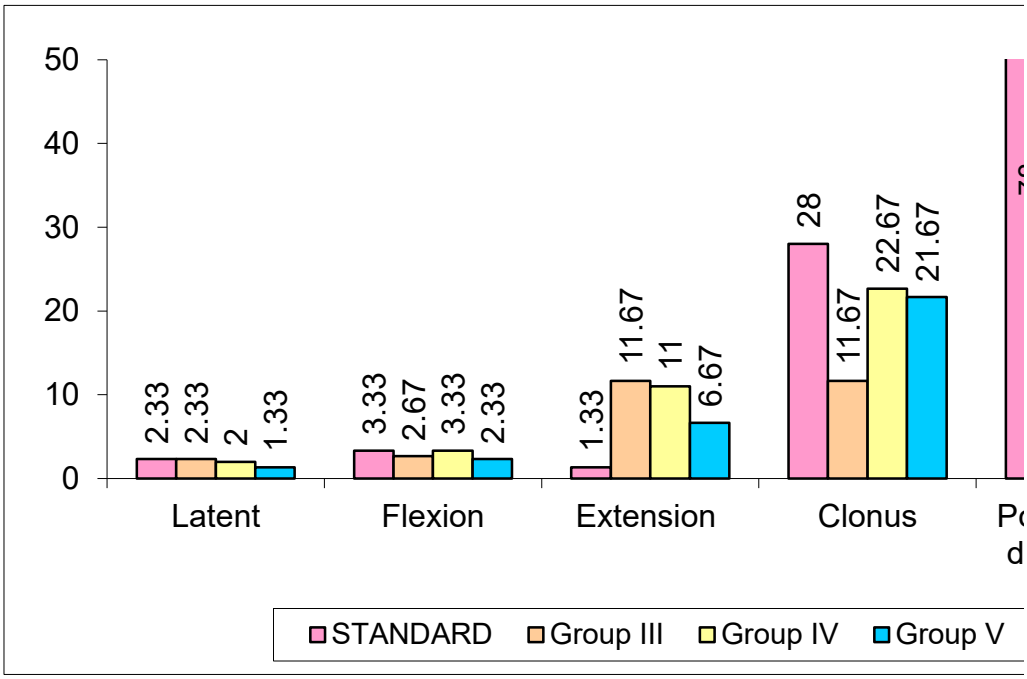
	Latent	Flexion	Extension	Clonus	Post seizure depressio
CONTROL	2.67	4.33	19.33	2.67	285.674
STANDAR	2.33	3.33	1.33	28	78
Group V	1.33	2.33	6.67	21.67	159.67




Post seizure depression
78
159

	Latent	Flexion	Extension	Clonus	Post seizure depressio
STANDAR	2.33	3.33	1.33	28	78
Group III	2.33	2.67	11.67	11.67	235.33
Group IV	2	3.33	11	22.67	219.33
Group V	1.33	2.33	6.67	21.67	159.67



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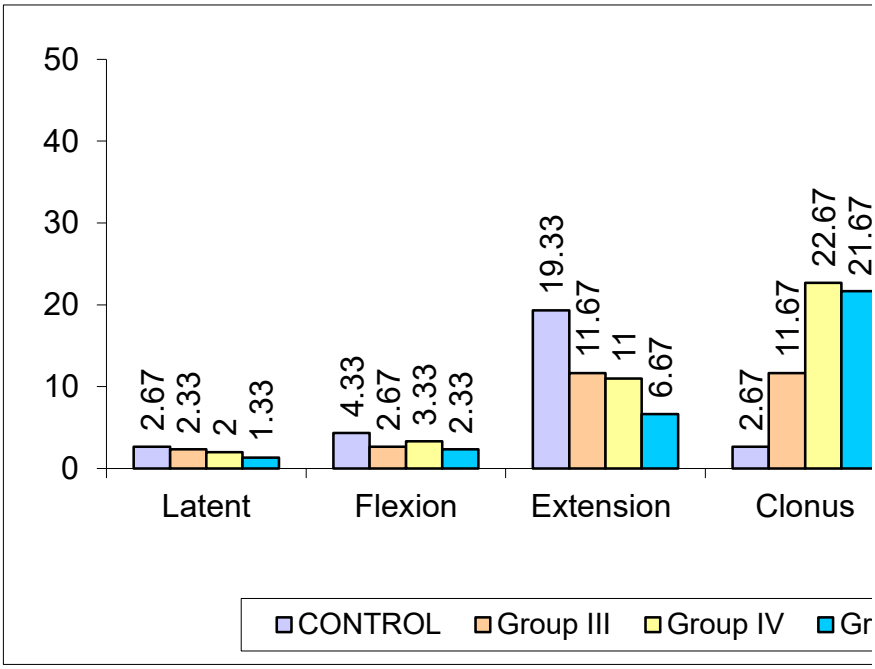
	Latent	Flexion	Extension	Clonus	Post seizure depressio
CONTROL	2.67	4.33	19.33	2.67	285.674
Group III	2.33	2.67	11.67	11.67	235.33
Group IV	2	3.33	11	22.67	219.33
Group V	1.33	2.33	6.67	21.67	159.67



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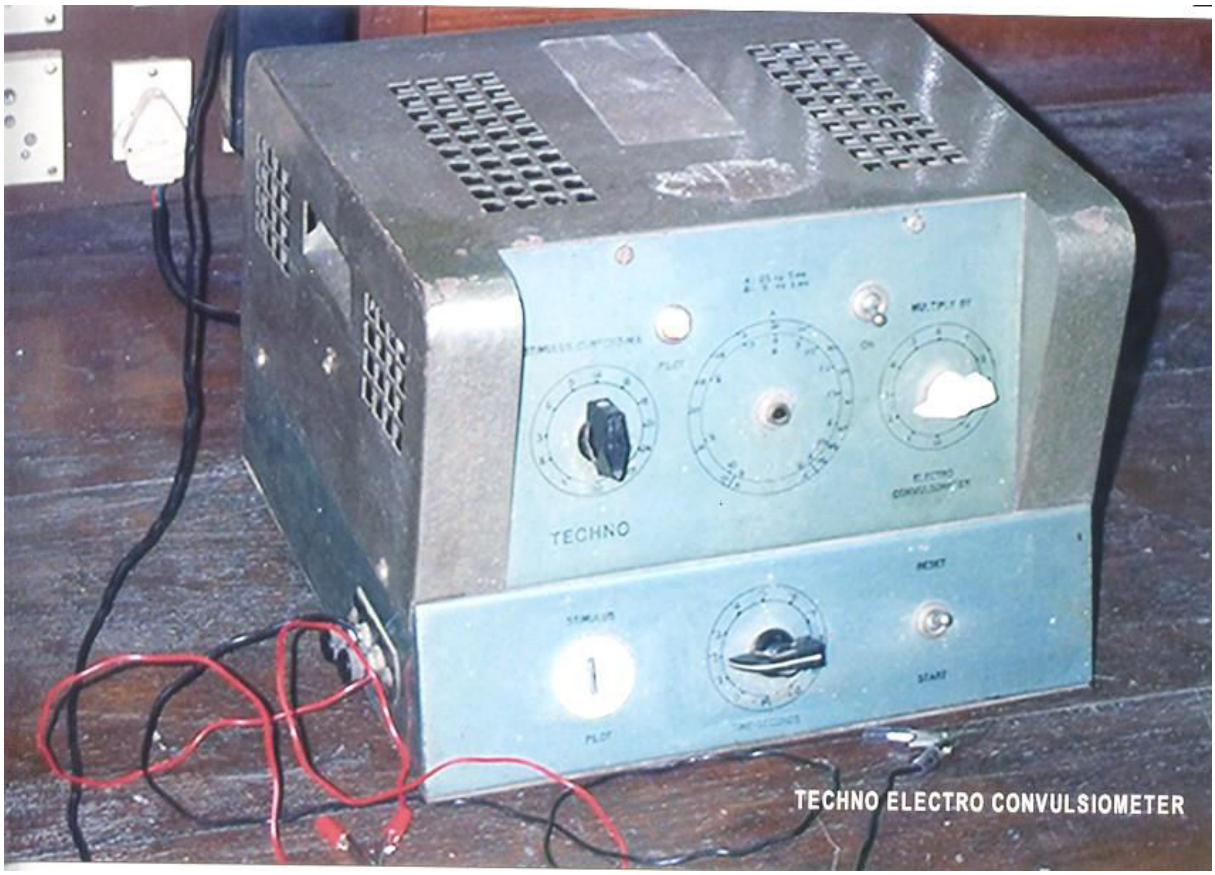
CISSUS QUADRANGULARIS

ELECTRO CONVULSIOMETER



MES INDUCED SEIZURES