EVALUATION OF ANTIEPILEPTIC ACTIVITY OF *Cucumis dipsaceus* FRUIT EXTRACT IN ANIMAL MODELS

A Dissertation Submitted to

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

MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted by

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Place: Coimbatore

DECLARATION

I hereby declare that the dissertation work entitled **"EVALUATION OF ANTIEPILEPTIC ACTIVITY OF *Cucumis dipsaceus* FRUIT EXTRACT IN ANIMAL MODELS"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment for the Degree of **Master of Pharmacy** in **Pharmacology** was carried out under the guidance of **DR. T. SENGOTTUVEL, M. Pharm., Ph.D.,** at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2018-2019.

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

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EVALUATION CERTIFICATE

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Examination Centre: KMCH College of Pharmacy, Coimbatore

Date:

Internal Examiner **External Examiner**

Convener of Examination

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The plant specimen brought by you for authentication is identified as *Cucumis dipsaceus* Ehrenb. ex Spach - CUCURBITACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

3.1.2019

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This is certify that the project title **Evaluation of Antiepileptic activity of Cucumis dipsaceus fruit extract in animal models** has been approved by the IAEC. Reg. No. 685/PO/Re/S/2002/CPCSEA.

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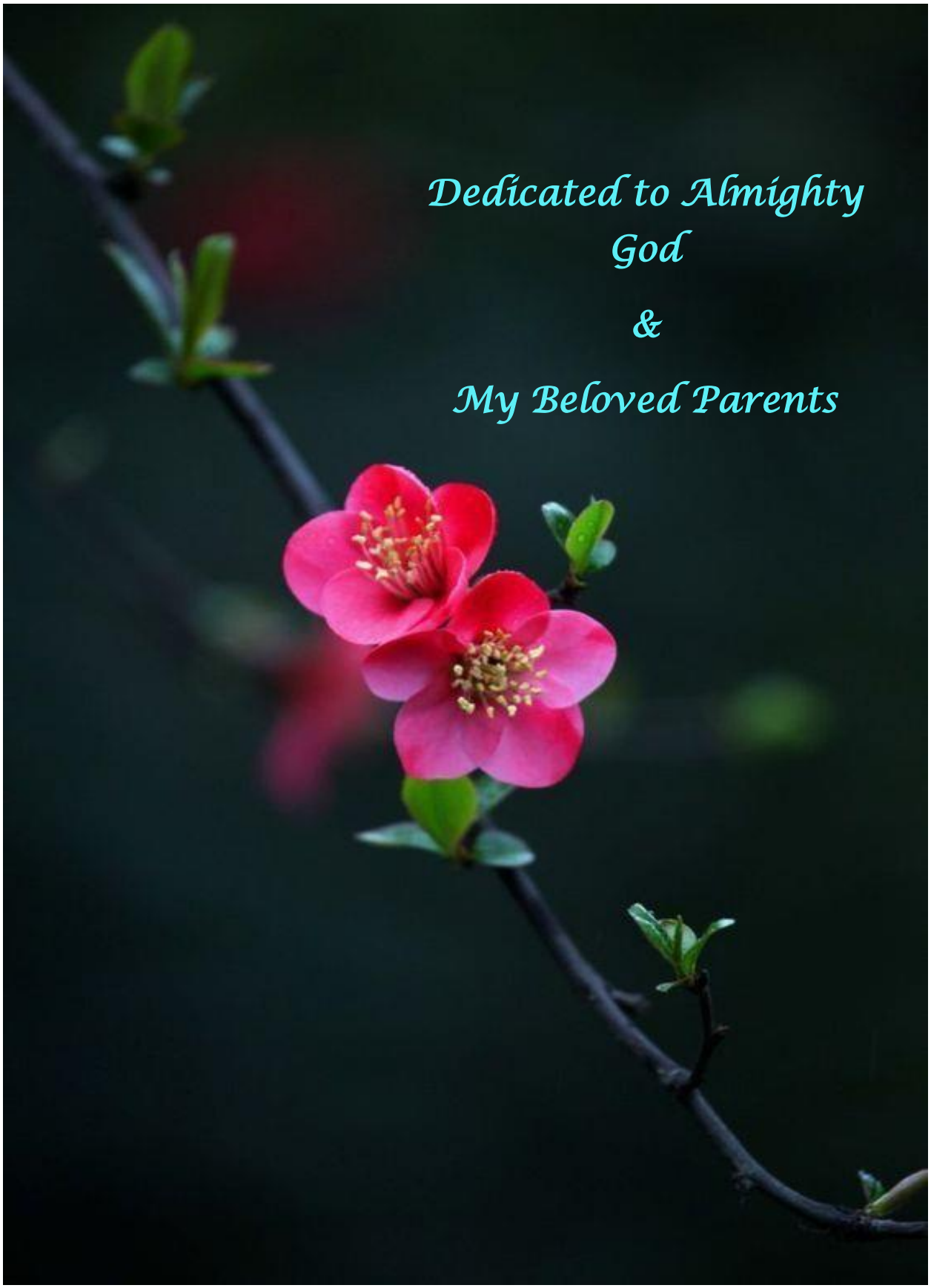
Dedicated to Almighty God
&
My Beloved Parents

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On this fruitful occasion of the successful completion of this dissertation, I bow my head to the **God almighty,** who is always showering blessings upon me and without whose blessings, I would not have been able to attain this stage in my life.

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Thank you all for the support and motivation

Reg No: 261725810

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ABBREVIATIONS

ABTS	2,2-azinobis-3- ethylbenzothiazoline-6-sulfonic acid
AED	Anti Epileptic Drugs
AMPA	α-amino-3-hydroxy-5-methyl4-isoxazoleproprionic acid
ANOVA	Analysis of variance
BSI	Botanical survey of India
CA	Cornu Ammonis
CPCSEA	Committee for the Purpose of Control And Supervision of Experiments on Animals
CT	Computed Tomography
DPPH	1,1-Diphenyl-2-picrylhydrazyl
DTNB	5,5'-DiThiobis-2-NitroBenzoic acid
EEG	Electroencephalogram
GABA	γ-aminobutyric acid
GC-MS	Gas Chromatography-Mass Spectrometry
GSH	Reduced glutathione
HAFEC	Hydro alcoholic fruit extract of cucumis dipsaceus
HPTLC	High performance thin layer chromatography
IAEC	Institutional Animal ethical Committee
IBE	International Bureau for Epilepsy
ILAE	International League Against Epilepsy
IUCN	International Union for Conservation of Nature
LPO	Lipid peroxidation
MES	Maximal electroshock
MDA	Malondialdehyde
MEG	Magenetoencephalography

MRI	Magnetic resonance imaging
MSI	Magnetic source imaging
NMDA	N-methyl-D-aspartate
OPT	O-phthaldialdehyde
PTZ	Pentylenetetrazole
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
SEM	Standard Error Mean
STD	Standard drug
SHFRF	Sustained, high-frequency, repetitive firing
TBA	Thio Barbituricacid
TCA	Tri Chloro Aceticacid
TLC	Thin Layer Chromatography
TP	Total protein
WHO	World Health Oragnisation

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1. INTRODUCTION

Epilepsy (Greek - to seize) is a neurological disorder afflicting ~65 million people worldwide. It is caused by aberrant synchronized firing of populations of neurons primarily due to imbalance between excitatory and inhibitory neurotransmission. Hence, the historical focus of epilepsy research has been neurocentric [1]. As per the current standard of the International League Against Epilepsy, an epilepsy diagnosis requires at least two unprovoked (or reflex) seizures occurring >24 h apart, one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures occurring over the next 10 years, or a diagnosis of an epilepsy syndrome [2]. Epileptic convulsions are expected to have negative consequences on the patient's psychological and social life such as relationships, education and employment [3]. More than half of the epileptics had some sort of cognitive problems with abnormal behavioral manifestations [4]. Despite of advanced medical management with modern anti epileptic drugs (AED), more than 30% of patients continue to have drug resistant epilepsy with frequent seizures [5]. Thus, an effective and safe therapy for epilepsy still remains a challenge and demand for new types of anti-epileptics still exists.

Natural plants have been a very much powerful source of drugs; particularly Indians have been using several plants, their parts and extracts as a medicine since Vedic days. According to WHO, 80% of world population, primarily those of developing countries rely on plant derived medicine for their healthcare. Even today, especially in rural India, people are still dependent on traditional herbal remedies for their primary health care. Therefore plants remain a rich source of discovery of new molecules and thus it has been exploited over decades for new drugs, to treat various diseases [6].

1.1. Global scenario of epilepsy

Epilepsy accounts for a significant proportion of the world's disease burden, affecting around 65 million people worldwide. The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people. Globally, an estimated five million people are diagnosed with epilepsy each year. In high-income countries, there are estimated to be 49 per 100000 people diagnosed with epilepsy each year. In low- and middle-income countries, this figure can be as high as 139 per 100000. This is likely due to the increased risk of endemic conditions such as malaria or neurocysticercosis; the higher incidence of road traffic injuries;

birth-related injuries; and variations in medical infrastructure, the availability of preventive health programmes and accessible care. Close to 80% of people with epilepsy live in low- and middle-income countries [7].

Globally, in 2016, there were 1·4 million idiopathic epilepsy cases in men and 1·3 million cases in women, with age-standardised incidence rates of 38·9/100000 person-years (32·7–45·7) for men and 37·1 per 100000 person-years (30·8–44·1) for women. Between 1990 and 2016, there were no significant changes in both age-standardised incidence rates and absolute number of people with incident idiopathic epilepsy. There were four times geographical variations in the age-standardised incidence rates of idiopathic epilepsy, with the highest rates observed in Ecuador (70·9/100000 person-years) and Mexico (56·0 per 100000 person-years) and the lowest rates in North Korea (17·0/100000 person-years) and China (19·7/100000 person-years). In 2016, the global age-standardised prevalence of all active epilepsy (idiopathic and secondary) was 621·5/100000 population. It varied from a low of 311·0 per 100000 population (253·4–370·5) in Japan to a high of 1287·7 per 100000 population (754·4–1791·3) in Cape Verde. The prevalence of idiopathic epilepsy was 326·7 per 100000 population (278·4–378·1). The prevalence was 329·3 per 100000 population (280·3–381·2) in men and 318·9 per 100000 population (271·1–369·4) in women. Highest prevalence was found in eastern, western, andsouthern sub-Saharan Africa regions, central Asia, central and Andean Latin America, and southeast Asia (Figure 1).[8]

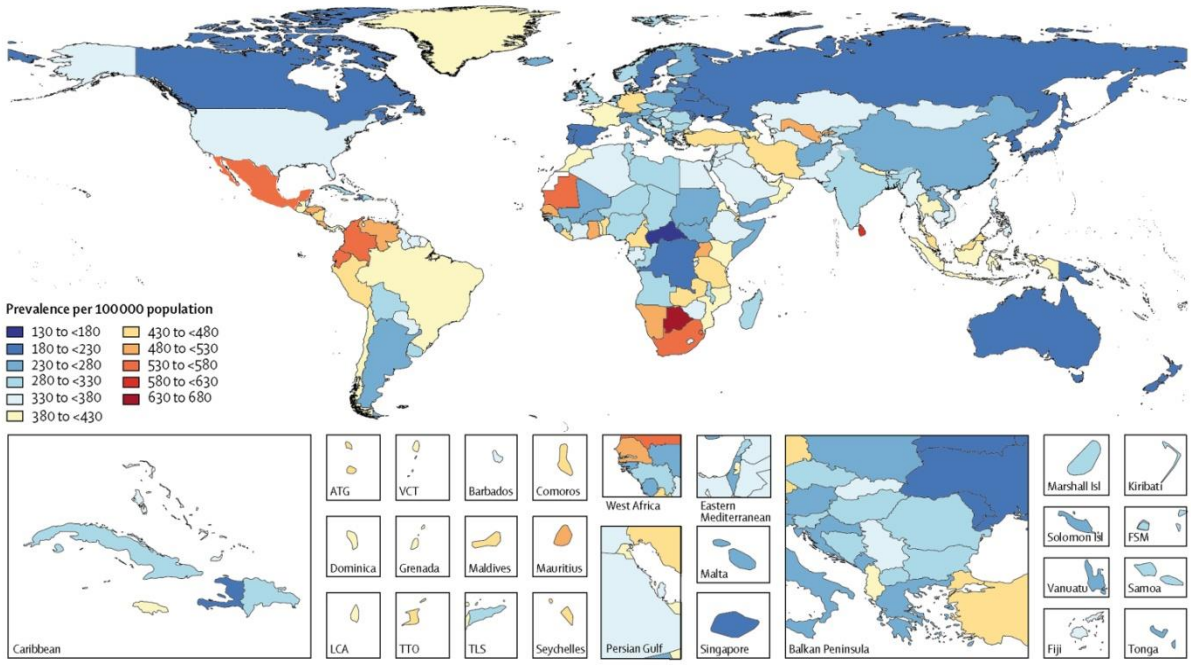

Figure 1: Global scenario of epilepsy per 100000 for both sexes, 2016

1.2. International Epilepsy Day

Despite being one of the world's oldest known neurological conditions, International Epilepsy Day was joint initiated in 2015 by the International Bureau for Epilepsy (IBE) and the International League Against Epilepsy (ILAE), [9] is a global event celebrated annually on the 2nd Monday of February, to promote awareness on epilepsy right around the world. With IBE and ILAE representation in more than 120 countries, this is a powerful opportunity to highlight the problems faced by people with epilepsy, their families and carers, in every region of the world [10].

1.3. Anatomy of hippocampus

The hippocampus is a major component of human brain. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. Like the cerebral cortex, with which it is closely associated, it is a paired structure, with mirror-image halves in the left and right sides of the brain. In humans and other primates, the hippocampus is located inside the medial temporal lobe, beneath the cortical surface. The hippocampus is composed of multiple subfields. It contains two interlocking parts, termed as dentate gyrus and the cornu ammonis (literally "Amun's horns", abbreviated CA). The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA_1, CA_2, and CA_3. Cut in cross section, the hippocampus is a C-shaped structure that resembles Ram's horns.

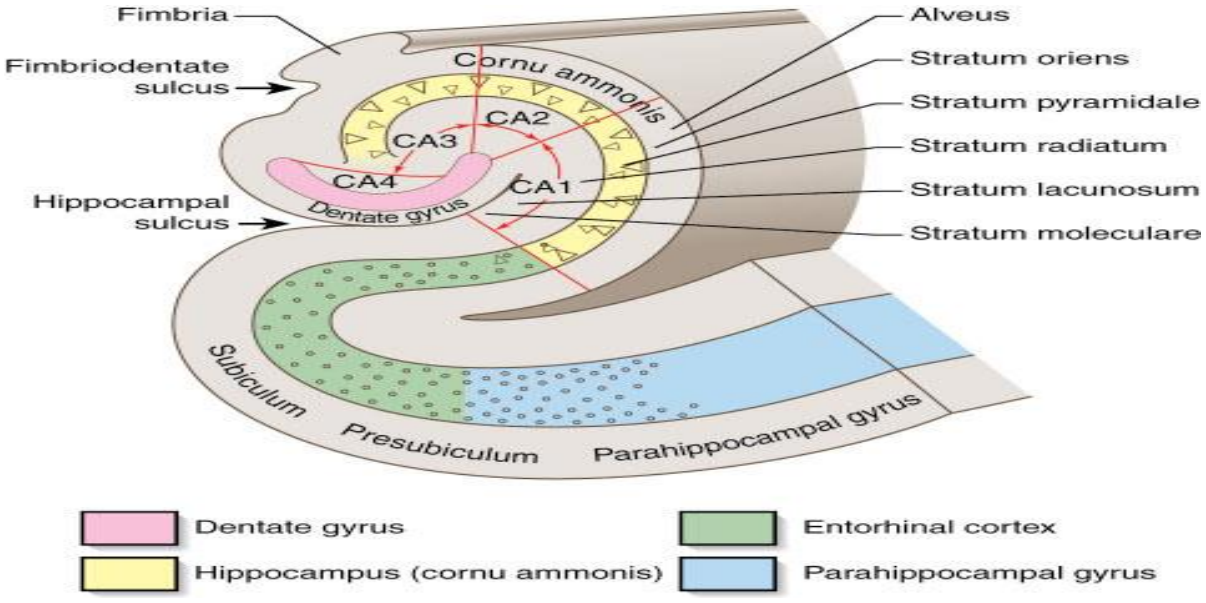

Figure 2: Anatomy of hippocampus.

As shown in (Figure 2), the structure itself is curved and subfields or regions are defined along the curve, from CA_4 through CA_1 (only CA_3 and CA_1 are labeled). The CA regions are also structured depth wise in clearly defined strata (or layers) such as alveus, Stratum oriens, Stratum pyramidale, Stratum lucidum, Stratum radiatum, Stratum lacunosum, Stratum moleculare, hippocampal sulcus.[11] Hippocampus is one of the most vulnerable brain area for epilepsy resultant from excitotoxicity [12] via NMDA and non-NMDA glutamate receptors (AMPA, kainate), and that the formation of black layer in hippocampal pyramidal neurons could be prevented by the blockage of glutamate receptors. [13]

1.4. Causes of epilepsy

Metabolic

- ✓ Hypoglycaemia
- ✓ Hypocalcaemia
- ✓ Electrolyte imbalance
- Pyridoxine deficiency
- Uraemia
- Phenylketonuria**

Infections

INTRACRANIAL	EXTRACRANIAL
✓ meningitis*	febrile illnesses (febrile convulsions)*
✓ encephalitis*	pertussis
✓ AIDS*	pertussis immunization
✓ cerebral malaria*	tetanus

Trauma

Anoxia

Toxic

- ✓ Alcohol and withdrawal from alcohol*
- ✓ Lead poisoning
- ✓ Organo-phosphorus insecticide poisoning
- Carbon monoxide poisoning
- Drugs (high dose i.v. penicillin)

Space-occupying lesions

- ✓ Haemorrhage
- ✓ Abscess
- Tuberculoma*
- Cysticercosis

Circulatory disturbances

Cerebro-vascular accident (stroke)

Sickle-cell crisis*

Congenital

Tuberous sclerosis (Bourneville disease)**

Neurofibromatosis (von Recklinghausen disease)**

Degenerative diseases

Niemann-Pick disease**

Dementias*

Cerebromacular degeneration**

** Genetic brain disorders with epilepsy*

***Idiopathic = genetic epilepsy without other disorders = epilepsy sui generis.*[14]

1.5. Classification of epilepsy

- ✓ Focal
  - Focal aware seizures
  - Focal impaired awareness seizures
  - Focal motor seizures
  - Focal non-motor seizures
  - Focal to bilateral tonic–clonic seizures
- ✓ Generalized
  - Absence seizures (petit mal)
  - Myoclonic seizures
  - Atonic seizures
  - Tonic seizures
  - Tonic-clonic seizures (grand mal)
- ✓ Combined Generalized and focal
- ✓ Unknown
- ✓ Unclassified
  - Neonatal seizures
  - Infantile spasms

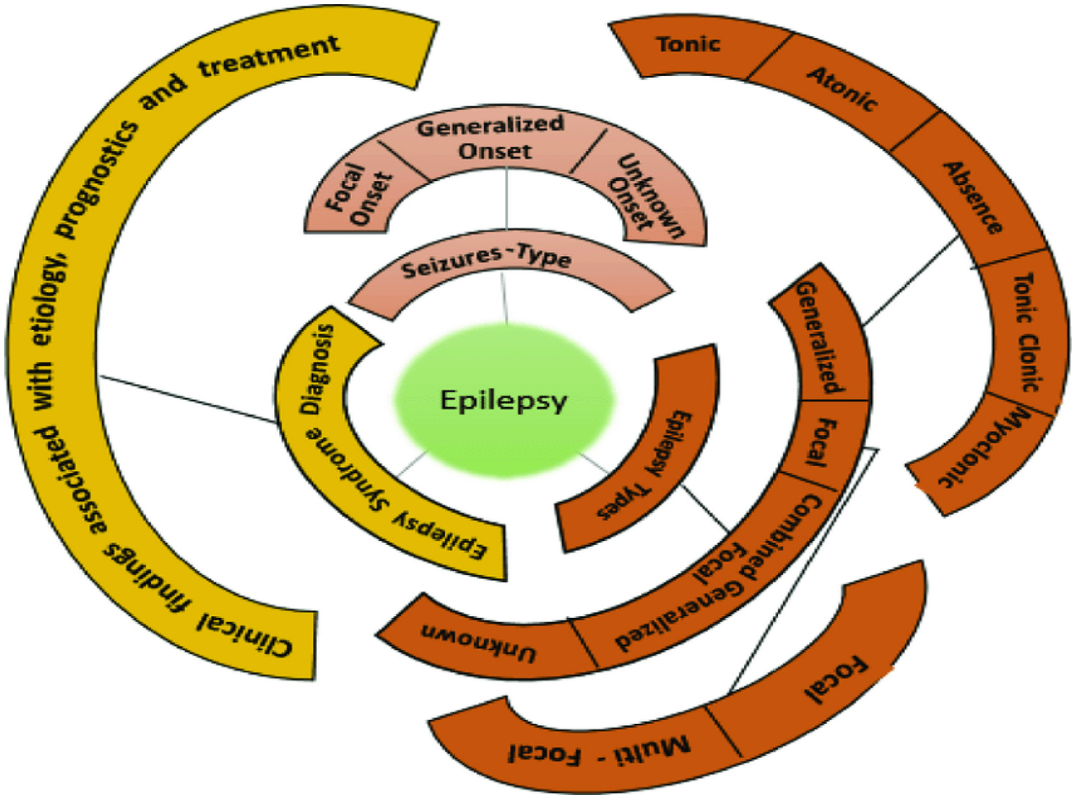

Figure 3: Classification of epilepsy according to the International League Against Epilepsy (ILAE)

1.5.1. Focal seizure

Seizures which start in one part of the brain and may affect a large part of one hemisphere or just a small area in one of the lobes.

Focal aware seizures

In these seizures the person is conscious (aware and alert), will usually know that something is happening and will remember the seizure afterwards.

Focal impaired awareness seizures

In these seizures, the person's consciousness is affected and they may be confused. They might be able to hear you, but not fully understand what you say or be able to respond to you.

1.5.2. Generalised onset seizures

Seizures which affect both sides of the brain at once and happen without warning.

Tonic clonic seizures

The seizure most people will recognise, where a person will jerk and shake as their muscles relax and tighten.

Atonic seizures and tonic seizures

In an atonic seizure (sometimes called drop attacks), the person's muscles suddenly relax and they become floppy, and they often fall, usually forwards. In a tonic seizure the person's muscles suddenly become stiff and they often fall, usually backwards.

Myoclonic seizures

Myoclonic means 'muscle jerk'. Muscle jerks are not always due to epilepsy (for example, some people have them as they fall asleep).

Absence seizures

Absence seizures (previously called petit-mal) are typical absences or atypical absences. During a typical absence the person becomes blank and unresponsive for a few seconds.

Atypical absences seizure

Similar to typical absences but they start and end more slowly, and last a bit longer [15].

1.6. Pathophysiology [16]

The neuronal cell membrane is populated by a variety of ion channels that convert chemical signals into electrical activity. As such, ion channels have traditionally been regarded as the "currency" of neuronal excitability. A variety of chemical signals act through

ligand-gated and voltage-gated ion channels to generate excitatory or inhibitory signals, which may be subthreshold or suprathreshold for subsequent action potential initiation which in turn give rise "downstream" to action potential propagated neurochemical signals. Traditionally, there are three major classes of molecular targets believed to be relevant for limiting epileptic activity. These include: (1) voltage-gated sodium and calcium channels; (2) γ-aminobutyric acid ($GABA_A$) receptors; and (3) ionotropic glutamate receptors. Clinically useful AEDs exert their effects principally on one or more of these targets.

1.6.1. Synaptic Excitation

Neuronal excitation in the central nervous system is mediated by ionotropic glutamate receptors (ie, α-amino-3-hydroxy-5-methyl4-isoxazoleproprionic acid or AMPA, kainate, and N-methyl-D-aspartate or NMDA) and metabotropic glutamate receptors (Figure 4). Inhibition of excitatory glutamatergic neurotransmission would appear the most obvious way to limit excessive excitatory activity, and this conceptual notion has been supported by many in vivo and in vitro preclinical studies.

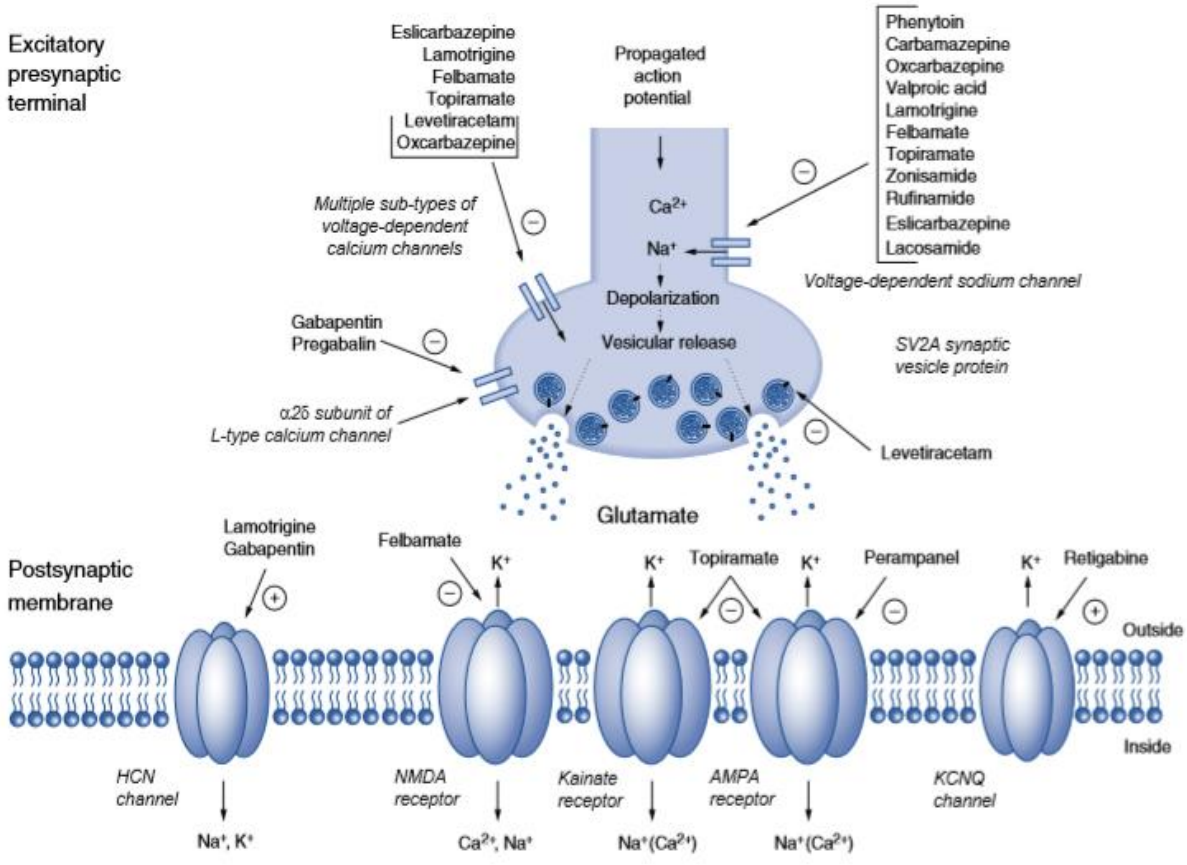

Figure 4: Mechanism of synaptic excitation

1.6.2. Synaptic Inhibition

Neuronal inhibition in the central nervous system is primarily achieved by the presynaptic release of the neurotransmitter GABA, which binds to ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. $GABA_A$ and $GABA_B$ receptors mediate fast and slow inhibition in the brain, respectively. During normal neuronal activity, communication between inhibitory GABAergic interneurons and excitatory glutamatergic neurons provides the critical balance necessary to prevent the development of synchronized epileptiform discharges-discharges that would naturally arise in an unchecked, recurrently connected excitatory network. However, the role of inhibition with respect to epileptiform activity is not simple. For example, it appears that inhibitory neurotransmission may actually participate in the synchronization of large populations of neurons and the subsequent propagation of seizures to adjacent brain regions. Thus, AEDs that act principally to augment GABAergic neurotransmission may prevent the initiation of epileptiform activity; however, such agents may also in some cases exacerbate the synchronization and spread of seizure activity, notably in thalamocortical circuits. There are several distinct ways that GABAergic inhibition can be augmented: (1) increased $GABA_A$ and $GABA_B$ receptor function; (2) enhanced GABA synthesis and decreased degradation; and (3) inhibition of GABA re-uptake into neuronal and glial cells (Figure 5).

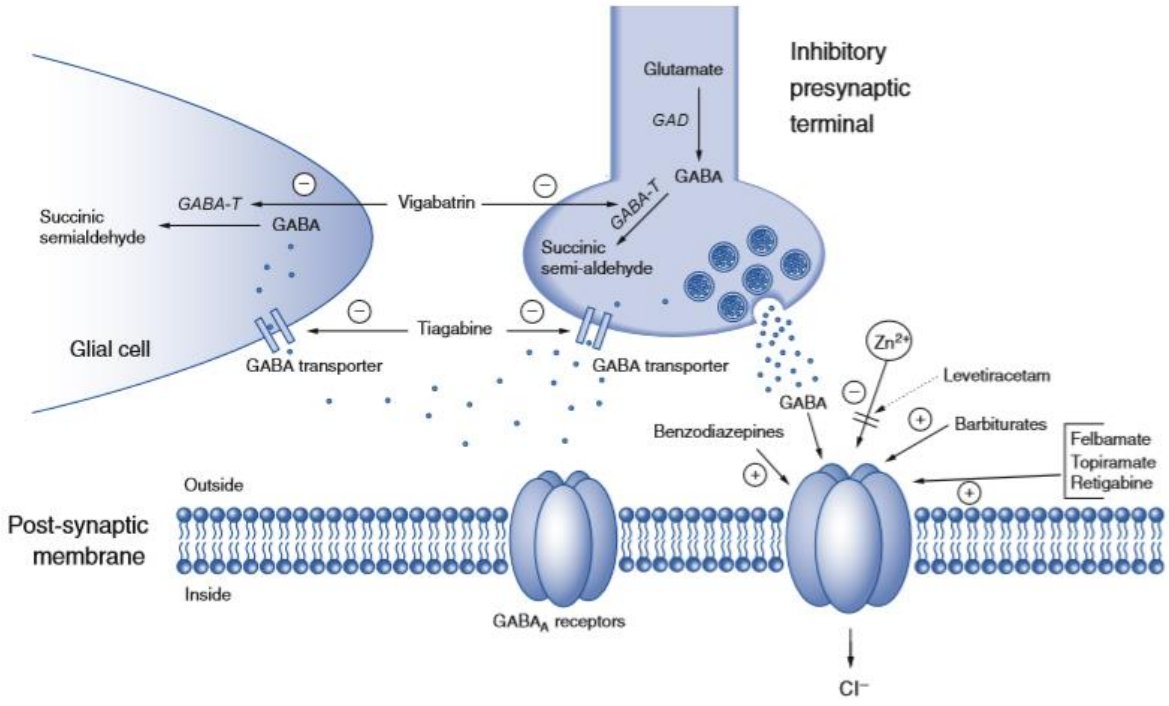

Figure 5: Mechanism of synaptic inhibition

1.7. Pharmacological treatment

Table 1: Antiepileptic drugs used for treatment of convulsion

Antiepileptic Drugs Approved for the Treatment of Seizures in the U.S.			
Generalized Tonic Clonic Seizures	Partial Seizures	Absence Seizures	Atypical Absence Myoclonic & Atonic Seizures
First-line agents			
Valproic acid Lamotrigine Topiramate	Carbamazepine Phenytoin Oxcarbazepine Valproic Acid	Valproic acid Ethosuximide	Valproic acid Lamotrigine Topiramate
Alternative agents			
Zonisamide+Phenytoin Carbamazepine Oxcarbazepine Phenobarbital Primidone Felbamate	Levetiracetam+ Topiramate Tiagabine+Zonisamide+Gabapentin Phenobarbital, Primidone, Felbamate, Eslicarbazepine, Vigabatrin, Lacosamide, Pregabalin, Rufinamide	Lamotrigine Clonazepam	Clonazepam Felbamate

1.7.1. Mechanism of action of antiepileptic drugs

Seizures result from episodic electrical discharges in cerebral neurons associated with prolonged depolarization, during which sustained, high-frequency, repetitive firing (SHFRF) occurs, followed by prolonged hyperpolarization. The goal of drug management is restoration of normal patterns of electrical activity. The mechanisms include:

- ↑ Inhibitory tone by facilitation of GABA-mediated hyperpolarization - barbiturates, benzodiazepines
- ↓ Axonal conduction by preventing Na^+ influx through fast Na^+ channels - carbamazepine, phenytoin; also, at high doses, barbiturates and valproic acid
- ↓ Presynaptic Ca^{2+} influx through type-T channels in thalamic neurons - ethosuximide and valproic acid
- ↓ Excitatory effects of glutamic acid-lamotrigine, topiramate (blocks AMPA receptors); felbamate (blocks NMDA receptors)

1.8. Diagnosis

- CT scan
- EEG (Electroencephalogram)
- MEG/MSI (Magenetoencephalography / Magnetic source imaging)
- PET (Positron emission tomography)
- MRI (Magnetic resonance imaging)
- SPECT (Single photon emission computed tomography).[17]

1.9. Other treatments

- Surgery
- Vagus Nerve Stimulator
- Ketogenic Diet [18]

1.10. Common epilepsy models [19-21]

Table 2: Various models for seizure induction in animals

Models	Methods to induce convulsion	Types of seizures
In vivo models	**Electrical stimulation:**	
	Maximal electroshock(MES)	Generalised tonic-clonic seizures
	Kindling	Chronic partial seizures
	Chemoconvulsants:	
	Pentylenetetrazole (PTZ)	Myoclonic and absence seizures
	Strychnine	Acute simple partial seizures
	Picrotoxin	Acute simple partial seizures
	Isoniazid	Clonic-tonic seizures
	Lithium pilocarpine	Status epilepticus
	Yohimbine	Clonic seizures
	Bicuculline	Acute simple partial seizures
	4-aminopyridine	Clonic-tonic seizures
	N-methyl d- aspartate	Status epilepticus
	Penicillin	Generalised tonic-clonic and Absence Seizures

In vitro models	Hippocampal slices,$GABA_A$ receptor binding Assay	Complex partial seizures
Genetic models	Photosensitive baboons Audiogenic seizures mice Totterer mice and seizures prone mouse strains Genetically epilepsy prone rats	Generalised tonic- clonic seizures

1.11. Scoring of animals [22]

Seizure scoring in developing animals were rated using a 5-point scoring system

0 = no change in behavior.

0.5 = scratching, chewing, tremors.

1 = 0.5 + myoclonic jerks.

2 = unilateral clonus and backing/shuffling.

3 = bilateral facial and forelimb clonus.

4 = clonic seizure with loss of righting.

4.5 = clonic seizure with running/bouncing or swimming like behavior.

5 = tonic extension (both forelimb and hind limb).

Table 3: Seizure scoring in animals

Score	Seizure	Type
0.5–3	clonic	"minor"
4-5	tonic–clonic	"major"

Latency to clonic or tonic–clonic seizure onset was defined as the time elapsed between Induction and the first observed clonic or tonic–clonic seizure response, respectively [23].

Most antiepileptic drugs do not prevent or reverse the pathological process that underlies epilepsy, hence continuing search for new therapies with fewer side effects and better efficacy [24]. Moreover, 30–40% of patients typically develop pharmacoresistant or intractable epilepsy [25]. In most cases, traditional healers are often the first line of contact in the search of therapy because of its link to supernatural powers [26], unavailability and high cost of conventional AEDs in developing countries [27].

Herbal medicine plays a very important role in meeting the primary health care needs of the population, with Africa and Asia being the continents with most of the users [28].

Stringer [29] states that more than 50 plants have shown some anticonvulsant activity whereas Xiao *et al.* [30] noted that 23 botanicals were used in Chinese traditional medicine to treat epilepsy but none of them has been developed into a standard medication for the treatment of seizures. Plants which have proven antiepileptic activity belongs to the Cucurbitaceae family are listed below,

- *Cucurbita pepo*
- *Melothria maderaspatana*
- *Momordica balsamina*
- *Momordica charantia* [31]

The therapeutic potential of herbal plants and some of their bioactive compounds has been the subject of extensive research. As with any other drug discovery, the process involves a screening step in animal models. Herbal medicines generally have a broad spectrum because they are an assortment of bioactive compounds. It is worth noting that most of the extracts tested were chosen based on knowledge that they are traditionally used against other ailments or as antiepileptics by traditional herbalists. Although herbs have been used for years and tested in animal trials, there is a lack of standardization and safety and efficacy studies, restricting their utilization in modern medicine [30].

For a substance to be an effective anticonvulsant, it must cross the blood-brain barrier. Selecting the appropriate model is a key factor in AED screening in the case of false-positive or false-negative results [32] as different models could simulate dissimilar kinds of epilepsy. The processes underlying epileptogenesis differ among models. Maximal electroshock (MES) and subcutaneous pentylenetetrazol (PTZ) are the two most widely used models in screening compounds for antiepileptic activity. Other less commonly used models exist, each modelling a particular form of epilepsy. Positive results in either model suggest that the test compound or its metabolite crossed the blood brain barrier and exerted its effect in the central nervous system [27].

2. REVIEW OF LITERATURE

2.1. Literature for plant

Madhua CS *et al.*, (2019) studied Fibrinogenolytic activity of serine proteases(s) from *Cucumis dipsaceus*. The aqueous *Cucumis dipsaceus* dialysate fraction (AqCDF) hydrolyzes casein and exhibiting a protease activity in a dose-dependent manner. The AqCDF shows a promising recalcification time up to 80% against trypsin. More interestingly AqCDF (300 µg) did not induce hemolysis even at higher concentration. These findings suggest the possible role of the dialyzed fraction of *Cucumis dipsaceus* in hemostasis and the wound healing process [33].

Suman L *et al.*, (2017) studied *In vitro* and *in vivo* hepatoprotective activity of flavonoids rich extracts on *Cucumis dipsaceus* Ehrenb. (Fruit). They evaluated the *in vitro* hepatotoxicity actyivity by performing cytotoxicity assay in HepG2 cell libne and *in vivo* hepatoprotective activity in CCl4 induced liver toxic rats. The results of her study revealed that the significant hepatoprotective activity of Flavonoids Rich Extracts of *Cucumis dipsaceus* Ehrenb fruits [34].

Nivedhini V *et al.*, (2014) reported the nutritional and antioxidant property of *Cucumis dipsaceus*. The results revealed significant amount starch (1.07 mg/g), proteins (85.9 mg/g), essential aminoacids and some most important minerals like calcium (14820 ppm) and nitrogen (6300 ppm). The phenolic (3.04 g GAE/100 g extract) tannin (1.66 g GAE/100 g extract) and flavonoid content (11.26 g RE/100 g extract) was found to be high in ethyl acetate, chloroform and methanol extract of fruit. This study clearly pointed out the nutritional and antioxidant properties of *Cucumis dipsaceus* which could support its use as a nutraceutical supplement in health promoting diets [35].

Urs SK *et al.*, (2013) studied the antioxidant activity of aqueous and methanolic extracts of *Cucumis dipsaceous* fruit. The results obtained in the *in vitro* models such as Reducing power assay, DPPH radical scavenging activity, Superoxide anion radical scavenging activity, Nitric oxide scavenging assay, and Metal ion chelating activity clearly suggest that, both the aqueous and methanolic extracts showed strong antioxidant activity when compared with different standards such as BHA, EDTA and Curcumin. In aqueous and methanolic extracts of *Cucumis dipsaceous*, 31.5 ± 2 and 43.1 ± 3.1 mg (gallic acid equivalent per gram) of phenols were

detected respectively. There is little correlation between antioxidant activity and phenol content [36].

Vasanth Kumar G *et al*., (2013) studied the phyto-chemical screening and antibacterial activity of *Cucumis dipsaceus* fruits. Phytochemical screening based on tests of colouration and precipitation were undertaken by methanol as a solvents. The tests carried out on friuts show presence of tannin, alkaloids, saponin, flaviniods, resins, steroids. However, absence of reducing sugar, carbohydrates and phenol observed. Antibacterial activity test carried out on three bacteria such as E-coli (-ve) Bacillus subtilis (+ve) Staphylococcus aureus (+ve). Maximum inhibition zone shown in Bacillus subtilis (+ve) and minimum in Ecoli (-ve) [37].

Salama AM *et al.*, (1999) studied the Analgesic, Anti-Inflammatory Activity of fruit of *Cucumis dipsaceus*. The extracts of petroleum ether, dichloromethane, methanolic and ethanolic *Cucumis dipsaceus* (fruit) showed high analgesic and anti-inflammatory activity as reported by authors but dichloromethane and methanolic extract showed highest analgesic effect and highest anti-inflammatory activity of dichloromethane extract [38].

2.2. Literature for anti-epileptic activity

Aaditya singh *et al.*, (2019) screened the anticonvulsant activity of *Rauwolfia tetraphylla* leaf extract using maximal electroshock seizure (MES) model and pentylenetetrazole (PTZ)-induced seizure model in Swiss albino mice. The ethanolic extract was also evaluated for rutin and gallic acid content by High performance thin layer chromatography studies. HPTLC results showed that the presence of antioxidants such as rutin and gallic acid was 15.60% and 7.81%respectively in the extract. Extract at different doses (200, 400, 600 mg/kg) showed reduction in duration much as standard showed complete abolition of extension phase in MES model. The onset of extensor phase increases with dose in PTZ model [39].

Barve KH *et al.*, (2019) evaluated the anti-epileptic activity of Methanolic extract (ME) and Acetone extract (AE) of *Sphaeranthus* flowers against Maximal electro shock (MES) seizures, Pentylenetetrazole (PTZ) induced convulsions and Picrotoxin induced convulsions.convulsions were experimentally induced by the administration of single subcutaneous dose of PTZ (80 mg/kg), Picrotoxin (3.5 mg/kg) after 1hour and MES (60Hz AC,150mA) for 0.2s using electrodes at the ear pinna after 45mins of vehicle, ME, AE and std administration. The results illustrate that mice pretreated with ME and standard showed increase in latency whereas AE

failed to show any increase in latency at both the doses in PTZ model. But both ME and AE did not show effect on picrotoxin as well as MES models [40].

Mahmoud RK *et al.*, (2019) explored the effect of pretreatment with hydroalcoholic extract of *Alpinia officinarum* rhizome on the severity of epilepsy and memory impairment in rat. The rats received *A. officinarum* extract at different doses (50, 100 and 150 mg/kg) as pretreatment for 10 days and on 10th day 30 minutes before PTZ injection. *A. officinarum* extract treated rats exerted significant increase in seizure latency and significant decrease in the frequency of total body seizure, frequent spinning, and jumping in PTZ-induced kindling model. Flumazenil (150 mg/kg) significantly inhibited the antiepileptic effects of *A. officinarum* extract in the rat receiving the extract. The results suggested that *A. officinarum* extract can inhibit PTZ-induced seizure and memory impairment [41].

Raafat K *et al.*, (2019) studied the anticonvulsant activity of *Ficus carica* methanol extract (Fc) and its oligosaccharides rich fraction (OFG). Both Fc and OFG reduced strychnine (STR) convulsion-action and fully protected the experimental-animals from STR-lethality. The intra cerebro ventricular-administration (ICV) of Fc or OFG in combination with glycine in ethanol-treated mice caused a dose-dependent returning to a 2nd-loss of righting-reflex (LORR), and was antagonized by STR. FC and OFG ICV injection counteracted STR-inhibition, confirming that Fc/OFG anticonvulsant mechanism of action was mediated by potentiation of glycine receptor [42].

Rahimi VB *et al.*, (2019) investigated the anticonvulsant effects of *Viola tricolor* (*V. tricolor*) on seizure models induced by pentylenetetrazol (PTZ) and maximal electroshock stimulation (MES) in mice. 30minutes after treatment with the hydroalcoholic extract of *V. tricolor* (VHE 100, 200, and 400 mg/kg) and its ethyl acetate (EAF 50, 100, and 200 mg/kg) and n-butanol (NBF 50, 100, and 200 mg/kg) fractions as well as diazepam (3 mg/kg), seizure was induced by PTZ (100 mg/kg) or by MES (50 Hz, 1 s and 50 mA). The results showed that *V. tricolor* and its ethyl acetate and n-butanol fractions possessed anticonvulsant effects as confirmed by the prolongation of latency to the first GTCs induced by PTZ and decrement in the incidence of HLTE induced by MES model [43].

Nkantchoua GCN *et al.*, (2018) demonstrated the anticonvulsant effects of *Senna spectabilis* decoction on seizures induced by MES (50 Hz, 30 mA, 0.2 s), PTZ (70.0 mg/kg), Pilocarpine PC

(42.6 to 426.0 mg/kg) and its possible action mechanisms in animal models using flumazenil (FLU), methyl-ß-carboline-3-carboxylate (BC) and bicuculline (BIC). *Senna spectabilis* decoction could interact with $GABA_A$ complex receptor probably on the GABA and benzodiazepines sites, at the dose of (106.5 and 213.0 mg/kg) antagonized completely tonic-clonic hind limbs of mice induced by MES and dose of (42.6 mg/kg) provided 100%of protection against PTZ (70 mg/kg) and 75% protection against PC models.Both FLU and BC antagonize strongly the anticonvulsant effects of this plant and are unable to reverse totally diazepam or the plant decoction effects on inhibiting seizures [44].

Nonato DT *et al.*, (2018) evaluated the anticonvulsant effect of a polysaccharide rich extract (PRE) from *Genipa americana* leaves was mediated through GABA receptor, decreasing the number of hippocampal black neurons. The results showed that FT-IR spectrum bands around 3417 cm−1 and 2928 cm−1, relative to the vibrational stretching of O-H and C-H, respectively, ^{1}H NMR spectrum signals at δ 3.85 (methoxyl groups) and δ 2.4 (acetyl)ppm and ^{13}C NMR spectrum signals at δ108.0 and δ61.5 ppm, corresponding to C1 and C5 of α-L-arabinofuranosyl residues. PRE presented central inhibitory effect, increasing the latency for PTZ-induced seizures by 63% (9 mg/kg) and 55% (27 mg/kg), and the latency to death by 73% (9 mg/kg) and 72% (27 mg/kg). Both effects were reversed by the association with flumazenil [45].

Kaushik A *et al.*, (2018) studied the anticonvulsant effect of the stem bark of *Anogeissus latifolia (Roxb.) by* MES (50 mA for 0.2 seconds through cornea) induced hind limb tonic extension and PTZ (80 mg/kg, *i.p.*) induced clonic convulsions in mice. Different concentrations of ethanolic extract of stem bark of *A. latifolia* (200, 400, and 600 mg/kg) were administered orally to mice. The ethanolic extract of stem bark of *A. latifolia* showed significant dose dependant protection against seizures in both MES and PTZ-induced convulsion models. These findings suggested that ethanolic extract of stem bark of A. latifolia exhibited anticonvulsant effect against MES and PTZ-induced convulsions in a dose-dependent manner which may be attributed to the presence of ellagic acid and other tannins [46].

Oscar HC *et al.*, (2018) determine the effect of *Cyperus articulatus L.*(CA) ethanolic extract on pentylenetetrazol (PTZ) induced seizures in Balb/C albino mice as well as measuring its antioxidant activity in vivo and in vitro. Administered 50, 150 and 300 mg/kg of CA extract, 30 min prior to each PTZ injection (80mg/kg, *i.p.*). The PTZ-CA 150 group showed lower seizure

scores (P < 0.01), latency (P < 0.01), frequency (P < 0.01) and duration (P < 0.01) than control group. The antioxidant activity of CA extract scavenged DPPH radical showed IC 50 = 16.9 ± 0.1 mg/ml and TEAC = 2.28 ± 0.08, mmol trolox/g of extract, the content of gamma amino butyric acid (GABA) and malondialdehyde (MDA) were significantly high (P < 0.01) at dose of 150 mg/kg (82 ± 1.2 ng/g tissue;1.0 ± 2.2 mol/g tissue, respectively) [47].

Shelar MK *et al.*, (2018) evaluated the anticonvulsant activity of ethanolic leaf extract of mangrove plant *Excoecaria agallocha* in swiss albino mice and male wistar rats against Maximal Electroshock (MES)(150mA for 0.2s using corneal electrodes on cornea) and Lithium-Pilocarpine [pilocarpine (30mg/kg, *i.p.*) 24h after lithium sulphate (3mEq/kg, *i.p.*)] convulsion method. The ethanolic leaf extract subjected to acute toxicity study showed LD_{50} of 2.12 g/kg of oral dose and 3.12 mg/kg of intraperitoneal dose. The extract (100, 200 and 400 mg/kg) showed significant dose dependant delay in onset of seizures in MES model and reduction in intensity of seizures in the Lithium-Pilocarpine epilepsy model [48].

2.3. Literature for HPTLC quantification

Ankita AP *et al.*, (2017) developed the simultaneous estimation of quercetin and gallic acid in, Vitaceae. The system was found to give well resolved bands for quercetin (R_f 0.63) and gallic acid (*Leea indica* R_f 0.45) from other constituents present in the extract of *L. indica*. The correlation coefficient was found to be 0.991 and 0.999 with relative standard deviation, 0.97–1.23% and 0.1–1.13% for quercetin and gallic acid respectively in the developed method. The proposed validated high performance thin layer chromatographic method offers a new, sensitive, specific and precise gauge for quantification of quercetin and gallic acid in *L. indica* [49].

Vishal RM *et al.*, (2016) developed HPTLC method for the quantitative estimation of gallic acid, rutin and querecetin from methanolic extract of *Portulaca quadrifida L*. The R_f values of gallic acid, rutin and quercetin are 0.41, 0.19 and 0.94 respectively. The total peak areas of the standards (gallic acid, rutin and querecetin) and the corresponding peak areas of extract were compared and the gallic acid, rutin and quercetin content were estimated to be 790.9, 2029.7 and 4326 [50].

2.4. Literature for *in vitro* antioxiant study

Ahmed D *et al.*, (2015) analyzed for phenolic and flavonoid contents, and antioxidant and antimicrobial potential in methanolic, hexanic, and aqueous extracts of *Adiantum caudatum*

leaves. Antioxidant activities of the extracts in reducing power, FRAP (ferric reducing antioxidant power), phosphomolybdate and ABTS assays follow the same order of methanolic > aqueous > hexanic. In the DPPH assay, however, the aqueous extract exhibited a slightly higher antioxidant activity than the methanolic one. Methanol is therefore a better solvent to extract most of the antioxidant components from *A. caudatum* leaves. In lipid peroxidation inhibitory assay, the extracts showed almost similar behavior and their activity decreased gradually with time. And concluded *A. caudatum* can potentially provide a remedy against disorders caused by oxidative stress and infections [51].

2.5. Literature for neurotransmitters level

Sameh MM *et al.*, (2019) investigated the anticonvulsant and neuroprotective activity of Hydroalcoholic *Phoenix dactylifera* fruit extract (HAPD) and *Pimpinella Anisum* Oil (PAO) against Pentylenetetrazole (PTZ) and Maximal Electric Shock (MES) induced a seizure in mice. Mice groups were treated with HAPD 1000 mg/kg, PAO 4ml/kg. The onset of a seizure and generalized seizure were measured, followed by GABA and antioxidant enzymes assessments. The potential of these substances to induce any neurological toxicity was also evaluated by using rotarod, forced swim test and horizontal screen test. HAPD and PAO delay onset of the seizure and generalized seizure, also elevated GABA and antioxidant enzymes level and haven't show any neurological toxicity [52].

Zadeh MM *et al.*, (2019) evaluated the role of serotonin and its receptors in the anticonvulsant effect of curcumin in mice. The results suggested that curcumin exerts inhibitory effect on seizure induced by PTZ through increasing the serotonin levels in the brain that influence receptors, including 5-HT1A, 5-HT2C, and 5-HT4 and likely through the reduction of 5-HT7 gene expression and/ or HTR1A, HTR2C and HTR4activation [53].

Zhang L *et al.*, (2018) elucidated the molecular mechanism of tempol in pentylenetetrazol-induced epilepsy in mice and the role of gamma-amino butyric acid, tumor necrosis factor-alpha, interleukin-1β and c-Fos. Mice pretreated with tempol (100 and 200 mg/kg) showed significantly ($P < 0.01$ and $P < 0.001$) delayed-onset on tonic-clonic convulsion, decrease the duration of convulsions and mortality in mice, significant increase in oxido-nitrosative stress, significantly increased ($P < 0.01$ and $P < 0.001$) in the levels of GABA and dopamine and Na+ K+ ATPase activity, whereas significantly decreased ($P < 0.01$ and $P < 0.001$) in xanthine oxidase activity in

tempol pretreated mice. PTZ-induced up-regulated mRNA expressions of tumor necrosis factor-alpha, interleukin-1 beta, and c-Fos were significantly inhibited ($P < 0.01$ and $P < 0.001$) by tempol [54].

Singh NK *et al.*, *(*2014) evaluated the anticonvulsant activity and probable mechanism of action of the methanol root extract from *Ichnocarpus frutescens* (MEIF) single dose (100, 200, and 400 mg/kg, *p.o.*) using maximal electroshock (MES), pentylenetetrazole (PTZ), and isoniazid (INH) induced convulsions models in rats. The levels of GABA, glutamate, GABA-transaminase activity and oxidative stress markers were measured in pretreated rat's brain homogenate. MEIF (200–400 mg/kg, *p.o.*) protected the animals in all the behavioral models used and significant restoration of GABA and glutamate level in the rat's brain. MEIF and vigabatrin (50 mg/kg, *i.p.*) reduced the PTZ-induced increase in the activity of GABA-T (46%) in the brain and reversed the PTZ-induced increase in lipid peroxidase (LPO) and decrease in reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities [55].

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3. AIM AND OBJECTIVES

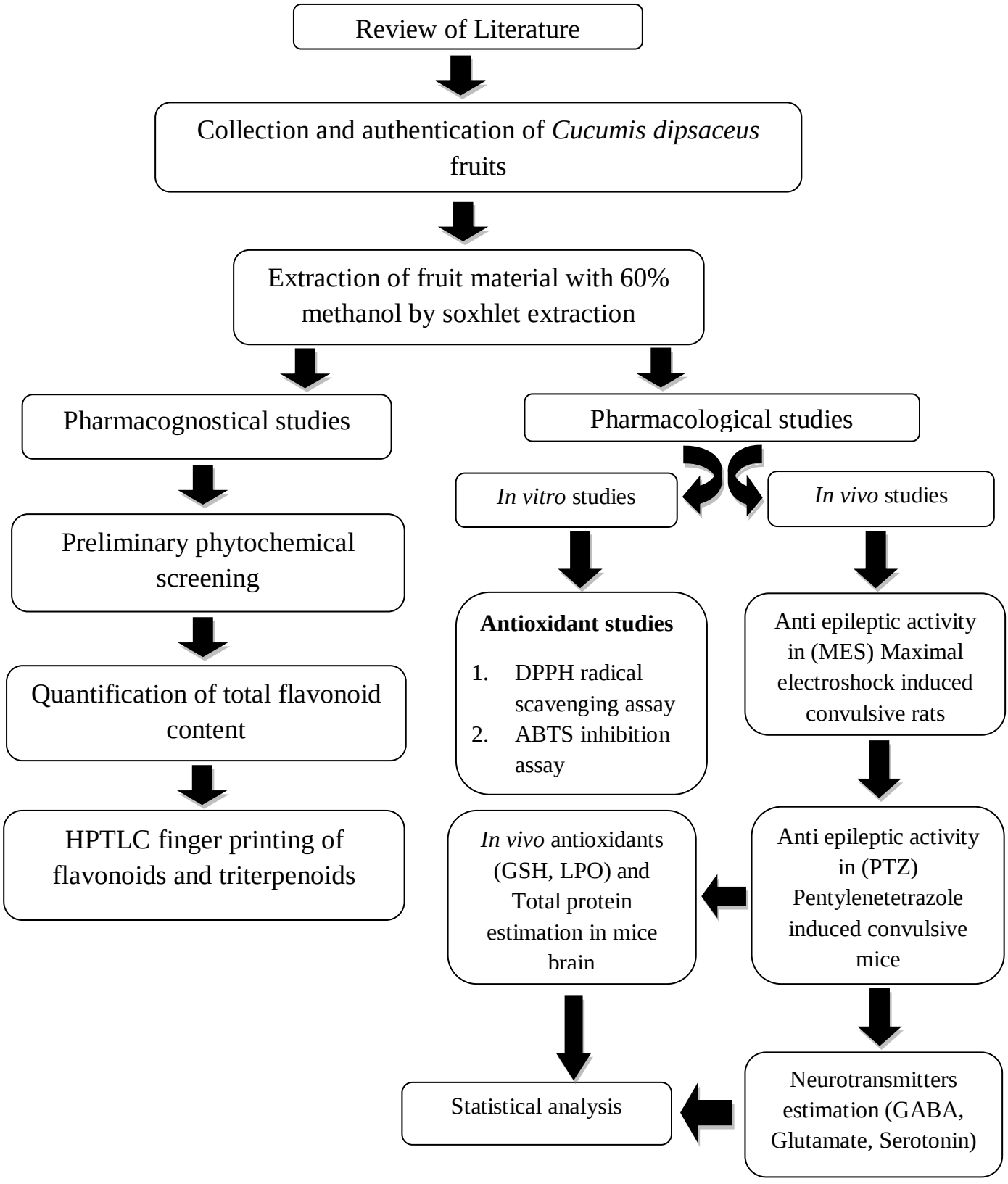
AIM

The aim of the present study is to evaluate the antiepileptic activity of *Cucumis dipsaceus* fruit extract in Maximal electroshock (MES) and Pentylenetetrazole(PTZ) induced convulsion in animal models.

OBJECTIVES

- To extract the fruit of *Cucumis dipsaceus* with hydroalcoholic solvent (60% methanol)
- To perform the phytochemical screening and identify the phytochemicals responsible for antiepileptic activity by HPTLC method.
- To perform the *in vitro* antioxidant study to evaluate antiepileptic activity
- To evaluate antiepileptic activity of *Cucumis dipsaceus* on MES induced convulsive rats and PTZ induced convulsive mice models
- To study the effects of plant extract and phenytoin on brain neurotransmitters (GABA, Glutamate and Serotonin) on PTZ induced convulsive mice.
- To estimate the *in vivo* antioxidant enzymes (GSH and LPO) and total protein level in brain of epileptic mice.
- To study the histopathological changes of brain in PTZ induced convulsive mice.

4. PLAN OF WORK



5. PLANT PROFILE

Figure 6: Fruit of *Cucumis dipsaceus*

Plant name : *Cucumis dipsaceus* Ehrenb ex. Spach

Family : Cucurbitaceae

Common Names

Arabian cucumber,

Hedgehog cucumber,

Mullampandri vellari,

Pepinodiablito,

Teasel gourd,

Scientific Classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Cucurbitales

Family : Cucurbitaceae

Genus : Cucumis L.,

Species : dipsaceus Ehrenb.

Morphological Characters

It is annual climbing herb by axillary tendrils, 1.5 m in length. Stems have branched, slender, angular, sulcate, tendrils simple. Leaves are simple, alternate, ovate, lobes obtuse, base cordiform, apex obtuse, margins dentate or entire. Inflorescence is solitary and axillary. Flowers are unisexual, monocieous; Berry ellipsoid or globose, densely spiny (spines ca. 1 cm long), 3-6.5 cm long, pale yellow; seeds numerous, elliptical, cream-colored, 4-5 mm long. IUCN Status is Exotic, naturalized, uncommon. Flowering season is September – November and fruiting season is November – January. But is Common in dry bushyland, especially like in disturbed woodland and wooded grassland and a weed of cultivation about 400 - 1,800 m [56].

Geographic distribution

It is cultivated in tropical region and Subtropical regions. It is found to be native in Tanga region, Uganda, Kenya, Africa, Ethiopia, Somalia, Sudan and Southern Egypt. This herb is also found in Western Ghats of foothills of Maruthamalai, Coimbatore (Tamil Nadu) and Mysore (Karnataka), India.

Chemical constituents

The fruit of this herb consist of phytoconstituents saponins, triterpenoids, fatty acids, flavonoids, glycosides (quercetin-3-rutinoside-7-rhamnoside) and phytosterols etc. GC-MS analysis revealed the presence of fatty acid esters, tetradecanal, tetradeconic acid, squalene and nonadecanoic acid fatty acids, fatty aldehydes, alkanes and triterpenoids [57].

Traditional uses

Various parts of the plant have been used in traditional treatment such as hemostasis, hemorrhoid, rabies, gallstone, constipation, diarrhea, etc. The leaves are consumed as a leafy vegetable and fruit juice is topically applied to prevent hair loss [33-38].

Pharmacological activity

Analgesic and anti-inflammatory activity, anti-bacterial activity, anti-cancer activity, anti-helminthic activity, anti-microbial activity, anti-oxidant activity, fibrinogenolytic activity, hepatoprotective activity, potential source of protease enzyme, trypsin inhibitory activity [58].

6.1. MATERIALS

6.1.1 Plant collection and authentication

The fruits of *Cucumis dipsaceus* were collected from the surrounding areas of Kalapatti, Coimbatore, Tamil Nadu, India, during the month of December-2018 and authenticated from Botanical Survey of India (BSI) Southern circle, Coimbatore, Tamil Nadu, India. The authentication certificate number is No.BSI/SRC/5/23/2019/Tech/3010.

6.1.1.1 Preparation of plant material

The collected fruits were cleaned, washed with distilled water, dried under sunshade in dark room, and powdered by using mechanical mixer. After size reduction fruits were sieved under sieve No. 40 and sieve No. 60, stored in airtight container at room temperature.

6.1.2. Glassware and chemicals

For the entire project, Borosil glasswares were used. They were soaked in chromic acid for 3 days, washed with tap water and rinsed with distilled water and dried over a hot air oven. Analytical grade chemicals supplied by SD Fine Chemicals, SRL, Hi Media, Merck India and Sigma Aldrich Chemicals were used for this research. List of chemicals were shown in Table 4.

6.1.3. List of Instruments

All the instruments were calibrated before use of experimentation, list of instruments used in the entire project were listed in Table 5.

Table 4: List of chemicals used in the studies

S.no	Chemical Name	Manufacturer
1.	1,1-Diphenyl-2-picrylhydrazyl	Hi Media
2.	1-amino-2-naphthol-4-sulphonic acid	Hi Media
3.	2,2-azinobis-3- ethylbenzothiazoline-6-sulfonic acid	Hi Media
4.	2,4 – Dinitrophenyl hydrazine	Hi Media
5.	5,5’-dithiobis-2-nitrobenzoic acid	Hi Media
6.	Acetic acid	S D Fine-Chem Limited
7.	Acetic anhydride	S D Fine-Chem Limited
8.	Benedict's reagent	S D Fine-Chem Limited

9.	Boric acid	Merck Life Science India Pvt. Ltd.
10.	Bovine serum albumin	Hi Media
11.	Butanol	Merck Life Science India Pvt. Ltd.
12.	Chloroform	Merck Life Science India Pvt. Ltd.
13.	Copper sulphate	S D Fine-Chem Limited
14.	Cysteine	Sigma Aldrich
15.	Dipotassium hydrogen phosphate	Merck Life Science India Pvt. Ltd.
16.	Disodium carbonate	Merck Life Science India Pvt. Ltd.
17.	Disodium hydrogen phosphate	Merck Life Science India Pvt. Ltd.
18.	Dragendorff's reagent	S D Fine-Chem Limited
19.	Ethanol	Merck Life Science India Pvt. Ltd.
20.	Ethyl acetate	Merck Life Science India Pvt. Ltd.
21.	Ethylene Diamine Tetra Acetic Acid	Hi Media
22.	Ferric chloride	Merck Life Science India Pvt. Ltd.
23.	Ferrous ammonium sulphate	Merck Life Science India Pvt. Ltd.
24.	Ferrous sulphate	Merck Life Science India Pvt. Ltd.
25.	Folin-Ciocalteu reagent	S D Fine-Chem Limited
26.	Folin's phenol reagent	S D Fine-Chem Limited
27.	Formic acid	Merck Life Science India Pvt. Ltd.
28.	Gallic acid	Sigma Aldrich
29.	γ-amino butyric acid	Sigma Aldrich
30.	Heptane	S D Fine-Chem Limited
31.	Hydrochloric acid	S D Fine-Chem Limited
32.	Hydrogen peroxide	S D Fine-Chem Limited
33.	Iodine	Merck Life Science India Pvt. Ltd.
34.	Ketamine hydrochloride	Sigma Aldrich
35.	L-glutamic acid	Sigma Aldrich
36.	Lead acetate	Merck Life Science India Pvt. Ltd.
37.	Magnesium chloride	Merck Life Science India Pvt. Ltd.
38.	Mercapto ethanol	S D Fine-Chem Limited
39.	Methanol	Merck Life Science India Pvt. Ltd.
40.	Molisch's reagent	S D Fine-Chem Limited
41.	Nicotinamide adenine dinucleotide	Hi Media
42.	Ninhydrin reagent	Sigma Aldrich
43.	O-Toluidine	Hi Media
44.	O-Phthaldialdehyde	Sigma Aldrich
45.	Pentylene tetrazole	Merck Life Science India Pvt. Ltd.
46.	Phenazine methosulphate	Sigma Aldrich

47.	Phenol	Merck Life Science India Pvt. Ltd.
48.	Phenytoin	Sigma Aldrich
49.	Potassium acetate	Merck Life Science India Pvt. Ltd.
50.	Potassium chloride	Merck Life Science India Pvt. Ltd.
51.	Potassium dichromate	Merck Life Science India Pvt. Ltd.
52.	Potassium dihydrogen phosphate	Merck Life Science India Pvt. Ltd.
53.	Potassium ferricyanide	Merck Life Science India Pvt. Ltd.
54.	Potassium hydroxide	SRL
55.	Potassium iodide	SRL
56.	Propanol	Merck Life Science India Pvt. Ltd.
57.	Quercetin	Sigma Aldrich
58.	Rutin	Sigma Aldrich
59.	Serotonin	Sigma Aldrich
60.	Sodium acetate	S D Fine-Chem Limited
61.	Sodium bicarbonate	S D Fine-Chem Limited
62.	Sodium bisulphate	S D Fine-Chem Limited
63.	Sodium carbonate	S D Fine-Chem Limited
64.	Sodium chloride	S D Fine-Chem Limited
65.	Sodium dihydrogen phosphate	S D Fine-Chem Limited
66.	Sodium fluoride	S D Fine-Chem Limited
67.	Sodium hydroxide	Merck Life Science India Pvt. Ltd.
68.	Sodium nitroprusside	Merck Life Science India Pvt. Ltd.
69.	Sodium phosphate	Merck Life Science India Pvt. Ltd.
70.	Sodium potassium tartrate	Merck Life Science India Pvt. Ltd.
71.	Sodium sulphate	S D Fine-Chem Limited
72.	Sodium sulphite	S D Fine-Chem Limited
73.	Sulphuric acid	Merck Life Science India Pvt. Ltd.
74.	Tartaric acid	Merck Life Science India Pvt. Ltd.
75.	Toluene	Merck Life Science India Pvt. Ltd.
76.	Thiobarbituric acid	Merck Life Science India Pvt. Ltd.
77.	Trichloro acetic acid	Sigma Aldrich

Table 5: List of instruments

S.no	Instruments	Manufacturer
1.	Analytical weighing balance	Shimadzu
2.	Cooling centrifuge	Remi
3.	Deep freezer (-800C)	Remi
4.	Homogenizer	Remi
5.	Hot air oven	Narang Scientific works
6.	HPTLC	Camag
7.	Microtitre plates	Tarsons
8.	pH meter	Eutech
9.	Spectrofluorometer	Jasco, FP-6200
10.	Ultra sonicator	Soltec
11.	UV Spectrophotometer	UV- 1700,Shimadzu
12.	Vortex	Spinix
13.	Magnetic stirrer	Remi
14.	Microtitre plate reader	Bio RAD U.S.A
15.	Rotary microtome	

6.1.4. Software

Statistical calculations [mean ± standard error mean (SEM)] were analyzed by one-way analysis of variance (ANOVA), and significant differences were determined by Dunnett's post hoc test using Graphpad Prism version 8.2.1(441) computer software.

6.2. METHODS

6.2.1. Extraction of the fruit material

Coarsely powdered fruits of *Cucumis dipsaceus* were extracted with 1L of 60% methanol (600 ml of methanol: 400 ml of water) solvent using soxhlet apparatus for about 72 h at 40°C. After that the sediment was filtered with Whatman no.1 filter paper (Whatman Ltd, England). The extract was further concentrated by heating at 40^0C using heating mantle. The obtained crude extract was weighed and stored at 4^0C for the further analysis. The percentage yield was calculated by using following formula

$$\textbf{Percentage yield}\left(\%\frac{w}{w}\right) = \frac{\textbf{Weight of extract obtained}(g)}{\textbf{Weight of plant material used}} \times 100$$

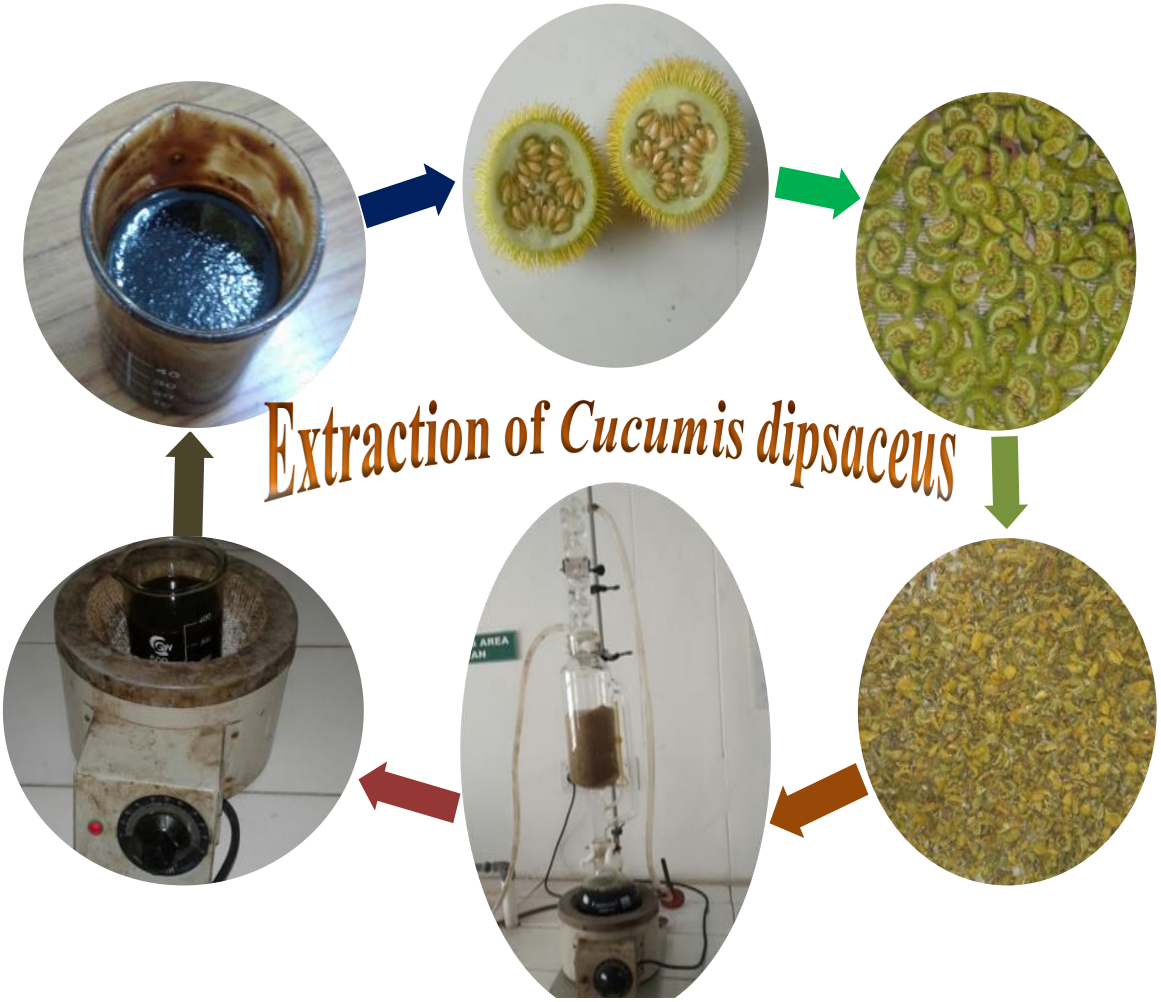


Figure 7: Extraction of *Cucumis dipsaceus* fruit using soxhlet apparatus.

6.2.2. Phytochemical analysis of *Cucumis dipsaceus*

The Hydroalcoholic Fruit extract of *Cucumis dipsaceus* (HAFEC) was subjected to phytochemical evaluation and identified the various phyto constituents present in the test sample both qualitatively and quantitatively. The following studies were carried out in phytochemical analysis.

- ❖ Qualitative chemical test (Preliminary phytochemical tests)
- ❖ HPTLC Analysis
- ❖ Estimation of total phenol and flavonoid

6.2.2.1. Qualitative phytochemical analysis of HAFEC

Preparation of test sample

A small quantity of extract was dissolved in 5 ml of distilled water and then filtered. The filtrate was tested to detect the presence of different phytochemical constituents in the sample [59].

6.2.2.1.1. Test for carbohydrates

Molisch's test

Few drops of Molisch's reagent was added to 2-3 ml of filtrate, followed by addition of concentrated sulphuric acid along the sides of the test tube. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's test

One ml of Fehling's-A (copper sulphate in distilled water) was added to 1 ml of Fehling's-B (potassium tartarate and sodium hydroxide in distilled water) solution, boiled for one minute. To this added 1 ml of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

Benedict's test

Few ml of filtrate was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex) and heated in boiling water bath for 5min. Formation of the reddish brown precipitate infers the presence of reducing sugars.

6.2.2.1.2. Test for alkaloids

Small amount of extract mixed with few ml of dilute hydrochloric acid. Shaken well and filtered. Following tests were performed with the obtained filtrate.

Dragendorff's test

A few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to 2-3 ml of filtrate. Orange red precipitate indicates the presence of alkaloids.

Mayer's test

A few drops of Mayer's reagent (potassium mercuric iodide solution) were added to 2-3 ml of filtrate. Cream (dull white) precipitate was formed.

Wagner's test

A few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to 2-3 ml of filtrate. Reddish brown precipitate was obtained.

Hager's test

A few drops of Hager's reagent (Picric acid) were added to 2-3 ml of filtrate. Yellow precipitate was obtained.

6.2.2.1.3. Test for triterpenoid

Libermann Burchard test

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green color, infers the presence of phytosterols and formation of deep red color indicates the presence of triterpenoids.

Salkowski test

A small quantity of the extract was treated with chloroform and few drops of concentrated sulphuric acid and allowed to stand for few minutes. Yellow colour at the lower layer indicates the presence of triterpenoids.

6.2.2.1.4. Test for glycosides

Legal's test

One ml of pyridine and 1 ml of sodium nitroprusside was added to 1 ml of extract. Pink to red color indicates the presence of glycosides.

Keller Killiani test

Glacial acetic acid was added to 2 ml extract, followed by the addition of trace quantity of ferric chloride and 2 to 3 drops of concentrated sulphuric acid. Reddish brown colour appears at the junction of two liquid indicates the presence of cardiac glycosides.

Baljet test

Two ml of extract was added to sodium picrate solution. Yellow to orange colour formation indicates the presence of glycosides.

6.2.2.1.5. Test for steroids and sterols

Liebermann Burchard reaction

Two ml of extract was mixed with chloroform. To that mixture added 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric acid along the sides of the test tube. The solution becomes; red, then blue and finally bluish green colour.

Salkowski reaction

Two ml of extract was mixed with 2 ml of chloroform and 2 ml concentrated sulphuric acid. Shaken well Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

6.2.2.1.6. Test for phenols

Ferric chloride test

One ml of the alcoholic solution of the extract was added to 2 ml of distilled water followed by few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

Lead acetate test

Diluted 1 ml of alcoholic solution of extract with 5 ml distilled water and to this added few drops of 1% aqueous solution of lead acetate. Formation of yellow colour precipitate indicates the presence of phenols.

6.2.2.1.7. Test for Tannins

Lead acetate test

A few drop of lead acetate was added to 5 ml of aqueous extract. Formation of yellow or red color precipitate indicates the presence of tannins.

6.2.2.1.8. Test for Saponins

Foam Test

One ml of test sample was diluted with 20 ml of distilled water and shaken it in a graduated cylinder for 3minutes. Foam of 1 cm after 10 min indicates the presence of saponins.

Froth test

Five ml of test sample was added to sodium bicarbonate solution. After vigorous shaking the mixture, kept it for 3 min. A honey comb like froth formation indicates the presence of saponins.

6.2.2.1.9. Test for flavonoids

Alkaline reagent test

A few drop of sodium hydroxide solution was added to the extract. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Shinodas test [Magnesium hydrochloride reduction test]

Alcoholic solution of extract was treated with a small piece of magnesium ribbon and a few drops of concentrated HCl was added and heated. Appearance of crimson red or occasionally green to blue colour infers the presence of flavonoid.

6.2.2.1.10. Test for proteins and amino acids

Biuret test

Three ml of test solution was added to 4% sodium hydroxide and few drops of 1% copper sulphate solution. Formation of violet colour indicates the presence of proteins.

Ninhydrin test

A mixture of 3 ml test solution and 3 drops of 5% Ninhydrin solution was heated in a boiling water bath for 10 min. Formation of purple or bluish colour indicates the presence of free amino acids.

6.2.2.2. HPTLC method for estimation of biomarkers in HAFEC

Principle

HPTLC is a valuable quality assessment tool for the evaluation of botanical materials. It is the powerful method equally suitable for qualitative and quantitative phytochemical analysis. HPTLC produces visible chromatogram about the entire extract of the plant material. The technique followed in this study was reversed phase technique. The basic principle of HPTLC is adsorption. Where the mobile phase used is non-polar and the stationary phase is polar. Chemical or active constituents present in the plant extract will move through the plate according to the relative solubility of the constituents in the two phases and will be separated. The non-polar compound will be eluted first and the more polar later. The compounds can be identified based on the R_f value [60, 61].

Experimental condition

Stationary phase	:	Aluminium plates precoated with Silica Gel 60 F_{254} (10×10×0.2mm thickness)
Mobile phase	:	Toluene : Ethylacetate : Formicacid : Methanol (3:6:1.6:0.4)
Sample	:	HAFEC and standards Quercetin, Rutin, Gallic acid, Ursolic acid
Sample application	:	Camag Linomat 5
Chamber type	:	Twin trough Chamber 10× 10cm
Chamber saturation	:	5 min
Development time	:	30 min
Development distance	:	7 cm
Detection	:	Camag Scanner 3
Data system	:	Win CATS Planar Chromatography Manager.

Instrumental Parameters

Number of track	:	8
Band length	:	6.0 mm
Application position	:	10 mm
Solvent front position	:	80.0 mm
Solvent volume	:	10 ml
Position of first track	:	10 mm
Distance between tracks	:	11.4 mm
Scan start position Y	:	5.0 mm
Scan end position	:	75.0 mm
Slit dimension	:	6.00 × 0.45 mm, Micro
Optimized optical system	:	Light
Scanning speed	:	20 mm/sec
Data resolution	:	100 μM/ step

Measurement table

Wavelength	:	254 nm
Lamp	:	D_2 & W
Measurement	:	Remission
Measurement mode	:	Absorption
Optical filter	:	Second order
Detector mode	:	Automatic

Preparation of standard

Stock solutions of Standard compounds were prepared by dissolving accurately weighed 100 mg of Gallic acid, Rutin and Quercetin in 100 ml of methanol (HPTLC grade), filtered by using Whatmann No.1 filter paper and stored in amber colored container at 40°C.

Preparation of Sample

Accurately weighed 100 mg of HAFEC was dissolved in 10 ml of methanol, filtered by using Whatmann No.1 filter paper and stored in amber colored container at 4°C.

Procedure

Five µl of Gallic acid, Rutin, Quercetin and 5,10 µl of HAFEC were spotted in form of bands with a Camag microlite syringe on pre-coated Silica Gel glass plate $60F_{254}$ (10×10 cm with 0.2 mm thickness) using a Camag Linomat 5 applicator. The plates were prewashed with methanol and activated at 60°C for 10 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber after chamber saturation with respective mobile phase. The optimized chamber saturation time for mobile phase was 5 min at room temperature. Linear ascending development was carried out and the plate was developed in the respective mobile phase up to 7 cm. The developed plate was then dried by hot air to evaporate solvents from plate and also forthe development of bands. The dried plate was observed under UV light at 254 nm and 366 nm and photo documentation was done. Densitometric scanning was performed on Camag TLC scanner 3 in the absorbance mode at 280 nm. The percentage of active constituents present in the both the extracts were compared with that of standard.

6.2.2.3. Quantification of total phenolic and flavonoid content

6.2.2.3.1. Estimation of total phenolic content

Reagents

- Folin-Ciocalteu's reagent
- Gallic acid (1 mg/ml)
- 20% sodium carbonate

Preparation of standard

Standard solution of Gallic acid was prepared by adding 10 mg of accurately weighed Gallic acid in 10 ml of distilled water.

Preparation of sample

Ten mg of the accurately weighed HAFEC was dissolved in 10 ml water and used for the estimation.

Procedure [62]

The total phenolic content of the HAFEC was determined by Folin-Ciocalteau assay method. To an aliquot 100 µl of HAFEC (1mg/ml) or standard solution of Gallic acid (10, 20, 40, 60, 80, 100 µg/ml) added 50 µl of Folin-ciocalteau reagent followed by 860 µl of distilled water and the mixture was incubated for 5 min at room temperature. 100 µl of 20% sodium carbonate and 890 µl of distilled water were added to make the final solution to 2 ml. It was incubated for 30 min in dark to complete the reaction after that absorbance of the mixture was measured at 725 nm against blank. Distilled water was used as reagent blank. The tests were performed in triplicate to get the mean values. The total phenolic content was found out from the calibration curve of Gallic acid, and it was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extract.

6.2.2.3.2. Estimation of total flavonoid content

Reagents

- 10% aluminium chloride
- 1M Potassium acetate

Preparation of standard

Standard solution of Quercetin was prepared by adding 10 mg of accurately weighed Quercetin in 10 ml of methanol.

Preparation of sample

Ten mg of the accurately weighed HAFEC was dissolved in 10 ml hydroalcohol (60% methanol) and used for the estimation.

Procedure [63]

The total flavonoid content of the HAFEC was determined by using Aluminium chloride by colorimetric method. To an aliquot of 1 ml of extract (1 mg/ml) or standard solutions of quercetin (10, 20, 40, 60, 80, 100 µg/ml) methanol was added separately to make up the solution upto 2 ml. The resulting mixture was treated with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. Shaken well and incubated at room temperature for 30 min. The absorbance was measured at 415 nm against blank, where a solution of 2 ml ethanol, 0.1 ml potassium acetate, 2.8 ml distilled water and

0.1 ml of aluminium chloride serve as blank solution. The total flavonoid content was determined from the standard quercetin calibration curve. And it was expressed as milligrams of quercetin equivalents per gram of extract.

6.2.3. *In vitro* Antioxidant Studies

Various methods were used to investigate the antioxidant property of samples. In the present study the antioxidant properties of various HAFEC was evaluated by *in vitro* methods. The antioxidant properties could not be concluded based on the single antioxidant test method. It is in practice that generally several *in vitro* test procedure are carried out to conclude the antioxidant properties of the sample. Among various free radical scavenging methods DPPH, ABTS, FRAP and Total antioxidant assays was carried out in the present study.

6.2.3.1. ABTS radical scavenging assay

Principle

In ABTS decolorization assay, the peroxidase substrate 2, 2'- azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS), forms a relatively stable radical ($ABTS^{+}$) upon one electron oxidation. This assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical and inhibits the absorption of the radical cation which has characteristic long-wavelength absorption spectrum showing maxima at 660, 734, and 820 nm. The relatively stable ABTS radical has a green color and is quantified spectrometricaly at 734 nm.

Reagents

- 7 mM ABTS
- 2.4 mM potassium persulfate
- Quercetin

Procedure

To determine ABTS radical scavenging assay, the method of Re *et al.,* [63] was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the

spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of quercetin and percentage inhibition calculated as ABTS radical scavenging activity (%) by using following formula.

$$\textbf{ABTS radical scavenging activity}\ (\%) = \frac{\textbf{Absorbance of sample} - \textbf{Absorbance ofcontrol}}{\textbf{Absorbanceof control}} \times \mathbf{100}$$

6.2.3.2. DPPH radical scavenging assay

Principle

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol/methanol solution centred at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is Z• + AH = ZH + A•.

Reagent

- 0.135 mM DPPH

Procedure

The effect of the extracts on DPPH radical was estimated using the method of Liyana-Pathiranan and Shahidi [64]. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin was used as standard. The ability to scavenge DPPH radical was calculated by the following equation

$$\textbf{DPPH radical scavenging activity}\ (\%) = \frac{\textbf{Absorbance of sample} - \textbf{Absorbance ofcontrol}}{\textbf{Absorbanceof control}} \times \mathbf{100}$$

6.2.4. EVALUATION OF ANTIEPILEPTIC ACTIVITY OF HAFEC

6.2.4.1. Maximal electroshock [MES] model [65]

Wistar albino rats 3 months of age, and 150-250 g body weight were purchased. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 h light/ 12 h dark cycle in the animal house. Animals were fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee) of KMCH, governed by CPCSEA, Government of India. (IAEC No. KMCHRET/M.Pharm/13/2019-20).

Table 6: Selection of animal for MES induced Convulsion evaluation

Species	Wistar albino rats
Age	3 months
Body weight	150-250 g
Gender	Both
No.of animals	30

Experimental design

Table 7: Maximal electroshock seizure model

Group	Number of animals	Group Specifications
Group 1	6	Vehicle control (normal saline, *i.p.*)
Group 2	6	Only MES
Group 3	6	MES + Phenytoin (25 mg/kg, *i.p.*)
Group 4	6	MES + HAFEC (100 mg/kg, *p.o.*)
Group 5	6	MES + HAFEC (200 mg/kg, *p.o.*)

Procedure

Animals in the control group (Group 1) were administered equivalent volume of normal saline by *i.p.* route. Animals in Group 2 were administered standard drug Phenytoin (25 mg/kg). In Groups 3 and 4, HAFEC low dose and high dose were administered by oral route as hydroalcoholic solution respectively. After 30 min of administration of above drugs, all the rats will be given electroshock with electro convulsiometer through eye electrodes (after moistening the eyes of animals with drop of normal saline) at intensity of 150 mA, 60 Hz for 0.2 seconds. There after various parameters were recorded.

6.2.4.2. Pentylenetetrazol [PTZ] model [66]

Swiss albino mice 3 months of age, and 25-30 g body weight were purchased. All the mice were kept at room temperature and allowed to acclimate in standard conditions less than 12 h light/ 12 h dark cycle in the animal house. Animals were fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee) of KMCH, governed by CPCSEA, Government of India

Table 8: Selection of animal for PTZ induced Convulsion evaluation

Species	Swiss albino mice
Age	3 month
Body weight	25-30 g
Gender	Male
No. of animals	30

Experimental design

Table 9: Pentylenetetrazole model

Group	Number of animals	Group Specifications
Group 1	6	Vehicle control (normal saline, *i.p.*)
Group 2	6	Only PTZ (80 mg/kg, *i.p.*)
Group 3	6	PTZ + Phenytoin (25 mg/kg, *i.p.*)
Group 4	6	PTZ + HAFEC (100 mg/kg, *p.o.*)
Group 5	6	PTZ + HAFEC (200 mg/kg, *p.o.*)

Procedure

Animals in the control group (Group 1) were administered equivalent volume of normal saline by *i.p.* route. Animals in Group 2 were administered standard drug Phenytoin (25mg/kg). In Groups 3 and 4, *Cucumis dipsaceus* low dose and high dose were administered by oral route as hydroalcoholic solution respectively. After 60 min of administration of above drugs, all the animals were given Pentylenetetrazol (PTZ) and various parameters were recorded.

6.2.5. ESTIMATION OF BRAIN NEUROTRANSMITTER

Preparation of tissue extracts

Reagents

- HCl - Butanol solution: (0.85 ml of 37% hydrochloric acid in 1L n-butanol)
- Heptane
- 1 M HCl: (0.85 ml conc. HCl up to 100 ml H2O)

Procedure

At the end of experiment, rats were sacrificed and the whole brain was dissected out. 0.25 g of tissue was weighed and was homogenized in 5 ml HCl - Butanol with motor driven Teflon coated homogenizer for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 ml) was removed and added to centrifuge tube containing heptane (2.5 ml) and 0.1 M HCl (0.31 ml). After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase was then taken either for 5-HT or Glutamate assay.

6.2.5.1. Estimation of Serotonin

The serotonin content was estimated by the OPT method

Reagents

O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.4 ml aqueous extract, 1.75 ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Concentrated HCI without OPT was taken as blank. Serotonin (1 mg/ml) at different concentration was used as standard [66].

6.2.5.2. Estimation of Glutamate

The level of Glutamate was estimated by the OPT method

Reagents

O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.4 ml aqueous extract, 1.75 ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 515nm in the spectrofluorimeter. Concentrated HCI without OPT was taken as blank. Glutamate (1mg/ml) at different concentration was used as standard [67].

6.2.5.3. Estimation of brain GABA content

Preparation of tissue homogenate

Animals were sacrificed by decapitation and the whole brain was rapidly removed. 0.5 g tissue was weighed and placed in 5 ml of ice-cold TCA (10% w/v). The tissue was then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. The supernatant was used for estimation of GABA content.

Reagents:

- Carbonate-bicarbonate buffer, 0.5 M (pH 9.95): 1.0501g sodium bicarbonate and 1.3249 g sodium carbonate dissolved in distilled water and made up to 25 ml. pH adjusted to 9.95 if necessary.
- 0.14 M ninhydrin solution: 499 mg ninhydrin dissolved in 0.5 M carbonate-bicarbonate buffer and made up to 20 ml.
- Copper tartarate reagent: 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid.

Procedure:

0.1 ml of tissue homogenate placed in 0.2 ml of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.95), and kept in a water bath at 60°C for 30 min. It was then cooled and treated was with 5 ml of copper tartarate reagent. After 10 min fluorescence at 377/455 nm in a spectofluorimeter was recorded [68].

6.2.6. *In vivo* antioxidant activity

6.2.6.1. Determination of Total protein (T.P)

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 catalyzes the oxidation reaction of aromatic amino acid by reducing

phosphomolybdotungstate to heteropolymolybdanum ,which leads to the formation of blue color and absorbance was measured at 640 nm.

Reagents

- Alkaline copper reagent
- Solution A: 2 % w/v of sodium carbonate in 0.1 N NaOH.
- Solution B: 0.5 % w/v copper sulphate in 1 % sodium potassium tartarate, 50 ml of solution A was mixed with 1 ml of solution B just before use.
- Folin's phenol reagent commercial reagent, 1:2 dilutions
- Bovine serum albumin (BSA).

Procedure

Lowry method was adopted for the estimation of total protein. To 0.1 ml of the liver homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added and allowed to stand in the room temperature for 10 min. To this 0.5 ml of Folin's reagent was added. After 20 min, the blue color developed, measured at 640 nm. The level of protein present was expressed as mg/g tissue or mg/dl [69].

6.2.6.2. Determination of reduced glutathione (GSH)

Principle

DTNB is a disulfide compound, which was reduced by sulphadryl groups present in GSH. This reduction leads to the formation of yellow color, which was measured at 412 nm.

Reagents

- 5% TCA
- 0.2 M Phosphate buffer, pH 8.0
- 0.6mM Ellman's reagent
- Glutathione reduced solution (1mg/ml)

Procedure

Reduced Glutathione was estimated by Ellman's procedure [70]. To 250 µl of tissue homogenate taken in 2 ml eppendorf tube, 1 ml of 5% TCA was added and the above solution was centrifuged at 3000 rpm 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.

6.2.6.3. Determination of lipid peroxidation (LPO)

Principle

This assay is based on the reduction of thiobarbituric acid with malonyldialdehyde which is a formed as a result of polyunsaturated fatty acid oxidation. This reaction leads to the formation of pink colored TBA-MDA complex which is measured at 532 nm.

Reagents

- 4 % (w/v) sodium dodecyl sulfate
- 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5)
- 0.8% thiobarbituric acid (TBA, pH 7.4)
- 1,1,3,3-tetra-ethoxypropane

Procedure

Lipid peroxidation was estimated by the method of Okhawa et al., (1979) [71]. One ml of brain homogenate was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85˚C for 1 h. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. The concentration was expressed as n moles of MDA per mg of protein using 1,1,3,3 – tetraethoxy propane as the standard.

6.2.7. Histological Examinations [72]

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 h 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:

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

7. RESULTS

7.1. Percentage yield and properties of Extracts

Table 10: Properties of HAFEC

Percentage yield of extract	17.27 %
Colour of extract	Brown
Solubility of extract	Soluble in water and alcohol

7.2. Qualitative phytochemical analysis

Preliminary phytochemical evaluation of HAFEC was shown in Table 11. The results revealed that HAFEC has alkaloids, flavonoids, triterpenoids, carbohydrates, saponins, glycosides, proteins and amino acids.

Table 11: Preliminary phytochemical evaluation of HAFEC

Phytoconstituents	**HAFEC**
Alkaloids	Present
Flavonoids	Present
Tannins	Absent
Steroids	Present
Triterpenoids	Present
Saponins	Present
Glycosides	Present
Proteins and aminoacids	Present
Phenols	Absent
Carbohydrates	Absent

7.3. Quantitative phytochemical analysis

7.3.1. Estimation of total Flavonoid content

Table 12: Estimation of total flavonoid content of HAFEC

S. no	Sample	Concentration(µg/ml)	Absorbance at 415nm
1	Standard (Quercetin)	10	0.1526
2		20	0.2352
3		40	0.2924
4		60	0.3665
5		80	0.4125
6		100	0.4963
7	HAFEC	100	0.3676

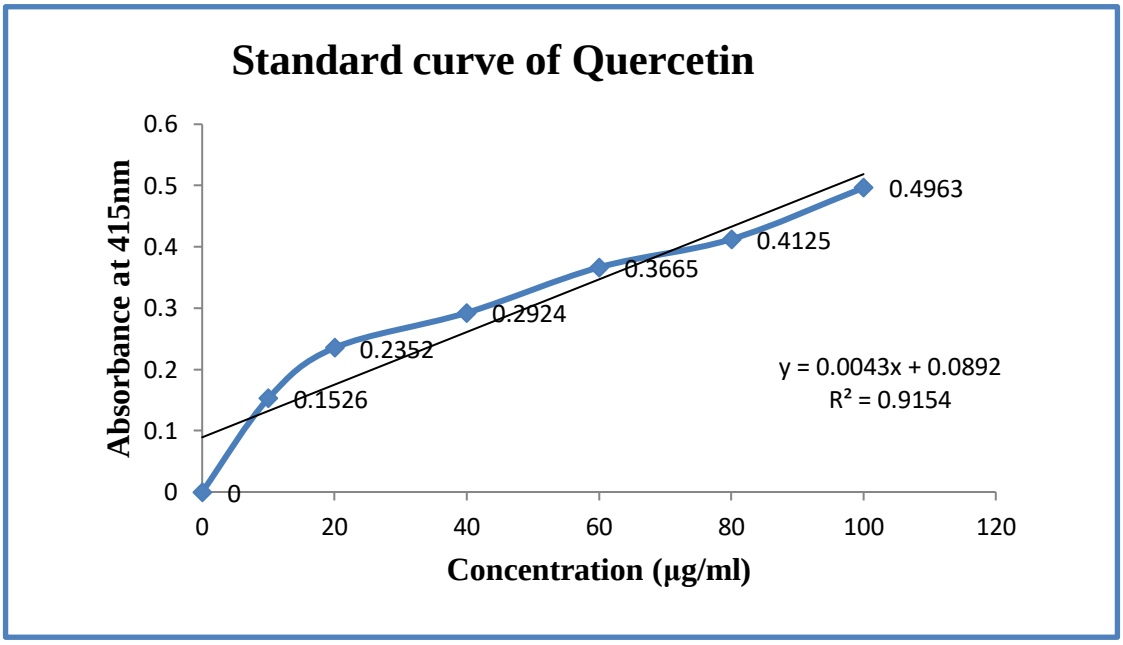


Figure 8: Standard graph of Quercetin for the estimation of total flavonoid content

The total flavonoid content in HAFEC was found to be 69.65 mg/g of extract calculated as quercetin equivalent.

7.4. HPTLC analysis of HAFEC

HPTLC study was carried out for the quantification of flavonoids, alkaloids and triterpenoids in HAFEC. After development the plate was scanned in densitometer under 254nm and the chromatogram obtained is depicted in Figure 9.

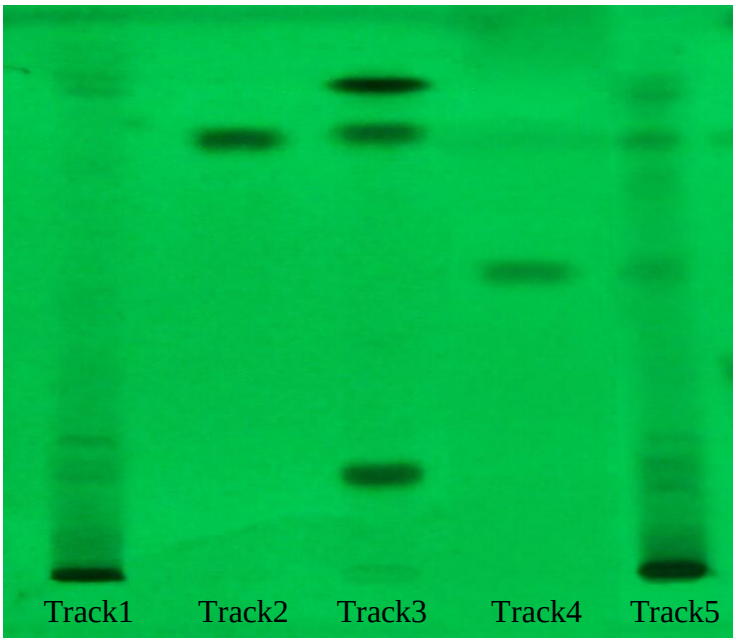


Figure 9: Detection of bands at UV light 254 nm

Table 13: Lists of spots applied on HPTLC plate

Track number/ Sample	Amount of sample applied in µl	R_f value	Name of marker present in sample
Track 1	5	0.18, 0.85	Rutin, Quercetin
Track 2	5	0.76	Catechin
Track 3	15	0.18, 0.77, 0.85	Rutin, Gallic acid, Quercetin
Track 4	5	0.50	Ursolicacid
Track 5	10	0.18, 0.48, 0.76, 0.85	Rutin, Ursolicacid, Catechin, Quercetin

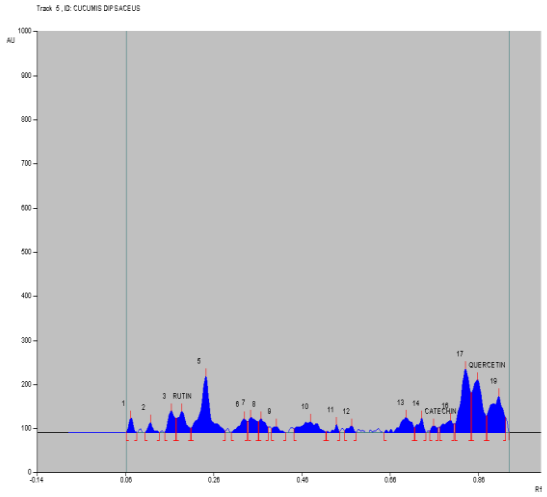


Figure 10: Track 1

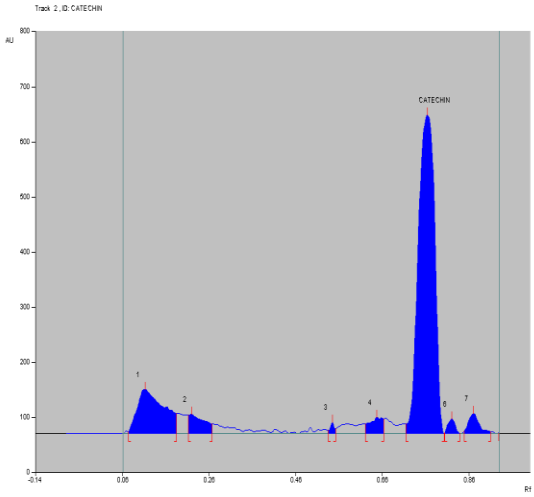


Figure 11: Track 2

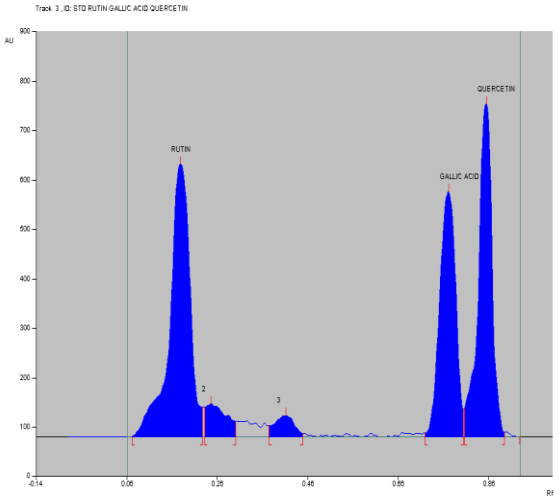


Figure 12: Track 3

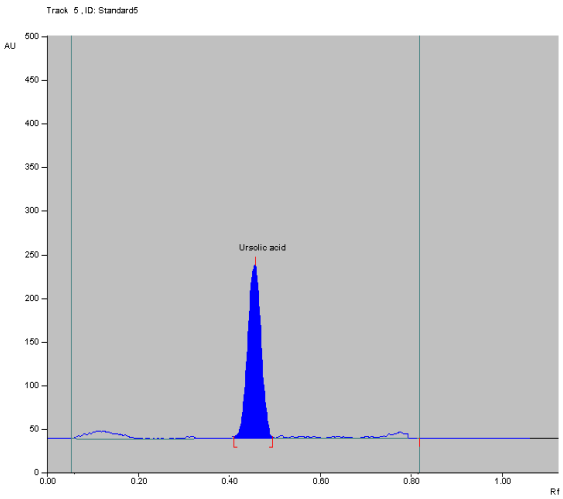


Figure 13: Track 4

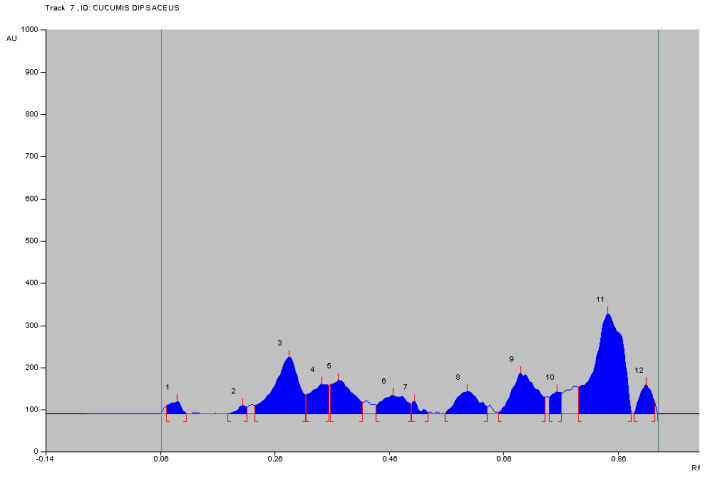


Figure 14: Track 5

Densitogram display of Standards and HAFEC

Table 14: Quantification of flavonoids and triterpenoid in HAFEC using HPTLC

Track number	R_f value	Area of peak	Amount of marker present in 10 mg/ml HAFEC (µg)	Percentage of standard marker present (%)
Track 1 HAFEC(5µl)	0.18-Rutin	640.7	0.16	3.2
	0.49- Ursolic acid	828.3	0.49	9.9
	0.85-Quercetin	1679.4	0.50	10
Track 2 Standard	0.76-Catechin	17758.1	5	100
Track 3 Standard	0.18-Rutin	20379.6	5	100
	0.77-Gallic acid	14420.5	5	100
	0.85-Quercetin	16707.1	5	100
Track 4 Standard	0.50-Ursolic acid	8324.6	5	100
Track 5 HAFEC(10µl)	0.18-Rutin	707.1	0.17	1.7
	0.48-Ursolic acid	759.7	0.91	9.1
	0.76-Catechin	156.4	0.04	0.4
	0.85-Quercetin	2095.0	0.63	6.3

7.5. *IN VITRO* ANTIOXIDANT ACTIVITY OF HAFEC

7.5.1. DPPH radical scavenging activity

Table 15: DPPH scavenging activities of HAFEC and standard Quercetin

S.no	Concentration (µg/ml)	Percentage inhibition of Quercetin (%)	Percentage inhibition of HAFEC (%)
1	10	75.97	72.64
2	20	79.72	76.91
3	40	84.88	84.15
4	60	90.01	85.42
5	80	92.16	86.65
6	100	94.63	88.28

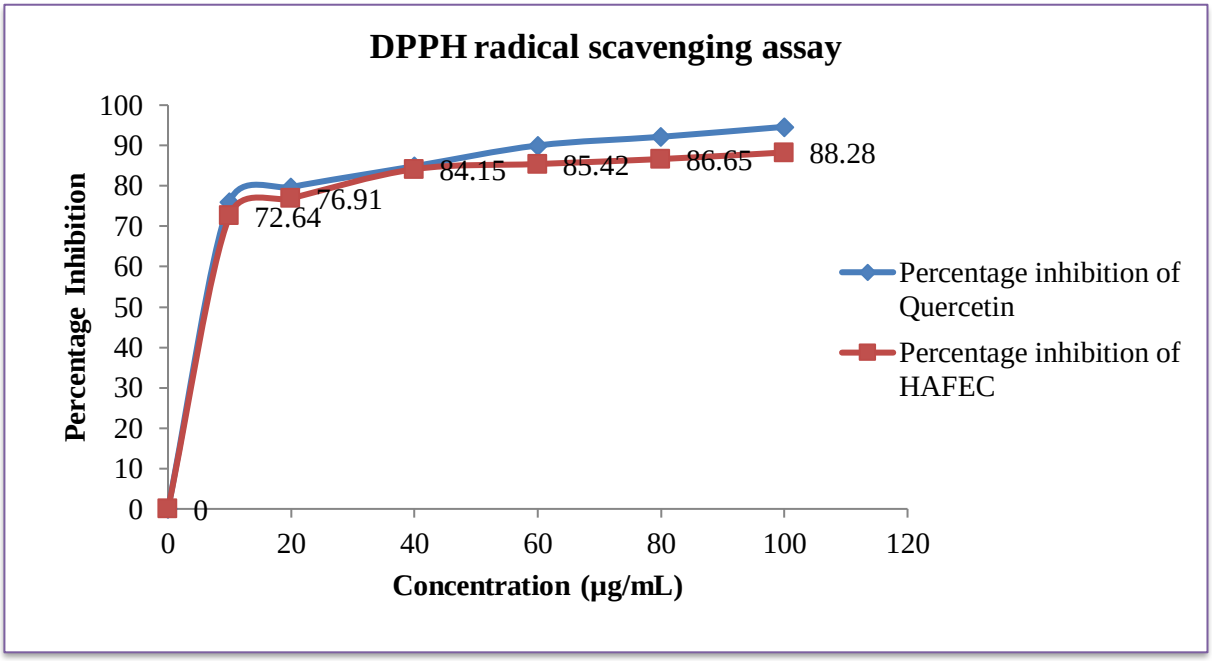


Figure 15: DPPH radical scavenging assay of HAFEC and standard Quercetin

IC_{50} value of standard (Quercetin) = 9.86 µg/ml

IC_{50} value of HAFEC = 13.03 µg/ml

7.5.2. ABTS radical scavenging assay

Table 16: ABTS scavenging activities of HAFEC and standard Quercetin

S.no	Concentration (µg/ml)	Percentage inhibition of Quercetin (%)	Percentage inhibition of HAFEC (%)
1	10	71.84	69.66
2	20	78.26	76.98
3	40	85.98	84.02
4	60	90.30	89.54
5	80	92.02	91.36
6	100	94.24	92.88

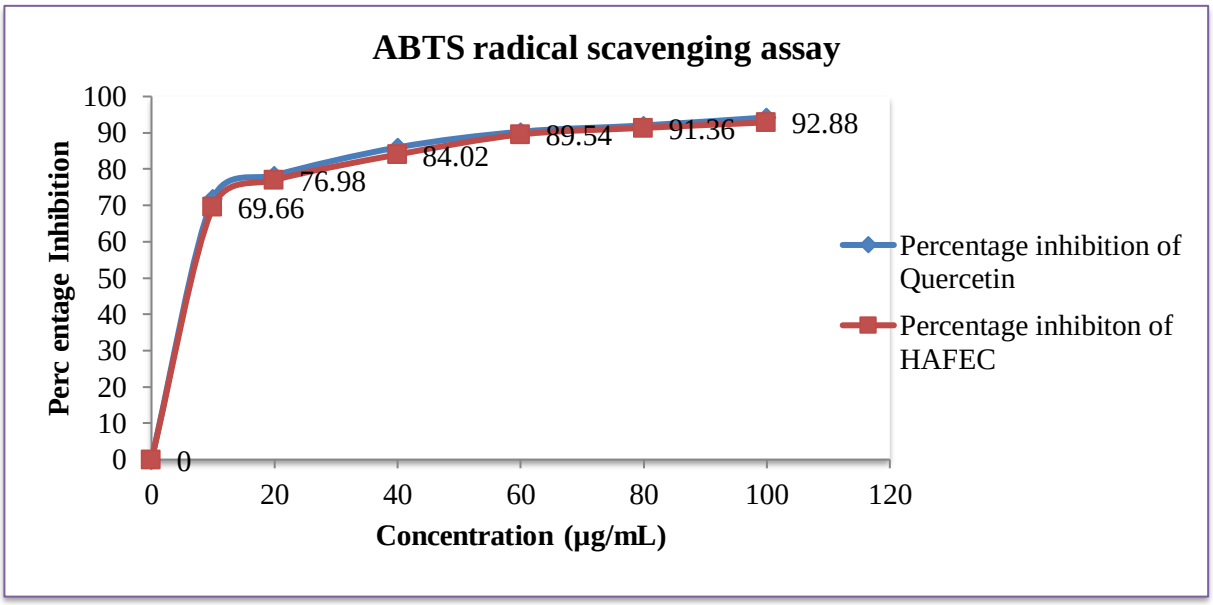


Figure 16: ABTS radical scavenging assay of HAFEC and standard Quercetin

IC_{50} value of Quercetin = 6.50 µg/ml

IC_{50} value of HAFEC = 7.04 µg/ml

7.6. EVALUATION OF ANTIEPILEPTIC ACTIVITY OF HAFEC

7.6.1. Maximal electroshock (MES) model

Table 17: Effect of HAFEC on duration of various phases in MES model

GROUPS	Various phases of convulsions (sec)				
	Flexion	Extensor	Clonus	Stupor	Recovery/ Mortality
MES	5.58±0.06	21.88±0.56	25.22±0.43	65.38±0.35	Recovery
MES+STD (Phenytoin)	1.49±0.05***	7.47±0.13***	0.75±0.24***	31.35±1.04***	Recovery
MES+HAFEC 100 mg/kg	3.62±0.07***	15.12±0.14***	4.82±0.28***	55.47±1.09***	Recovery
MES+HAFEC 200 mg/kg	2.43±0.06***	10.95±0.15***	1.90±0.29***	38.73±0.95***	Recovery

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

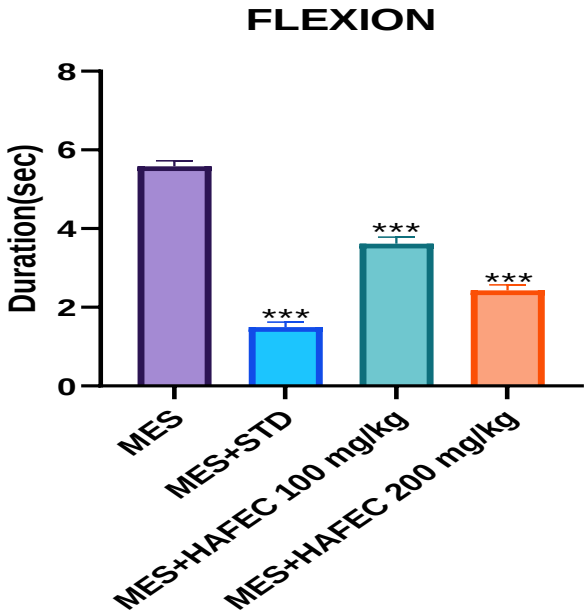


Figure 17: Effect of HAFEC on duration of flexion after MES

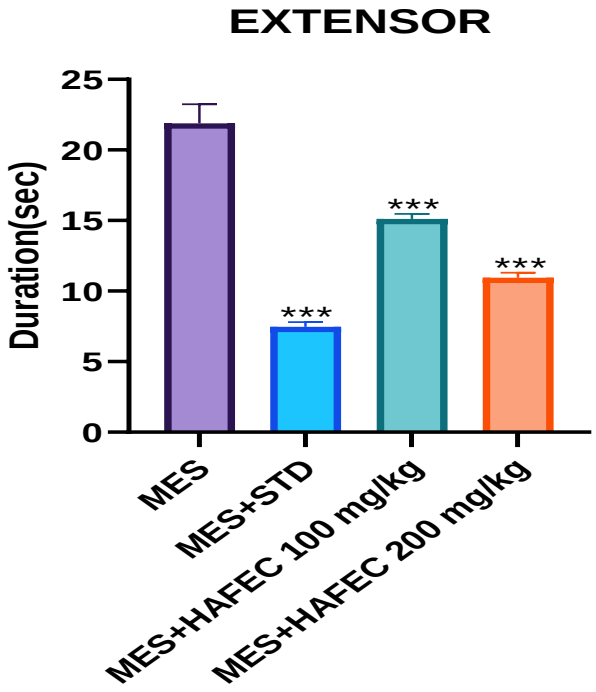


Figure 18: Effect of HAFEC on duration of extensor after MES

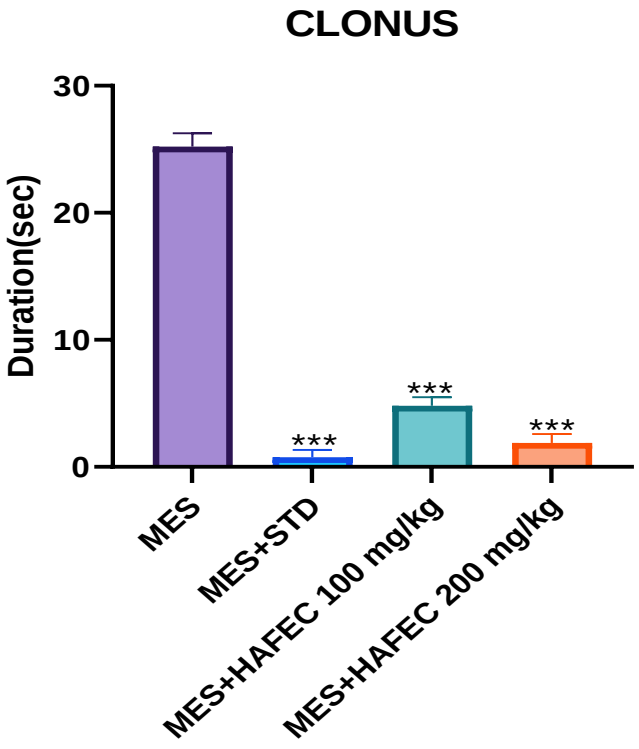


Figure 19: Effect of HAFEC on duration of clonus after MES

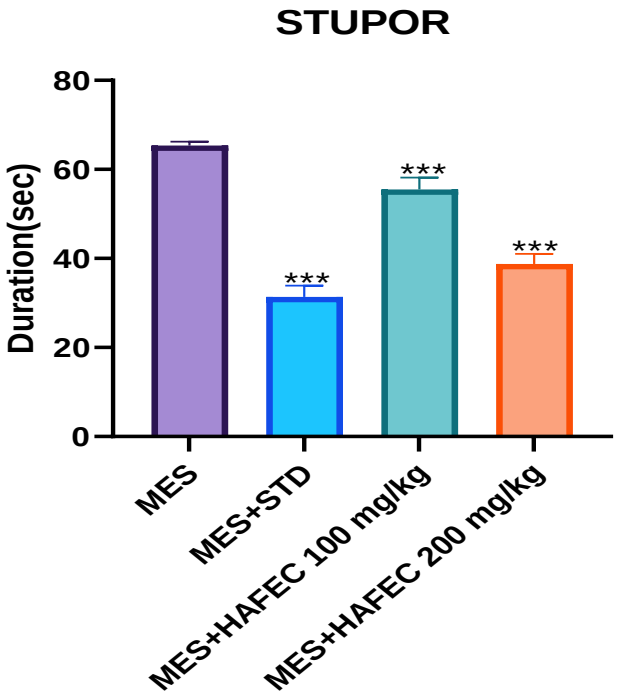


Figure 20: Effect of HAFEC on duration of Stupor after MES

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

7.6.2. Pentylenetetrazole (PTZ) model

Table 18: Effect of HAFEC on PTZ induced seizure model

GROUPS	Onset of action (sec)	Jerky movements (sec)	Recovery/ Mortality
PTZ	101.7±1.97	260.5±1.93	Mortality
PTZ+STD (Phenytoin)	273.8±4.19***	123±3.59***	Recovery
PTZ+HAFEC 100 mg/kg	160.3±3.41***	225±2.87***	Mortality
PTZ+HAFEC 200 mg/kg	250.3±3.72***	175.83±2.92***	Recovery

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

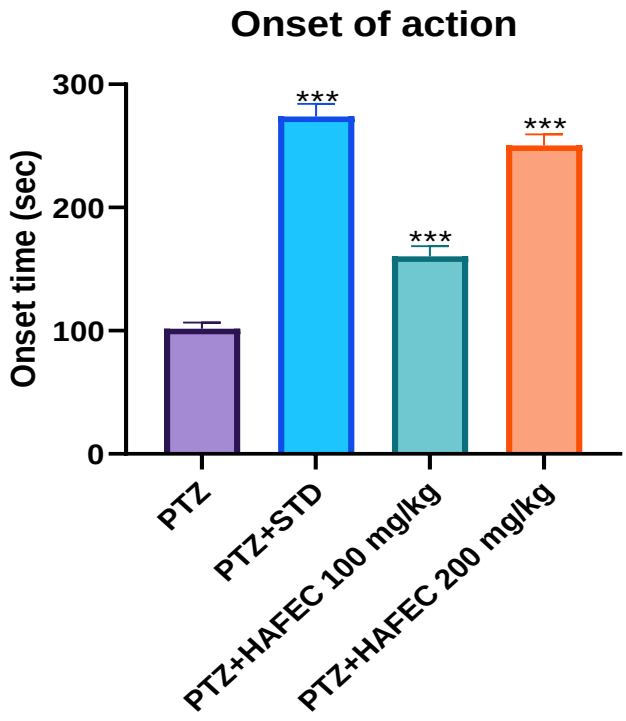


Figure 21: Effect of HAFEC on onset of convulsion in PTZ induced seizure model

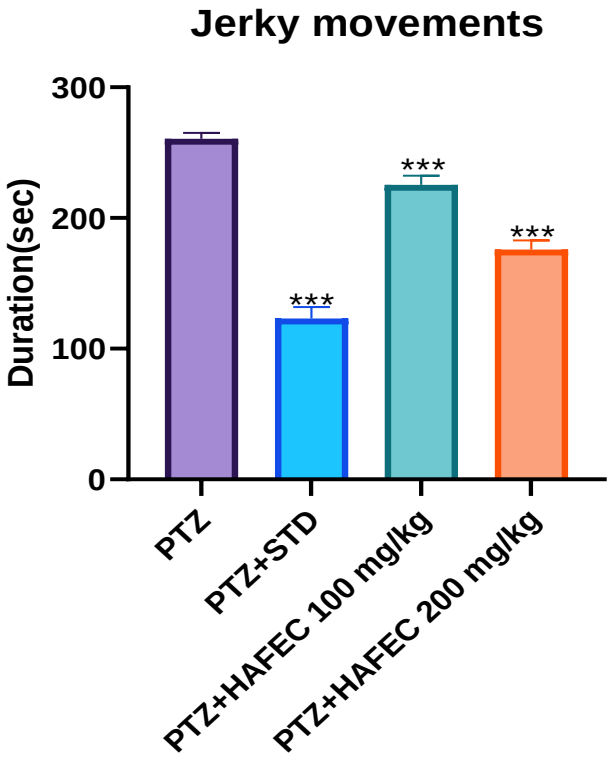


Figure 22: Effect of HAFEC on duration of jerk in PTZ induced seizure model

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

7.7. ESTIMATION OF TOTAL PROTEIN

Table 19: Estimation of total protein in brain tissue

Groups	Total protein (mg/dl)
Normal	3.327±0.023
PTZ	4.535±0.048###
PTZ + STD (Phenytoin)	3.405±0.025***
PTZ + HAFEC 100 mg/kg	4.103±0.031***
PTZ + HAFEC 200 mg/kg	3.602±0.012***

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

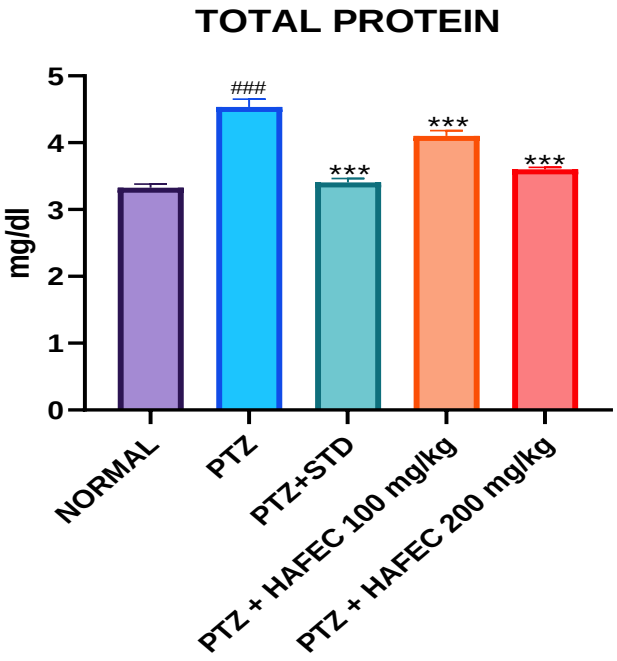


Figure 23: Effect of HAFEC on total protein in brain tissue

7.8. *IN VIVO* ANTIOXIDANT STUDY

Table 20: Estimation of *in vivo* antioxidants in brain tissue

Groups	GSH (µg/mg of tissue extract)	LPO (nMoles of MDA released/ mg protein)
Normal	5.527±0.058	6.46±0.054
PTZ	2.460±0.056###	10.10±0.044###
PTZ + STD (Phenytoin)	5.020±0.070***	7.37±0.051***
PTZ + HAFEC 100 mg/kg	3.457±0.045***	9.73±0.073**
PTZ + HAFEC 200 mg/kg	4.127±0.023***	8.24±0.11***

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

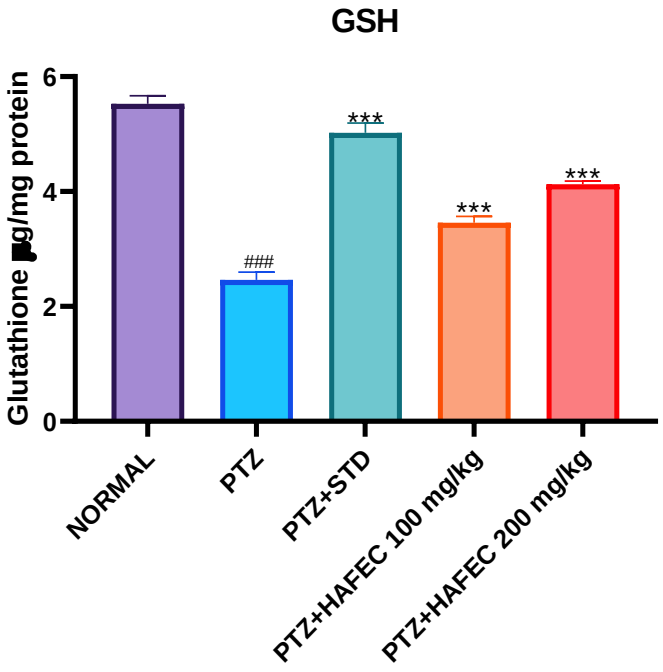


Figure 24: Effect of HAFEC on GSH in brain tissue

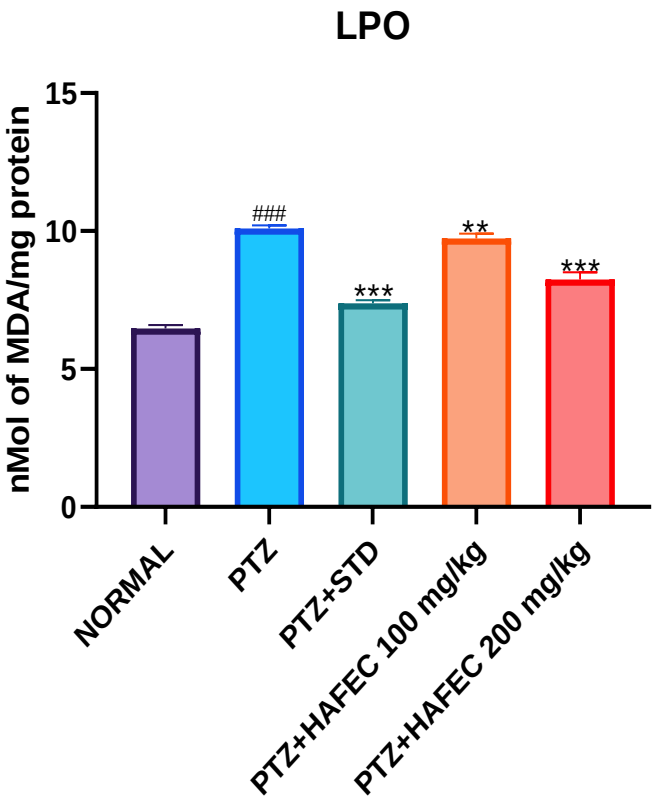


Figure 25: Effect of HAFEC on LPO in brain tissue

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

7.9. ESTIMATION OF BRAIN NEUROTRANSMITTERS

Table 21: Effect of HAFEC on brain neurotransmitters in PTZ model

GROUPS	GABA (ng/g tissue)	GLUTAMATE (ng/g tissue)	SEROTONIN (ng/g tissue)
NORMAL	317.56±3.18	143.78±2.32	146.80±0.81
PTZ	235.10±5.51###	204.70±4.08###	103.18±3.32###
PTZ + STD	302.73±5.32***	156.68±5.42***	137.07±0.75***
PTZ + HAFEC 100mg/kg	258.18±8.32*	181.53±3.49**	115.15±3.32**
PTZ + HAFEC 200mg/kg	270.50±6.68**	154.96±3.62***	124.50±2.19***

Data is expressed as mean ± SEM

n=6, One way ANOVA followed by Dunnett's test

ns = non-significant

Compared with normal control; # p<0.05, ## p<0.01, ### p<0.001

Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001

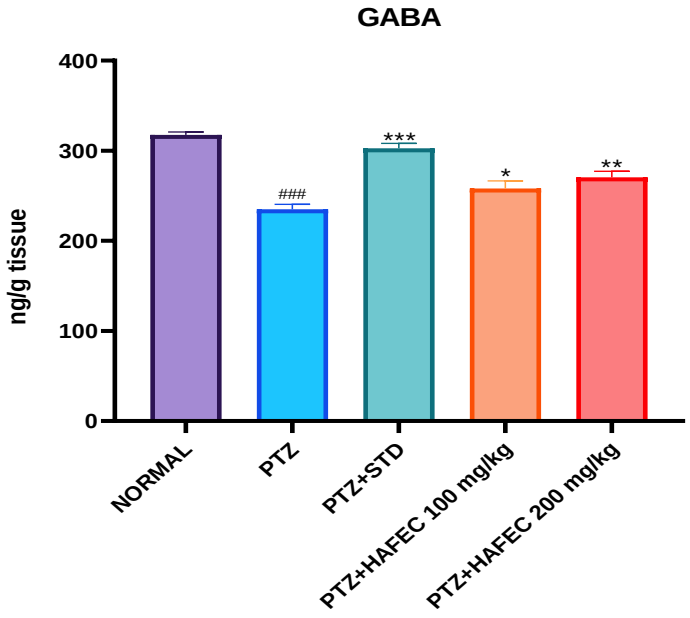


Figure 26: Effect of HAFEC on GABA level in brain tissue

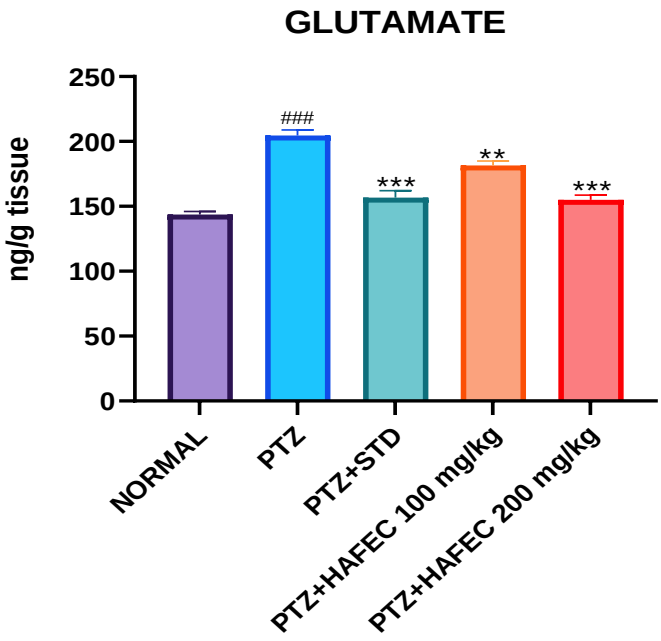


Figure 27: Effect of HAFEC on glutamate level in brain tissue

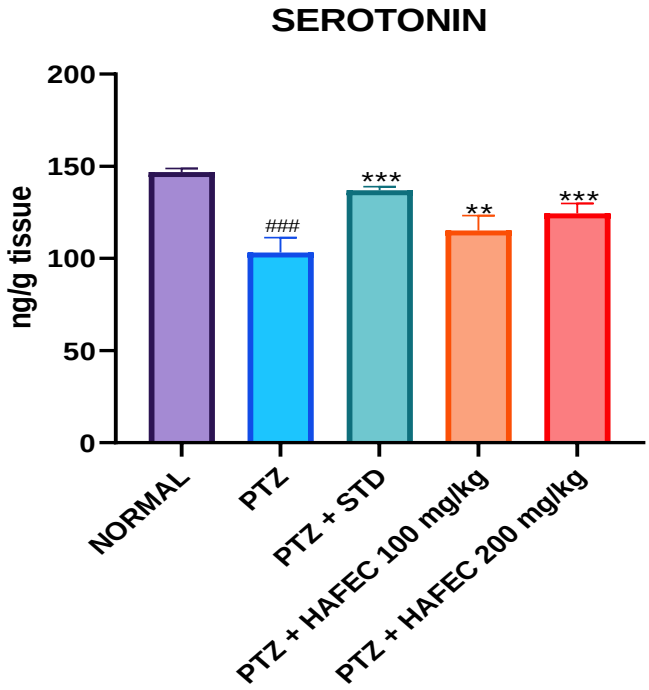


Figure 28: Effect of HAFEC on serotonin level in brain tissue

7.10. HISTOPATHOLOGY OF BRAIN

7.10.1. Histopathological evaluation of PTZ induced mice

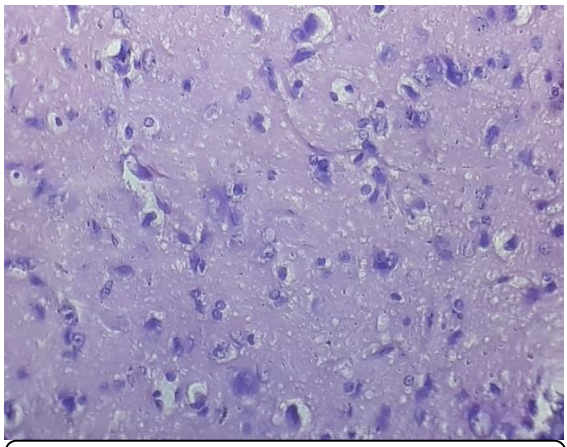


Figure 29: Group 1 Normal

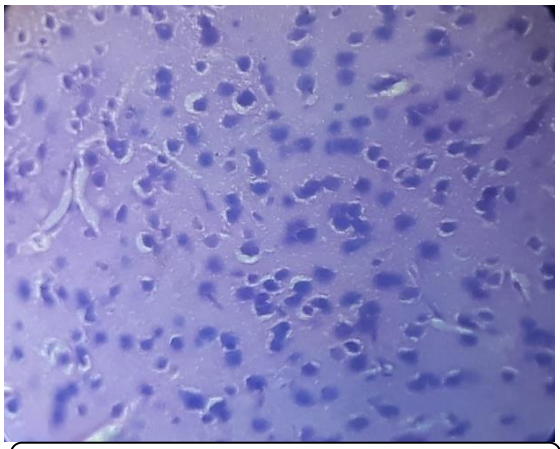


Figure 30: Group 2 PTZ (80mg/kg)

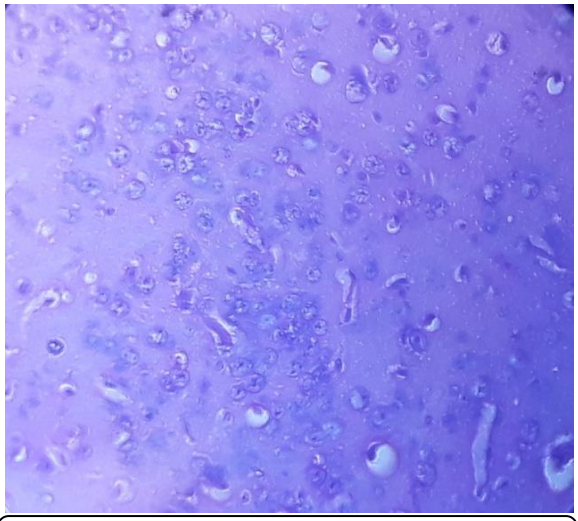


Figure 31: Group 3 PTZ+Phenytoin (25mg/kg)

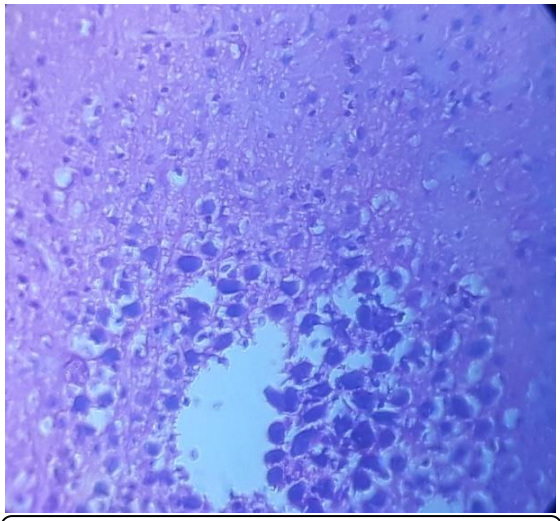


Figure 32: Group 4 PTZ + HAFEC (100mg/kg)

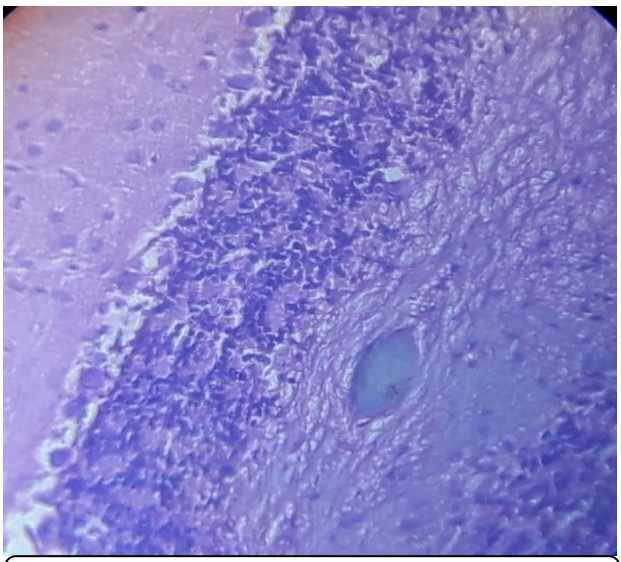


Figure 33: Group 5 PTZ + HAFEC (200mg/kg)

Histopathology Report

Histopathological sections of all the groups were compared with control. No significant changes were observed. Sections at 40X shows that cerebellum and hippocampus regions are with normal histology. Neuropil texture also normal. Cortical dysplasia or hippocampus sclerosis, are the major indicators of epilepsy, which were not seen in the brains of mice induced with epilepsy. No signs of abnormalities related to cell texture was noticed.

8. DISCUSSION

Epilepsy affects around 65 million people worldwide, a significant proportion of the world's population. Treatment with modern antiepileptic drugs elicits more side effects and their cost of interest also too high. Hence, discovering new alternative medicine from natural sources has increased. Some plants belong to the Cucurbitaceae family which has proven antiepileptic activity. But antiepileptic effect of the fruit pulp of *Cucumis dipsaceus* has not been studied so far. So, this study is designed to evaluate the anticonvulsant activity and possible mechanisms of action of *Cucumis dipsaceus* in animal models for the first time.

Plants and plant derived products contain a wide range of phytochemicals, such as alkaloids, flavonoids, glycosides, terpenoids which are thought to have an antiepileptic role. Phytochemical analysis of HAFEC revealed the presence of various phytoconstituents like steroids, alkaloids, saponins, triterpenoids, proteins and flavonoids in HAFEC.

HPTLC finger printing analysis of HAFEC revealed the presence of markers rutin, quercetin and ursolic acid with Rf values at 0.18, 0.85 and 0.50 respectively (Figure 9). The HPTLC quantification suggests that HAFEC has higher flavonoid and triterpenoid content. Few reports stated that flavonoids act on $GABA_A$-Cl^- channel complex due to structural similarity to benzodiazepines, potentiating its effects [73].

Oxidative stress is 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. Hence, antioxidants such as flavonoids play an important role in prevention of oxidative stress. HAFEC restrains a good quantity of flavonoid content in it.

The doses of HAFEC used in study were selected based on the results of the acute toxicity study and histopathological effects of fruit extract of *Cucumis dipsaceus* on rat, Lata *et al.,* [34] reported that the median lethal dose to be 500 mg/kg for methanolic extract and 2000 mg/kg for water extract.

The present study revealed that the HAFEC attenuated both MES-induced tonic and PTZ-induced clonic seizures indicating that HAFEC possesses anticonvulsant effects. The effect was comparable with standard anticonvulsant drug, phenytoin. The hydroalcoholic fruit extract of *Cucumis dipsaceus* was evaluated in chemical and electroshock induced seizure models in mice and rats respectively. The animal models used in this study were selected to

represent different human seizure types (generalized tonic, clonic and myoclonic seizures; simple and complex partial seizures). The ability of HAFEC to delay the onset of convulsions and/or shorten the frequency and duration of convulsions in two various models were considered as an indication of anticonvulsant activity. Inhibition of seizures induced by PTZ and maximal electroshock in laboratory animals is the most common predictive screening test used for characterizing potential anticonvulsant drugs [74, 75].

The maximal electroshock induced convulsion in animals represents grand mal type of epilepsy. MES induced convulsion model causes the activation of Ca^{2+} and Na^{+} channels and drugs inhibiting this influx can prevent MES induced tonic hind limb extension. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure. It permits evaluation of the ability of a compound to prevent seizure spread through neural tissue [76]. The result of the present study shows that HAFEC at doses 100 mg/kg and 200 mg/kg significantly ($p<0.001$) delayed the onset of tonic hind limb extension and reduced the duration of tonic hind limb extension. And also both doses completely diminished the phases of convulsion in MES induced convulsion model. HAFEC is likely to have efficacy against human generalised tonic clonic seizures and may also prevent seizure spread in the brain.

Mice pretreated with HAFEC demonstrated a statistically significant ($p<0.001$) increase in latency to convulsions as well as a decrease in the duration and frequency of convulsion when compared with the vehicle-treated group in PTZ induced convulsive model. This effect increased dose dependently at doses of 100 and 200 mg/kg of HAFEC, but HAFEC at the dose of 100 mg/kg, shows mortality. The extract at 200 mg/kg also exhibited anticonvulsant effect comparable to phenytoin at 25 mg/kg. Pentylenetetrazole (a non-competitive antagonist of $GABA_A$ receptors) exerts its convulsant effects presumably by impairing GABA mediated inhibition by an action mostly via the t-butylbicyclo-phosphorothionate (TBPS) site of the $GABA_A$ receptor [77, 78]. PTZ enhances the glutamatergic neurotransmission by activating the NMDA receptors [79, 80] which results in neuronal toxicity, thus accelerating the onset of convulsions, prolonging the convulsive phase and finally causing mortality. The extracts helped in reversing these symptoms suggestive of probable modulation of glutamatergic neurotransmission. PTZ-induced seizures can be prevented by 2 groups of drugs: (1) $GABA_A$ agonists including benzodiazepines and Phenobarbital and (2) drugs that reduce T-type Ca^{2+}currents such as ethosuximide [81]. Anticonvulsant activity against PTZ seizures also identifies compounds that can raise seizure

threshold in the brain [75]. The anticonvulsant actions exhibited in the PTZ model therefore suggests that it may be effective against generalized myoclonic and absence seizures. HAFEC may also have the ability to increase seizure threshold in the brain.

The present study showed the protective effect of HAFEC on PTZ induced epilepsy. In this study, the 14 days pretreatment was given to mice before the single administration of PTZ. It has been found that the PTZ can increase the free radicals in the brain and can lead to neuronal damage which can lead to epilepsy [82]. The oxidative stress causes an increase in lipid peroxidation level in the brain while a decrease in GSH level in the brain. The imbalance of these levels in the brain could result in the decreased removal of the free radicals and can cause the harmful effects that can lead to neuronal tissue damage [83].

Increasing dose of HAFEC (100 mg/kg and 200 mg/kg) and phenytoin resulted in a decrease ($p<0.01$ & $p<0.001$) in MDA levels in brain tissue. The activities of reduced GSH were also increased significantly ($p<0.001$) in both treatment groups. Pretreatment with HAFEC showed increased activity of these enzymes, which suggests that HAFEC may have the ability to prevent the deleterious effects induced by free radicals. A significant effect on the free radical detoxification by HAFEC suggests the protective effect of HAFEC in epilepsy induced by PTZ in mice.

The results of TP contents in mice brain tissues showed significantly decreased ($p<0.001$) in HAFEC high dose and low dose pretreated groups than PTZ induced group. Since PTZ causes bursting activity in Euhadra neurons by altering the ionic conductance of sodium and potassium channels by changes in intracellular Ca^{2+} related processes [84]. The cell itself has capacity to protect oxidative destruction via scavenge, sequester or neutralize Reactive Oxygen Species (ROS) but in presence of phenytoin, HAFEC (200 mg/kg) and HAFEC (100 mg/kg), the process of damage is low. Total protein also restored to normal level when compared to PTZ induced group.

GABA is the major inhibitory neurotransmitter in the central nervous system and even slight deficiencies in GABAergic transmission may lead to hyperexcitability and pathological neuronal discharges leading to epilepsy. GABA is an endogenous agonist at $GABA_A$receptor (ionotropic receptor) thereby opening the channels to Cl^-ions in the neuronal membrane. An enhancement of GABAergic inhibitory transmission is responsible for the antiepileptic effects of drugs that directly bind and activate $GABA_A$ receptors or influence GABA release, transport and metabolism [85]. HAFEC exerted a positive effect on GABA level, that is,the

level of GABA was found to be significantly increased (P<0.01) at the dose of 200 mg/kg comparable to the standard drug phenytoin.

At the core of the pathophysiology of epilepsy, there is an imbalance between excitatory and inhibitory neurotransmission. Glutamate is a main player of over excitation in epilepsy. mGlu receptors are G protein-coupled receptors activated by glutamate and are involved in the excitatory synaptic transmission in the central nervous system (CNS). Drugs that inhibit mGlu1 and mGlu5 receptors have consistently shown protective activity in models of convulsive epilepsy [86]. HAFEC exerted a modulatory effect on glutamate level, that is, the level of glutamate was found to be high in PTZ induced group which was significantly reduced (p<0.01 & p<0.001) at the dose of HAFEC 100 mg/kg and HAFEC 200 mg/kg respectively.

PTZ induced convulsive mice had decreased serotonin levels when compared to the normal group. The serotonin level observed in PTZ induced convulsive mice, when compared to the normal group (p<0.01). Mice which were pretreated with standard drug, HAFEC 100 mg/kg and 200 mg/kg showed statistically significant increase in serotonin levels (p<0.01 & p<0.001) respectively, when compared with the PTZ induced convulsive mice shown in Table 21.

PTZ induction caused Leptomeningeal deformation (Cellular damage in meninges), Neocortex I region damage, Loss of gray matter (arrow) is observed in the mice brain. Large numbers of pykonic cells are seen. Large number of reactive astrocytes are observed. But low number of nerve cells in neocortex I region. This observation was in line with the previous studies [86]. It was found that HAFEC reversed the histopathological changes caused by PTZ. This may be due to the potential antiepileptic effect of HAFEC.

CONCLUSION

The present study described the evaluation of antiepileptic activity of the hydroalcoholic fruit extract of *Cucumis dipsaceus* (HAFEC) on MES induced tonic himd limb extension in rats and PTZ induced myoclonic convulsion in mice. To the best of our knowledge, it was the first time that HAFEC tested for its antiepileptic activity. The related flavonoid and triterpenoid compounds present in HAFEC may be responsible for its observed antiepileptic activity. The scope for future study is to isolate the active principle and understand the exact molecular mechanism responsible for its antiepileptic activity.

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ABSTRACT

Epilepsy is one of the severe neurological disorders in the world, characterized by recurrent spontaneous seizures due to an imbalance between cerebral excitability and inhibition. Currently available antiepileptic drugs (AEDs) raise several concerns related to lack of efficacy in a significant proportion of epilepsy patients and adverse events as well as their limited supply in Countries with poor resources and high costs. Over the years, traditional herbal medicines have occupied a prominent role in the treatment and management of epilepsy. Many of the plants belonging to the cucurbitaceae family exhibits antiepileptic activity but still there is no scientific evidence for the antiepileptic activity of *Cucumis dipsaceus.* Therefore the current study is aimed to investigate the antiepileptic activity of hydroalcoholic fruit extract of *Cucumis dipsaceus* (HAFEC) against MES and PTZ induced seizures in animal models. PTZ induced convulsive swiss albino mice have shown reduced GABA, Serotonin levels and elevated Glutamate levels, which are the signs of excitotoxicity. HAFEC (200 mg/kg and 100 mg/kg) reduced these signs dose dependently similar to that of phenytoin but with lesser magnitude. The result may be attributed to the chemical constituents such as triterpene, flavonoids and alkaloids present in it which may be due to their individual or cumulative effect that enhanced antiepileptic activity. These findings could justify the inclusion of *Cucumis dipsaceus* in the management of epilepsy.

Keywords: HAFEC, Maximal electroshock, Pentylenetetrazole, GABA, Flavonoid.

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