EVALUATION OF PREVENTIVE ROLE ON MICROANATOMICAL CHANGES IN BRAIN AND ANTICONVULSANT PROPERTIES OF CALCIUM CHANNEL BLOCKERS IN EXPERIMENTAL ANIMAL MODELS

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In

MEDICAL ANATOMY

By

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LIST OF ABBREVIATIONS USED

ACh	Acetyl choline
AEDs	Antiepileptic drugs
AGS	Audiogenic seizures
AMPA	α-Amino-3-hydroxy-5-Methyl-4-isooxazolepropionic Acid
ANOVA	Analysis of Variance
BW	Body Weight
Ca	Calcium
CAT	Catalase
CCB	Calcium Channel Blocker
cm	Centimetre
CPCSEA	Committee for the Purpose of Control and Supervision of Experiment on Animals
CPS	Complex partial seizures
DA	Dopamine
DPH	Diphenyl Hydantoin
DPX	Dibutylphthalate Polystyrene Xylene
DTNB	5,5- Dithiobis-(2- Nitrobenzoic Acid)
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram
fig.	Figure
FL	Forelimb
gm	Gram
GLU	Glutamate
GPX	Glutathione Peroxidase
GSH	Reduced Glutathione
GTCs	Generalised Tonic-Clonic seizures
H_2O	Dihydrogen monoxide
Hcl	Hydrochloric Acid
hr	Hour
HSP	Heat Shock Protein
H & E	Haematoxylin and Eosin

Hz	Hertz
5-HT	5-Hydroxytryptamine [Serotonin]
i.p	Intraperitoneal
IAEC	Institutional Animal Ethical Committee
ILAE	International League Against Epilepsy
kg	Kilogram
LPO	Lipid peroxidase
M	Molarity
mA	Milliampere
MES	Maximal Electroshock
mg	Milligram
mg/dl	Milligram/Decilitre
mg/kg	Milligram Per Kilogram
mg/ml	Milligram/ Millilitre
min	Minute
mL	Millilitre
mM	Millimole
mm	Millimetre
µg	Microgram
µL	Microlitre
MPO	Myeloperoxidase
MRI	Magnetic Resonance Imaging
N	Normality
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium hydroxide
ng/g	Nanogram per Gram
nm	Nanometer
NMDA	N - Methyl – D - Aspartate
p.o	Per Oral
^{o}C	Degree Centigrade
^{o}F	Degee Fahrenheit
PTZ	Pentylenetetrazole

rpm	Revolutions per minute
s.c	Subcutaneous
sec.	Seconds
SEM	Standard error mean
SOD	Superoxide Dismutase
SPS	Simple partial seizures
SPSS	Statistical Package for the Social Sciences
TCA	Tricyclic Antidepressant
THLE	Tonic Hind Limb Extension
V	Voltage
%w/w	Percentage Weight/Weight
WHO	World Health Organization

ABSTRACT

Background and objectives

Drug-to-drug interactions and drug toxicity are among the many downsides of currently available epilepsy drugs. Many pathophysiological pathways have a role in the onset of seizures. Excess calcium entry into neuronal cells can cause the cells to depolarize, which can lead to seizures. Based on this basic knowledge, calcium channel blockers were assumed to be able to prevent seizures.

The goal of this study was to see how the anti-convulsant properties of diltiazem, nimodipine, and flunarizine functioned in experimental animal models. To find out how diltiazem, nimodipine, and flunarizine work to stop animals from having seizures, three animal models were chosen: PTZ, Pilocarpine, and MES.

Materials and Methods

Wistar albino rats [180 - 250 grams], were selected for the research study. The Institutional Animal Ethics Committee [IAEC] approved the study proposal. Throughout the study, all national and international standard guidelines[CPCSEA] were followed. In wistar albino rats, the anticonvulsant properties of diltiazem (20 mg/kg), nimodipine (20 mg/kg), and flunarizine (10 mg/kg) were evaluated using the PTZ, Pilocarpine, and Maximal Electroshock Seizure (MES) test models. The onset, duration, number, and severity of seizures were recorded. After recording seizure parameters as per CPCSEA guidelines, experimental animals were sacrificed, and the brain tissue was preserved for antioxidant, neurotransmitter and inflammatory marker assays (one hemisection) and also for histological and immunohistochemistry evaluations (another hemisection). A "P" value of 0.05 was considered statistically significant when one-way ANOVA and its non-parametric variant were used to look at the data.

Results

When compared to the respective negative control groups, the drugs diltiazem, nimodipine, and flunarizine were able to increase the onset of seizures and decrease the duration, number and the scores of seizures. These findings were also comparable to those of the standard drug groups. When compared to the negative control groups, the drugs diltiazem, nimodipine, and flunarizine were able to increase the anti-oxidant enzymes (SOD, GPx, GSH, and CAT) and decrease the lipid peroxidation, having results that were comparable to the standard drugs. When compared to the corresponding standard drug groups, the test drug groups showed an increase in the neurotransmitter levels (Serotonin, DA, and GABA). However, as compared to the negative control group, the test drug groups showed lower levels of neurotransmitters (Glutamate and ACh).

The experimental drugs diltiazem, nimodipine, and flunarizine showed favourable histopathological findings that were comparable to the standard drugs. Similarly, the experimental drugs diltiazem, nimodipine, and flunarizine delivered positive immunohistochemistry results that were comparable to the standard drugs.

Conclusion

In the PTZ, Pilocarpine, and MES models, diltiazem, nimodipine, and flunarizine significantly improved seizure parameters. In the PTZ, Pilocarpine, and MES models, diltiazem, nimodipine, and flunarizine considerably improved oxidative stress, neurotransmitter, and antiinflammatory conditions, as well as the neuroprotective favourable scores for the histopathological and immunohistochemistry evaluations.

Keywords: Epilepsy, Calcium channel blockers, Seizures, Animal models of epilepsy.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

Background of the study

Seizures are brief occurrences of involuntary movement affecting either a portion of the body (partial) or the entire body (generalised), which may be followed by loss of consciousness and control of bowel or bladder function.[1] A seizure is characterised by transient signs and/or symptoms caused by abnormal brain neuronal activity. Epilepsy is a neurological disease that causes people to have long-term epileptic seizures, as well as the neurological, cognitive, psychological, and social implications.[2]

A seizure is an unproven event, whereas epilepsy is a disease characterised by frequent unexplained seizures. At least one epileptic episode is required for epilepsy to be diagnosed.[2]

In response to task force group suggestions, the International League Against Epilepsy (ILAE) recently formulated an operational definition of epilepsy. The following conditions are included in the operational definition of epilepsy:

- At least two spontaneous seizures happened within 24 hours of duration
- One unprovoked seizure with a risk of a second unprovoked seizure comparable to the risk of subsequent seizures (in the next 10 years)[3]

Epilepsy is a serious neurological condition that affects up to 5% of the world's population at some point in their lives. Adverse effects, dose-related and chronic toxicity, as well as teratogenic implications, are all common side effects of modern antiepileptic drug therapy, and around 30% of patients continue to have seizures while on current antiepileptic drug therapy.

Statement of the problem

One estimate says that epilepsy affects more than 50 million people globally. The majority of these people live in poor countries. Epilepsy affects more than ten million people in India.[4] People with epilepsy do not acquire appropriate and complete treatment for their ailment due to under-diagnosis and social stigma. As a result, there is a significant therapeutic gap and subsequent problems. Lack of awareness, limited access to health care, cultural beliefs, social stigma, and a lack of health care providers who specialise in epilepsy are just a few of the many causes of underdiagnosis and undertreatment. Infections play a substantial part in the etiopathogenesis of epilepsy in India, unlike in many developed countries.

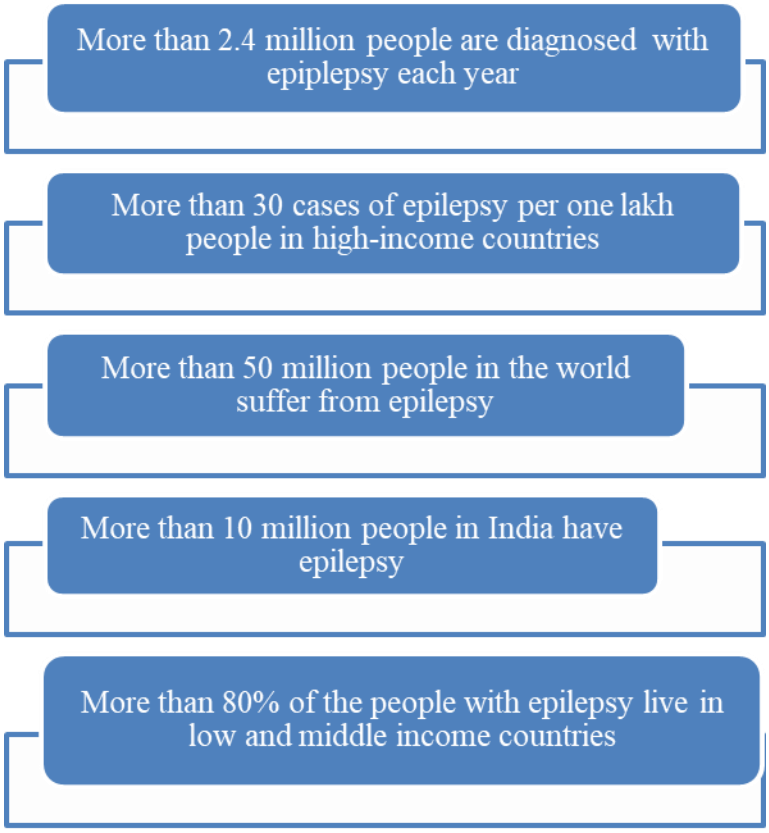

Figure 1: Global burden of the disease

According to the World Health Organization (WHO), epilepsy accounts for 0.6 percent of the global health burden. Years of life lost due to premature death and time lived in less than full health make up the global burden of illness. As a result, epilepsy has major economic consequences. It's 20 times more common for people with epilepsy to die suddenly without a reason than for people in the general public.[5]

Theoretical foundation

For example, clinicians now have access to a wide range of antiepileptic medications, including carbamazepine, phenytoin, and valproate, as well as other newer additions such as gabapentin, lacosamide, lamotrigine, levetiracetam, rufinamide, tiagabine, topiramate, and zonisamide. With so many alternatives to epilepsy pharmacotherapy, it's understandable for anyone to expect complete remission or symptom-free duration once treatment begins. Despite these numerous drugs, however, clinical outcomes are not entirely optimistic. The current therapy's tolerability and efficacy remain serious flaws. The problems of current epilepsy treatment occur due to a lack of understanding of the etiopathogenesis of the disease process.[6,7] Significant and ongoing efforts are being made to develop new drugs for epilepsy pharmacotherapy, as well as to study drugs that have already been used to treat epilepsy.

Animal model preclinical research serves a critical role in the identification of new therapies and the development of new indications for older treatments used in epilepsy. The animal model is used to examine not just the efficacy of newer drugs, but also their safety and tolerability. The seven important features are represented by animal models.[8] (Figure 2)

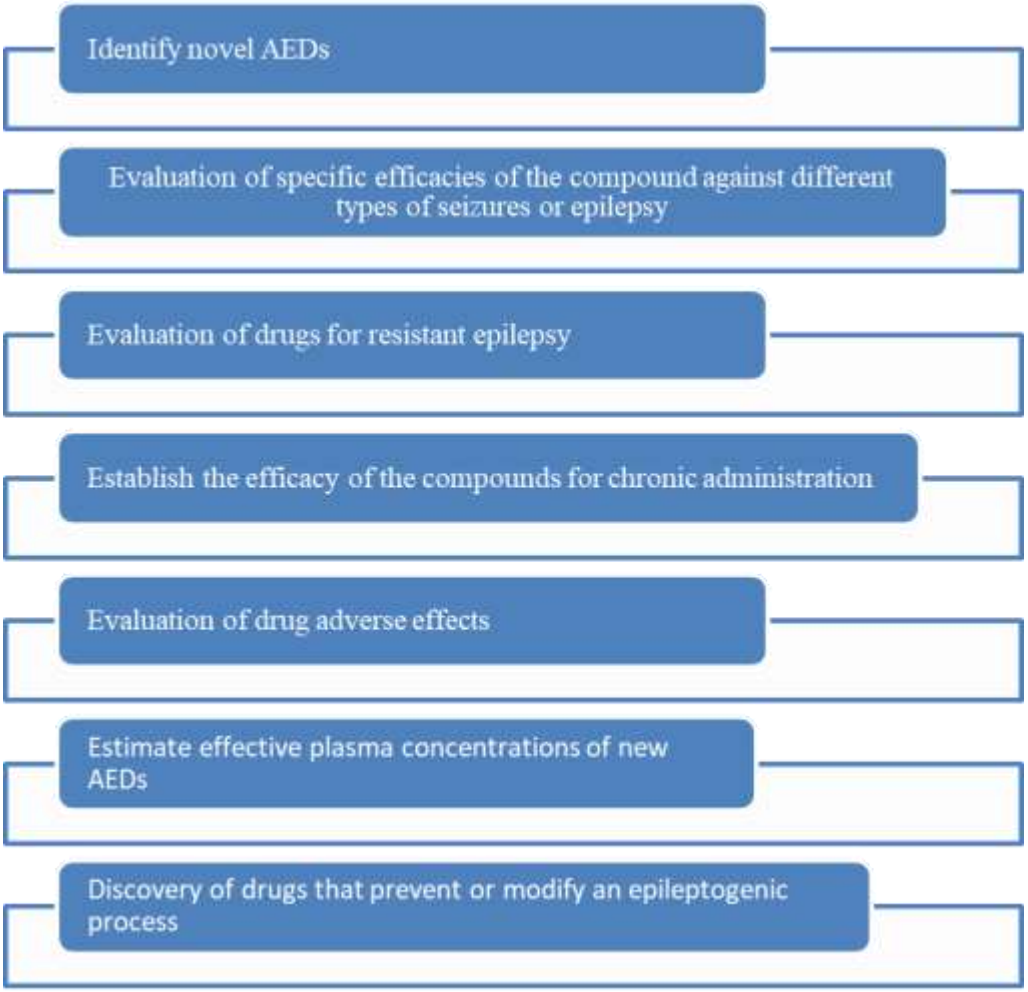

Figure 2: Uses of animal models in evaluating epilepsy pharmacotherapy

There are a variety of drugs available on the market today to treat epilepsy. Antiepileptic drugs work through a variety of methods, including prolonging Na+ channel inactivation, inhibiting Ca^{2+} current, facilitating GABA mediated Cl^- channel opening, boosting K^+ channel opening, blocking NMDA receptors, blocking AMPA receptors, and blocking kainic acid receptors.[2] Due to their toxicity profile, drug-drug interactions, and resistance in some individuals, current antiepileptic medicines have some limitations.[1]

Many investigations[3,12] have established that neuroinflammation and immune-mediated insults play a significant role in seizure initiation and epileptogenesis. In

mouse experiments, inflammation has been shown to enhance the permeability of the blood-brain barrier as well as the excitability of nervous tissue.[7]

The significance of oxidative stress in the epileptogenesis process has been outlined in several experimental studies.[13,16] Mitochondrial-related oxidative stress has an essential role in epileptogenesis in the lithium-pilocarpine model of temporal lobe epilepsy in mice.[13] In some animal models, antioxidants can reduce the brain harm produced by oxidative stress and hence prevent the onset of seizures.[14]

Calcium channel blockers [CCBs] are commonly given for the treatment of hypertension, angina, and cardiac arrhythmias by inhibiting L-type and T-type calcium channels found in different tissues, including the brain. Calcium channel blockers include phenyl alkylamine [verapamil], benzothiazepine [diltiazem], and dihydropyridines [nifedipine, felodipine, amlodipine, nitrendipine, nimodipine, lacidipine, lercanidipine, and benidipine].[17] Arrhythmias are treated with phenyl alkylamine and the benzothiazepine class of CCBs, whereas angina and hypertension are treated with dihydropyridines.[17] CCBs have the advantage of being safe to use in individuals with chronic obstructive pulmonary disease and peripheral vascular disease. And other clinical uses of CCBs are - hypertrophic cardiomyopathy, early labour, migraines and nocturnal cramps.[17]

Anti-inflammatory[18,19] and antioxidant[20,21] properties of calcium channel blockers have been demonstrated in several experimental animal models. Calcium channel blockers have several advantages over currently available antiepileptic drugs, includes no enzyme induction or inhibitory capabilities, no sedation, no impact on quality of life, a broad therapeutic range, even best suited for the elderly, stroke prevention, and a preventive function in hypertensive or diabetic nephropathy[17]

Kalita et al.[22] conducted a study on swiss albino mice to evaluate the microanatomical damage caused by pentylenetetrazol administration. On histological inspection, pentylenetetrazol induced neuronal injuries such as meningeal cellular destruction, loss of grey matter, the presence of pyknotic cells, and a huge number of reactive astrocytes (gliosis).

On histoanatomical examination of mouse brain tissue, Borges et al.[23] found hippocampal sclerosis, total loss of hilar neurons, variable mortality of pyramidal cells, mossy fibre sprouting, astrogliosis, and thalamic axonal death in a mouse pilocarpine model. After a single dose of pentylenetetrazol [60 mg/kg], Jandova et al.[24] identified a neurological insult in young rats, specifically in the hippocampus and dentate gyrus regions of the brain.[25] In another study which reviewed microanatomical abnormalities in the human brain in the temporal lobe epilepsy, including hippocampal sclerosis, that is marked by glial response, aberrant growth of interneuron networks, and selective loss of pyramidal neurons in the CA1 and CA3 regions.[25]

As a result, further study into the anticonvulsant characteristics of calcium channel blockers in various experimental animal models, as well as their preventive roles on microanatomical changes in the brain, is essential to improve epilepsy pharmacotherapy.

HYPOTHESIS

HYPOTHESIS

Calcium channel blockers (Diltiazem, Nimodipine and Flunarizine) exhibit considerable anticonvulsant activity as well as a protective effect on microanatomical changes in the brain in experimental animal models induced by Pentylenetetrazole (PTZ), Pilocarpine and Maximal Electroshock Seizure (MES).

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

Aim of the study

The aim of the present study was to explore the CCB's [Diltiazem, Nimodipine and Flunarizine] for their anti-convulsant properties and their preventive role on the microanatomical changes in the brain of the experimental animal models [in wistar albino rats].

Objectives

i. To evaluate and compare the anticonvulsant activity among Diltiazem, Nimodipine and Flunarizine and with the standard antiepileptic drugs in individual Pentylenetetrazol, Pilocarpine and Maximally electroshock induced seizure models.

ii. To evaluate and compare the preventive role of Diltiazem, Nimodipine and Flunarizine by assaying antioxidant, neurotransmitter and inflammatory marker levels in the brain on individual Pentylenetetrazol, Pilocarpine and Maximally electroshock induced seizure models.

iii. To evaluate and compare the preventive role of Diltiazem, Nimodipine and Flunarizine on microanatomical changes of brain on individual Pentylenetetrazol, Pilocarpine and Maximally electroshock induced seizure models.

iv. To evaluate and compare the effect of Diltiazem, Nimodipine and Flunarizine on immunohistochemistry of brain in individual Pentylenetetrazol, Pilocarpine and Maximally electroshock induced seizure models.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Terms used in epilepsy

Traditionally, including many neurology textbooks define epilepsy as disorder or family of disorders. The term ‘disorder’ does not elicit required seriousness to address the underlying condition of epilepsy. ILAE recommends use of term disease for epilepsy, simply as this term indicates a more lasting derangement in the neurological functions and calls for more stringent and meaningful measures to address the etiopathological basis of the neurological condition.

Two seizure episodes are required for the diagnosis of epilepsy. In addition, in those individuals having pathological seizure propensity, with one episode of unprovoked seizures, epilepsy diagnosis to be considered. Absence of temporary or reversible factors lowering the seizure threshold is taken as ‘unprovoked’. In the presence of clear precipitating factor, such as concussion, fever, alcohol withdrawal, a single episode of seizure do not diagnose epilepsy.[2]

Single seizure episode occurring after a month of stroke or concussion constitute high recurrence risk. Similarly, in a child single seizure with structural or symptomatic etiology and electroencephalogram (EEG) study constitutes high recurrence risk.[2] However, a single seizure presenting as status epilepticus do not indicate a diagnosis of epilepsy. A single seizure episode with an epileptiform EEG does not per se qualify for the diagnosis of epilepsy. In the absence of these clear information about the recurrence risk, diagnosis of epilepsy can be made only with a second unprovoked seizure.[2]

Epilepsy syndrome, if present, then the diagnosis of epilepsy is evident. The syndromes include juvenile absence syndrome, juvenile myoclonic seizures, Angelman syndrome, Rolandic syndrome, hypothalamic hamartoma, ring

chromosome - 20 and many more. Presence of this syndrome association, epilepsy diagnosis shall be clear and pressing.[6]

Finally, a seizure is a sudden surge of electrical activity in the brain. A convulsion is a condition in which body muscles contract and relax rapidly and repeatedly, results in an uncontrolled shaking of the body.

Animal models of epilepsy

Three main categories of animal models are as follows:

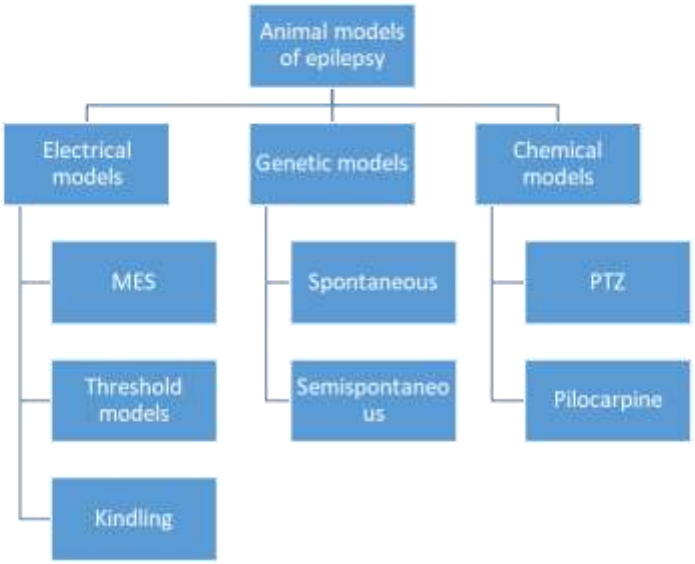

Figure 3: Animal models of epilepsy

Electrical models

Electrically induced seizures have three variants namely, maximal electroshock (MES) model, threshold model and kindling.[9]

In the **maximal electroshock (MES)** induced seizures, the animals used are mice or rats. An external device stimulator/convulsiometer is used to deliver an electrical stimulus strong enough to cause maximal (tonic extension) seizures of the hind limb. A supra maximal current strength, i.e. a stimulus about 5-10 times higher

than the individual electrical seizure threshold of the animals is used (50 mA in mice or 150 mA in rats and for 0.25 sec.). The stimulus is applied via corneal or ear clip electrodes. Drugs like phenytoin, carbamazepine, phenobarbitone and primidone are highly active in this test while ethosuximide is ineffective. The fact that the stimulus is so intense is a major flaw in this test. As a result, certain potentially beneficial agents may be overlooked. To obviate this, threshold seizures are employed. MES seizures remain the primary screen for potential anti-epileptic activity.[10]

In **threshold test (threshold models)**, the ability of a drug to alter the seizure threshold for tonic hind limb extension is determined as the current or voltage inducing hind limb extension in 50% of the animals. This test is a better predictor of generalized seizures of grandmal type and is much more sensitive to drugs than the MES test. Furthermore, threshold tests allow detection of proconvulsant effects of a given drug.

In **kindling**, repeated administration of an initially subconvulsive electrical stimulus results in a progressive intensification of seizure activity, culminating in a generalized seizure. The modification induced is robust and permanent. The seizures evolve through five stages i) immobility, eye closure, twitching of vibrissae, ii) facial clonus and head nodding, iii) unilateral forelimb clonus (contralateral to the focus), iv) rearing often accompanied by bilateral forelimb clonus, v) rearing and falling accompanied by generalized clonic seizures. Stages ‘i’ and ‘v’ correspond to complex partial (limbic or temporal lobe seizures) while stages ‘iii’ to ‘v’ signify limbic seizures evolving to generalized motor seizures. Kindling is a relatively time consuming procedure, requires chronic implantation of stimulation & recording electrodes and regular electrical stimulation.

Genetic models of epilepsy

Animals with chronically recurring, spontaneous seizures represent ideal models for human epilepsy. Some species and strains are prone to spontaneous seizures on a regular basis, or have a very low threshold for epileptogenic agents including stress and sensory stimulation. The genetic models may be classified into two groups. i) Spontaneous and ii) Semi spontaneous.

With newer genetic technics such as genetic linkage maps, microsatellite markers, single nucleotide polymorphism markers and genome wide sequencing. More than 27 inbred rat strains are reported, that can be used for genetic model of epilepsy. More than 650 rats strains are commercially available for genetic model studies.[11]

Chemically induced seizure models

Numerous chemicals and drugs cause seizures in experimental animals at toxic dosages, and many of these can be used to trigger epileptic seizures. Chemoconvulsants can be administered systemically (centrally) or topically.

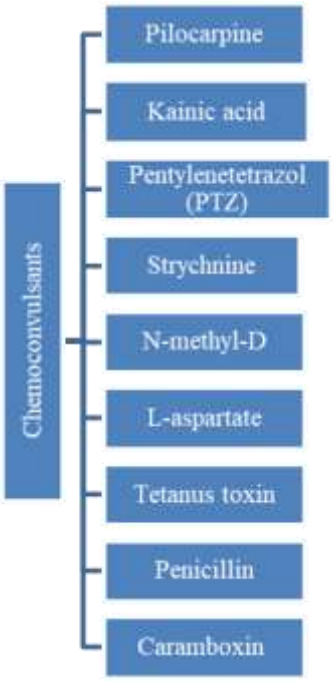

Figure 4: Important chemoconvulsants for animal models of epilepsy

Mainly, the chemical model epilepsy resembles temporal lobe epilepsy. Therefore, it is most likely mimic the human form of the disease including status epilepticus. Among many chemicals that induce epilepsy in animals, kainic acid was first to be used experimentally. Pilocarpine is a muscarinic acetylcholine receptor agonist. Administration, either systematically or intracranially results in seizures.[12] Injection of PTZ in rats or mice produces clonic convulsions that are prevented by drugs effective in myoclonic and absence seizures. Activity in this model represents action on seizure focus itself.

Epilepsy

Epilepsy implies a periodic recurrence of seizures with or without convulsions. A seizure results from an excessive discharge of cortical neurons and is characterized by changes in the electrical activity as measured by the electroencephalogram (EEG). A convulsion implies a violent, involuntary contraction(s) of the voluntary muscles. Epilepsy is a very common disorder, characterised by seizures, which take various forms due to the result from episodic neuronal discharges, and the form of the seizure is depending on the part of the brain affected.[1]

Improvements have been steady rather than spectacular, and epilepsy remains a difficult problem, despite the fact that controlling reverberative neuronal discharges would seem, on the face of it, to be a much simpler problem than controlling those aspects of brain function that determine emotions, mood and cognitive function. The site of the primary discharge and the extent of its spread determine the symptoms that are produced, which range from a brief lapse of attention to a full convulsive fit lasting for several minutes, as well as odd sensations or behaviours. The particular symptoms produced depend on the function of the region of the brain that is affected.

Thus, involvement of the motor cortex causes convulsions, involvement of the hypothalamus causes peripheral autonomic discharge, and involvement of the reticular formation in the upper brain stem leads to the loss of consciousness.

Nature of epilepsy

The characteristic event is the seizure, which may be associated with the convulsions but may take other forms. An asynchronous high frequency discharge of a group of neurons, starting locally and spreading to a varying extent to affect other parts of the brain constitutes seizures. In absence seizures, the discharge is regular and oscillatory. If only part of the brain is involved, then it results in partial seizures. It may involve mainly motor, sensory or behavioural phenomena. Unconsciousness occurs when the reticular formation is involved. Generalised seizures affect the whole brain. Two common forms of generalised seizure are the tonic - clonic seizure and the absence seizure. Status epilepticus is a life-threatening condition in which seizure activity is uninterrupted. Partial seizures can become secondarily generalised if the localised abnormal neuronal activity subsequently spreads across the whole brain. To evaluate and reproduce these disease processes, many animal models have been devised, including electrically and chemically induced generalised seizures, production of local chemical damage and kindling. These provide good prediction of antiepileptic drug effects in humans. The neurochemical basis of the abnormal discharge is not well understood. It may be associated with the enhanced excitatory amino acid transmission, impaired inhibitory transmission or abnormal electrical properties of the affected cells. Several susceptibility genes, mainly encoding neuronal ion channels, have been identified. Repeated epileptic discharge can cause the neuronal death (excitotoxicity).[13]

Classification of epilepsy:

The new ILAE classification of epilepsy considers three key features.[1] They are

- Focus of seizure in the brain
- Awareness during a seizure
- Other features of seizures

Based on these key features, epilepsy is divided into the following types

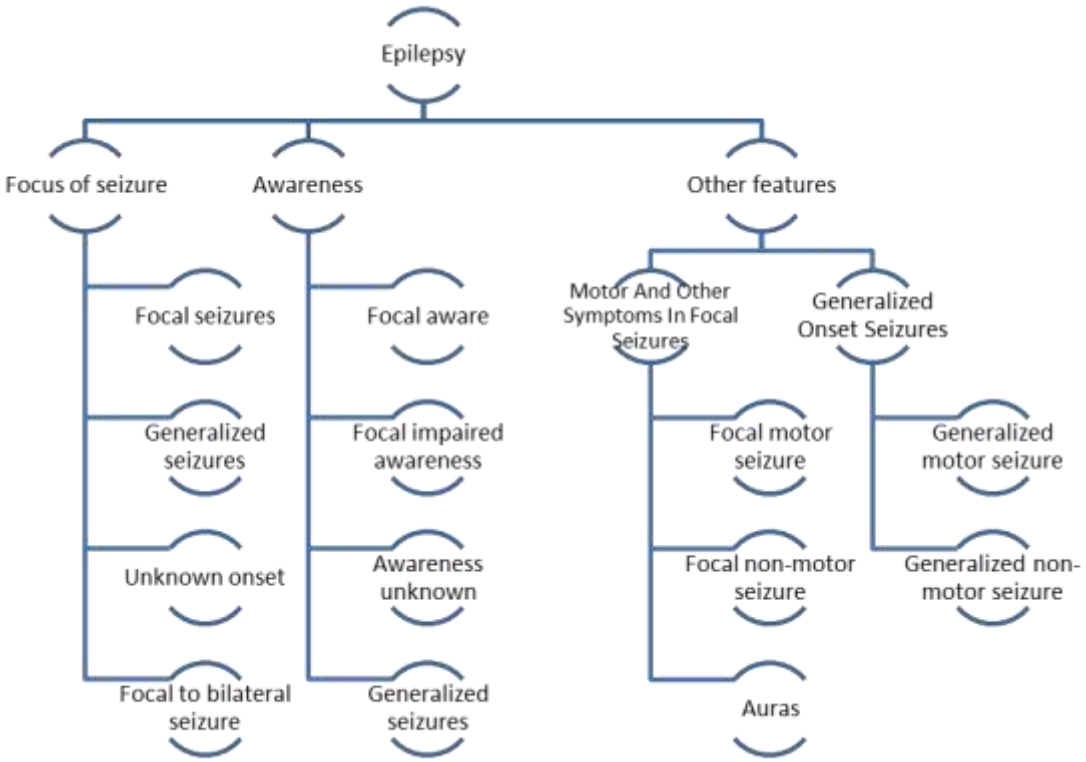

Figure 5: Three key features of epilepsy to be considered during classification of epilepsy

For more than a century, epilepsy was classified into two broad categories, grand mal and petit mal. Since three decades, these crude ways of defining epilepsy, has changed to partial and generalized seizures. Partial can be simple partial and complex partial. Recently, international league against epilepsy (ILAE) has described in detail regarding the classification of the epilepsy (Figure:6) based on the above mentioned three key features.[1]

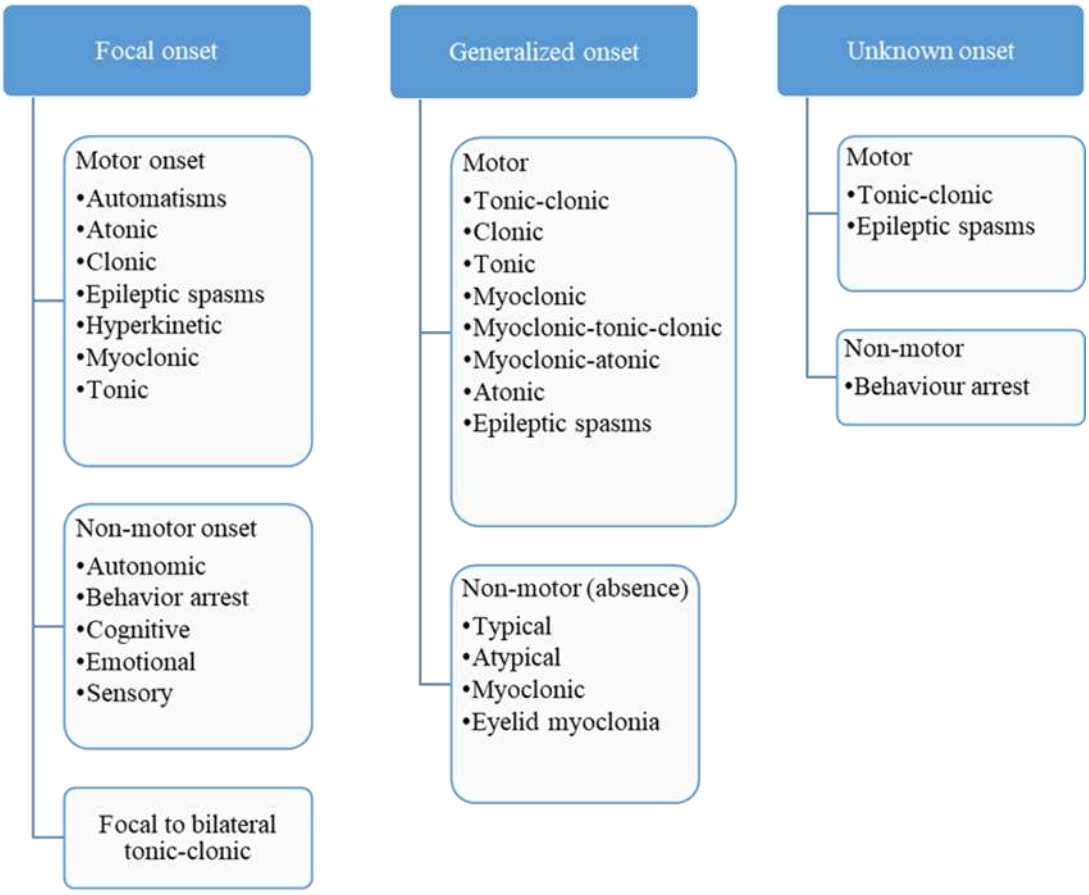

Figure 6: Classification of epilepsy according to International League Against Epilepsy (ILAE) [1]

This framework has been slightly expanded to include even more sub varieties of seizures.

Overall, seizures can be classified by observing symptoms and signs / based on the history and narrations of the patients and bystanders. Other inputs can be obtained from the video recordings and EEG. Brain imaging through MRI, some of the neuro-markers and genetic tests can also add to the diagnosis.[1]

- **Generalised tonic-clonic seizures (GTCS, Major epilepsy, Grand mal):**

  A tonic-clonic seizure consists of an initial strong contraction of the whole musculature, causing a rigid extensor spasm and an involuntary cry. Respiration stops, and defaecation, micturition and salivation often occur. This tonic phase lasts for about one minute, during which the face is suffused and becomes blue (an important clinical distinction from syncope, the main disorder from which fits must be distinguished, where the face is ashen pale), and is followed by a series of violent, synchronous jerks. The entire process may extend for two minutes to maximum of four minutes. The patient stays unconscious for a few more minutes and then gradually recovers, feeling ill and confused. Injury may occur during the convulsive episode. The EEG shows generalised, continuous and high-frequency activity in the tonic phase and an intermittent discharge in the clonic phase.[14]

- **Absence seizures (Minor epilepsy, Petit mal):**

  Prevalent in children, lasts about half minute or thirty seconds momentary loss of consciousness; patient apparently freezes and stares in one direction, no muscular component or little bilateral jerking. EEG shows characteristic three cycles per second spike and wave pattern. Patients are unaware of their surroundings and recover abruptly with no after effects. The rhythmicity appears to be due to oscillatory feedback between the cortex and the thalamus, the special properties of the thalamic neurons being dependent on the T-type calcium channels.[15]

- **Atonic seizures (Akinetic epilepsy):**

  Unconsciousness with relaxation of all muscles due to excessive inhibitory discharges. Patient may fall.

- **Myoclonic seizures:**

  Shock-like momentary contraction of muscles of a limb or the whole body.

- **Infantile spasms (Hypsarrhythmia):**

  Seen in infants. Probably not a form of epilepsy. Intermittent muscle spasm and progressive mental deterioration. Diffuse changes in EEG during the inter seizure period are noted**.**

Partial seizures

A partial seizure originates in one cerebral hemisphere, and the patient does not lose consciousness during the seizure.

- **Simple partial seizures (SPS, Cortical focal epilepsy):**

  Lasts ½ -1 min. Often secondary. Convulsions are confined to a group of muscles or localized and the sensory disturbance depending on the area of cortex involved in the seizure, without loss of consciousness.

- **Complex partial seizures (CPS, Temporal lobe epilepsy, Psychomotor):**

  Attacks of bizarre and confused behaviour and purposeless movements, emotional changes lasting 1-2 min. along with impairment of consciousness. An aura often precedes. The seizure focus is located in the temporal lobe.

- **Simple partial or complex partial seizures secondarily generalized:**

  The partial seizure occurs first and evolves into generalized tonic-clonic seizures with the loss of consciousness.

Neurobiology of seizures

Epileptic seizures are caused by synchronous neuronal discharges within a particular group of neurons, or seizure focus, which is often located in the cerebral cortex. However, similar foci may be found in the other parts of the brain. These impulses gradually spread to the other parts of the brain and produce abnormal movements, sensations, or thoughts. The neuronal mechanisms that initiate a seizure is not fully understood, but growing evidence indicates the involvement of excessive excitatory neurotransmission mediated by glutamate. Investigators believe that the excessive activation by glutamate of N-methyl-D-aspartate (NMDA) receptors displaces Mg^{2+} ions from the NMDA receptor - calcium ion channel and thereby, facilitates the calcium entry into the neurons. Calcium contributes to the long-term potentiation of excitatory glutamate neurotransmission by activating the synthesis of nitric oxide. Nitric oxide is a gas that can diffuse backward to the presynaptic neuron, where it facilitates glutamate release via stimulation of a G protein that activates the synthesis of cyclic guanosine monophosphate. These actions further increase NMDA receptor activation and calcium influx, which are believed to contribute to the depolarization shift that is observed in the seizure foci. The depolarization shift consists of abnormally prolonged action potentials (depolarizations) that have spikelets. The shift recruits and synchronizes depolarizations by surrounding neurons and thereby, initiates a seizure.[16]

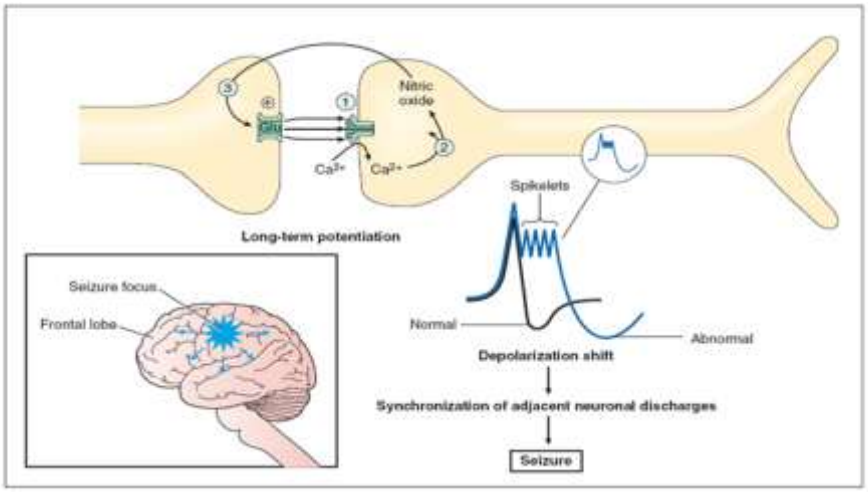

Figure 7: Illustration of epileptogenic focus and spread of impulses[17]

Pathophysiology

Epileptic seizure are produced by abnormal discharges of neurons that may be caused by any pathological process which affects the brain. The idiopathic epilepsies are those in which there is a clear genetic point, and probably account for a third of all the new cases of epilepsy. The likely aetiology of epilepsy depends upon the age of the patient and the type of seizure. The commonest causes in young infants are hypoxia or birth asphyxia, intracranial trauma during birth, metabolic disturbances and congenital malformations of the brain or infection. In young children and adolescents, idiopathic seizures account for the majority of the epilepsies, although trauma and infection play a major role. In this age group, particularly in children aged between six months and five years, seizures may occur in association with febrile illness. These are usually short, generalised tonic - clonic convulsions that occur during the early phase of a febrile disease. That must be distinguished from the seizures that are triggered by the central nervous system infections that produce fever.

The range of causes of adult onset epilepsy is very wide. Both idiopathic epilepsy, and epilepsy due to birth trauma may also begin in early adulthood.[17] Other important causes are head injury, alcohol abuse, cortical dysplasia, brain tumors and

cerebrovascular diseases. Brain tumors are responsible for the development of epilepsy in up to a third of the patients between the ages of 30 and 50 years.

Seizures result from the excessive excitation, or from disordered inhibition of a population of neurons. A small proportion of neurons fire abnormally at first. Normal membrane conductance and inhibitory synaptic currents are interrupted, causing excitability to spread locally (focal seizure) or more widely (generalised seizures).

Mechanisms that may contribute to synchronous hyper excitability include:

- Modulation of second messaging systems and gene expression
- Modulation of ion channels in neuronal membranes
- Biochemical alterations of receptors
- Changes in the concentrations of extracellular ions
- Glial cell changes in neurotransmitter absorption and metabolism
- Alteration of inhibitory circuits' ratio and function
- Imbalances in local neurotransmitters (for example: glutamate, -amino butyric acid [GABA], acetylcholine, nor-epinephrine, and serotonin,)

Large numbers of generalized tonic-clonic (GTC) seizures (more than 100) and multiple episodes of status epilepticus may be associated with the neuronal damage. In particular, continued exposure to glutamate may contribute to the neuronal damage.

Pharmacotherapy of epilepsy

Three main mechanisms that are involved in the pharmacotherapy of epilepsy are as follows:

- Limit the repetitively firing neurons by inactivating the voltage activated sodium channels
- Increase the γ-amino butyric acid (GABA) mediated synaptic inhibition. Both partial seizures and generalized seizures are targeted with this mechanism.
- Inactivation of voltage activated calcium channels responsible for calcium currents; absence seizures, are targeted by this mechanism.

Figure 8: Classification of epileptic seizures and existing antiepileptic drugs

Current therapy of epilepsy (Antiepileptic drugs - AEDs)

The treatment of choice depends on the type of epilepsy and on drug-specific adverse effects and patient preference, is a suggested algorithm for the treatment of epilepsy. Begin with monotherapy; about 50% to 70% of patients can be maintained on one antiepileptic drug (AED), but all are not seizure free. Up to 60% of the patients with epilepsy are noncompliant, and this is the most common reason for the treatment failure. Drug therapy may not be indicated in the patients who have had only one seizure or those whose seizures have minimal impact on their lives. Patients who have had two or more seizures should generally be started on AEDs.

Classification

i. Barbiturate: Phenobarbitone
ii. Deoxybarbiturate: Primidone
iii. Hydantoin: Phenytoin, Fosphenytoin
iv. Iminostilbene: Carbamazepine, Oxcarbazepine
v. Succinimide: Ethosuximide
vi. Aliphatic carboxylic acid: Valproic acid, Divalproex
vii. Benzodiazepines: Clonazepam, Diazepam, Lorazepam, Clobazam
viii. Phenyltriazine: Lamotrigine
ix. Gabapentin, Pregabalin -Cyclic GABA analogues
x. Topiramate, Zonisamide, Tiagabine -Newer drugs

Mechanism of action

The currently available anticonvulsant agents are thought to act by three main mechanisms:

i. Reducing electrical excitability of cell membranes, mainly through use-dependent block of sodium channels

ii. Enhancing GABA mediated synaptic inhibition; this may be achieved by an enhanced post synaptic action of GABA, by inhibiting GABA transaminase or by inhibiting GABA uptake into neurons and glial cells

iii. Inhibiting T-type calcium channels (important in controlling absence seizures).

- Newer drugs act by other mechanisms, largely yet to be elucidated.
- Drugs that block ionotropic glutamate receptors are effective in animal models but are unsuitable for clinical use.

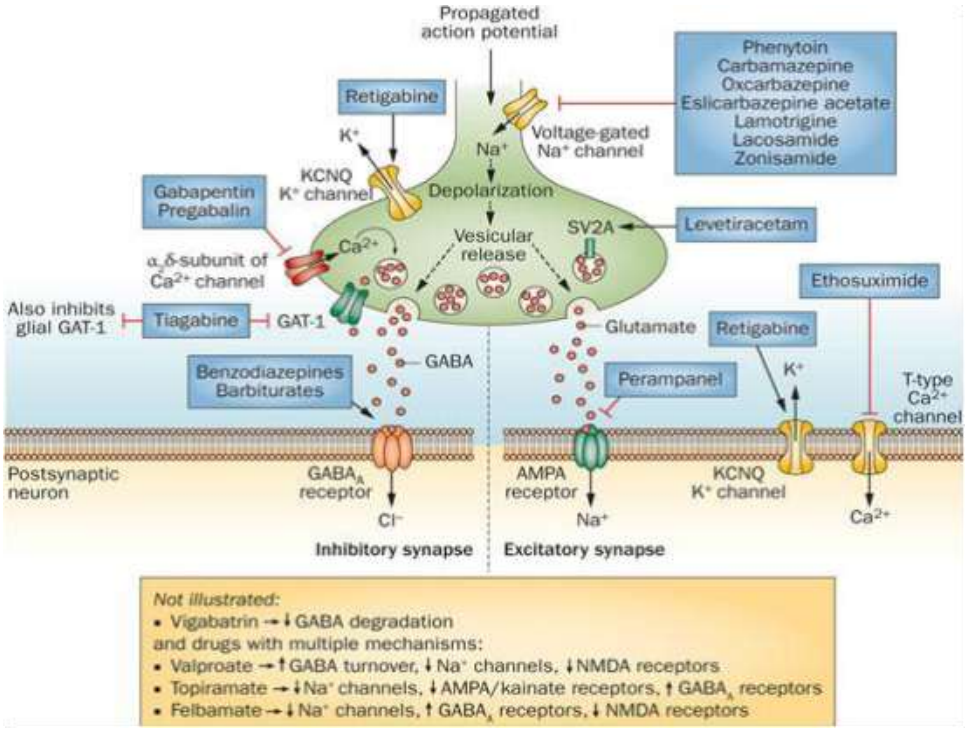

Figure 9: Mechanism of action of anti-epileptic drugs[17]

Surgery to treat underlying conditions

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

Surgery to remove a seizure focus

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

Indications of surgery

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

Contraindications for surgery

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

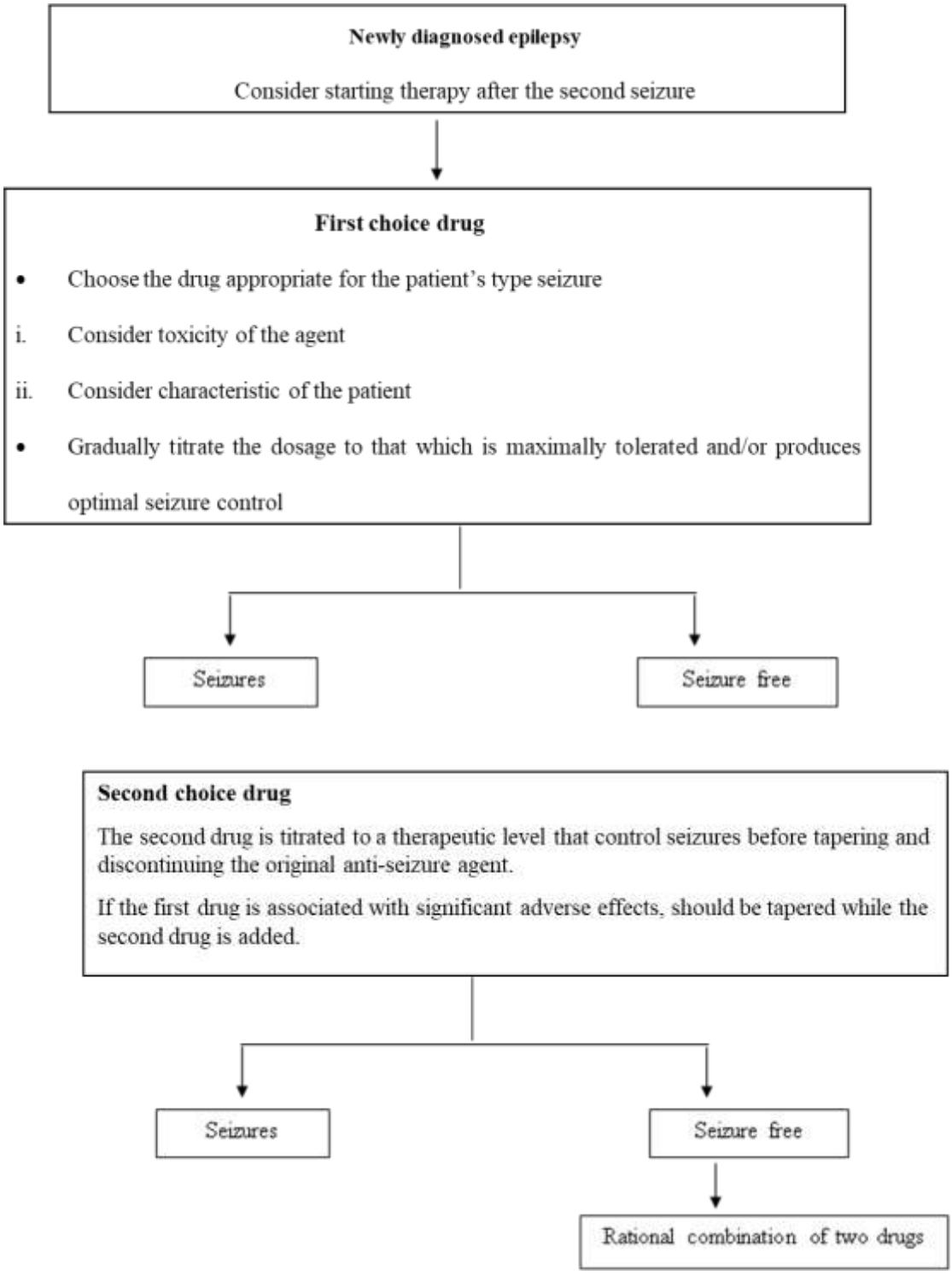

Figure 10: Therapeutic strategies for managing newly diagnosed epilepsy

Previous studies evaluating the antiepileptic effects of calcium channel blockers

Hadizadeh et al., studied the anticonvulsant effect of two dihydropyridine derivatives[diethyl-1,4-dihydro-2,6-dimethyl-4-(4-fluorobenzyl-2-methylthio-5-imidazolyl)-3,5-pyridine dicarboxilat (A) and diethyl-1,4-dihydro-2,6-diethyl-4-(4-fluorobenzyl-2-methylthio-5-imidazolyl)-3,5-pyridinedicarboxilate(B)] by pentylenetetrazole (PTZ) and electroshock in mice was evaluated.[26] By measuring hind limb tonic extensions (latency and duration), they have shown that these two compounds have got significant antiepileptic action along with nifedipine in PTZ and MES models.

Chattopadhyay et al., have evaluated the effects of verapamil, nifedipine and diltiazem in MES model. In this study, reduction of duration of tonic extension was considered as the index for the antiepileptic effect of these drugs. They concluded that, these calcium channel blockers do not individually have antiepileptic action. However, they potentiated the effects of phenytoin.[27]

Sahadevan et al., compared the anticonvulsant activity of three different groups of calcium channel blockers (CCB) nifedipine, diltiazem and flunarizine in different doses on maximum electroshock seizures (MES) and audiogenic seizures (AGS) in albino mice. In both the models, nifedipine and flunarizine has shown promising results by reducing the duration of tonic extensor phase, and overall the mortality reduction.[28]

Khanna et al., investigated the effects of nifedipine and nimodipine on acute (maximal electroshock and pentylenetetrazol model in rats) and chronic (pentylenetetrazol induced kindling in rats and mice) models of epilepsy. They concluded that, both nifedipine and nimodipine, possess anticonvulsant activity in acute models. However, in chronic model studies positive results were noted with

nifedipine only. One study gave peculiar outcome among mice than rats when the observed results were analysed.[29]

Brahmane RI et al., studied the effect of cinnarizine and nifedipine on MES induced and PTZ induced convulsions, and also their effect in combination with the conventional antiepileptic drugs. They found that the addition of these drugs to valproate therapy is beneficial in the animal model studied.[30]

Addition of flunarizine to phenytoin and valproate has shown to have beneficial effects in the MES and PTZ models by Khobragade A et.al.[31]

Kalita P et.al., noted the loss of cellular architecture in the neocortex of the rats induced with PTZ. They postulate that the excitatory neurotransmitter overloaded in axon terminal may be responsible for the neuronal degeneration.[22]

Chowdhury B et al., evaluated the anti-convulsant potential of aqueous and ethanol extract of Glycyrrhizaglabra among PTZ induced rats. They found that this plant extract has anti-convulsant potential. In addition, this extract decreases the free radical induced neuronal damage.[32]

De Sarro et al., evaluated the anticonvulsant activity of some calcium entry blockers in DBA/2 mice. They found that out of all the calcium channel blockers, flunarizine and dihydropyridine had the most potent anti-epileptic actions. This was followed by diltiazem potency. Verapamil and methoxy - verapamil were ineffective as AEDs.[33]

Amano T et al., investigated the effects of single and repeated administrations of S-312-d[methyl-4,7-dihydro-3-isobutyl-6-methyl-4-(3nitrophenyl)thieno (2,3 pyridine-carboxylate], a newly synthesized L-type Ca^{2+} channel blocker, on tonic convulsions and absence-like seizures in the spontaneously epileptic rat (SER: zi / zi,

tm/tm), a genetically based animal model of human epilepsy. They found that this new compound has antiepileptic actions.[34]

Ullal GR et al., evaluated the effect of antiepileptic drugs and the calcium channel blockers on hyperthermic seizures in rats. They found that in this setup, diphenylhydantoin and nifedipine failed to completely supress the seizure activity.[35]

Kalabharathi et al., evaluated the antiepileptic activity of calcium channel blockers in albino mice. They concluded that the combination of nifedipine/amlodipine along with routine AEDs during the therapy reduces the toxic adverse effects.[36]

Picrorhizakurroa leaves extracts (Dilnawaz et al.) has been proven to possess anticonvulsant activity against Pentylenetetrazole, Maximal electroshock and Picrotoxin induced convulsions in mice by Dilnawaz et al.[37]

MATERIALS & METHODS

MATERIALS & METHODS

Ethical Clearance

The Institutional Animal Ethics Committee, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state approved the study (Ref. No. 32/16 on 16/01/2016)

Study design: *In-Vivo and In-Vitro study* in experimental animal models [wistar albino rats]

Duration of the study: 36 months

Number of groups to be studied: 16

Sample size in each group: 6

Total sample size of the study: 96

Scientific basis of sample size used in the study:

Minimum number of animals in each group is six to draw the valid statistical conclusion.[38,39]

Sampling technique used in the study: Convenient sampling

Experimental animals

Experiments were carried out on 3 to 4 week old inbred healthy wistar albino rats of either sexes weighing 180 - 250 gm. All of the rats brought from the central animal house. During the experiment, the animals were housed in groups of six in large, spacious, hygienic cages with unlimited access to water and food. With a relative humidity of 41.55 %, the temperature was regulated at 25°C. During the experiment, a 12:12 light:dark cycle was used. The experiment lasted from 1200 to 1400 hours. Food and water were freely available to the animals. Food was denied for 8 hours before and during the experiments, but not water.

Figure 11: Grouping & caging of the animals [wistar albino rats] in the central animal house

Figure 12: Free access to food & water for the grouped animals [wistar albino rats] in the central animal house

Figure 13: Weighing of wistar albino rats

Inclusion criteria:

Wistar albino rats should be:

- Adult [3 years]
- Healthy
- Males or Females
- Weighing 180 - 250 gm

Exclusion criteria: Rats that had insufficient induction of epilepsy as judged by the examination were excluded. All dead animals during the induction of epilepsy and during the experimental period were excluded from the analysis

Placebo used in study: In vehicle control group [Group I] equivalent volume of normal saline was administered

Drugs used in the study:

Table 1: Details of test drugs used in the study[17]

Sl. No.	Drug	Company Pvt. Ltd.	Wistar albino rats	
	Generic Name	Sigma Aldrich (USA) (Analytical Grade)	Dose [mg/kg]	Route of Administration
1.	Diltiazem		20	i.p
2.	Nimodipine		20	i.p
3.	Flunarizine		10	i.p

Table 2: Pharmacokinetic properties of Calcium Channel Blockers

PK Properties	Diltiazem	Nimodipine	Flunarizine
Bioavailability	40 - 60 %	10 - 30 %	20 %
Volume of Distribution (Avd) (L/Kg)	3	7 - 15	10
Clearance [CL] (L/Hour/Kg)	0.7	1.76	5
Plasma Half Life (Hour)	3 - 6	8 - 9	5 - 15

There had been only a minimal attempt to analyse a group of drugs at the same time in order to build a corpus of comparison data. As a result, this study was intended to test three drugs in three rat models at the same time. This was planned to offer comprehensive information about the effects of these drugs [CCBs] in rat epilepsy models. Anticonvulsant activity, oxidative stress enzyme assay, neurotransmitter assay, inflammatory marker assay, histopathology, and immunohistochemistry of three drugs in three animal models were evaluated.

Table 3: Details of experimental and standard drugs used in the study[38,40,41,42]

Drug	**Wistar albino rats**	
Generic Name	**Dose [mg/kg]**	**Route of administration**
Pentylenetetrazol	70	s.c
Pilocarpine	360	i.p
Sodium Valproate	150	i.p
Phenobarbitone	25	i.p
Diphenylhydantoin	25	i.p
Lithium carbonate	15	i.p
Urethane	1.5*	s.c
Carboxymethyl cellulose	Not applicable	

*Urethane: 1.5 g/kg of wistar albino rats; s.c - Subcutaneous; i.p – Intraperitoneal

Detailed description of the groups:

A total of 96 wistar albino rats were selected and divided into 16 groups, one vehicle control group and five groups each for PTZ, Pilocarpine and MES models.

Vehicle control group

The vehicle control group consisted of six healthy adult wistar albino rats (Group I). Normal saline was administered intraperitoneally to these rats at the same time as the experimental groups received the drug. Antioxidant assays, neurotransmitter estimations, inflammatory marker estimation, brain histopathology examinations, and immunohistochemistry studies were performed in the same way as the experimental groups.

To limit the number of animals used in the study, the same Group I (vehicle control) was used in all three animal models.

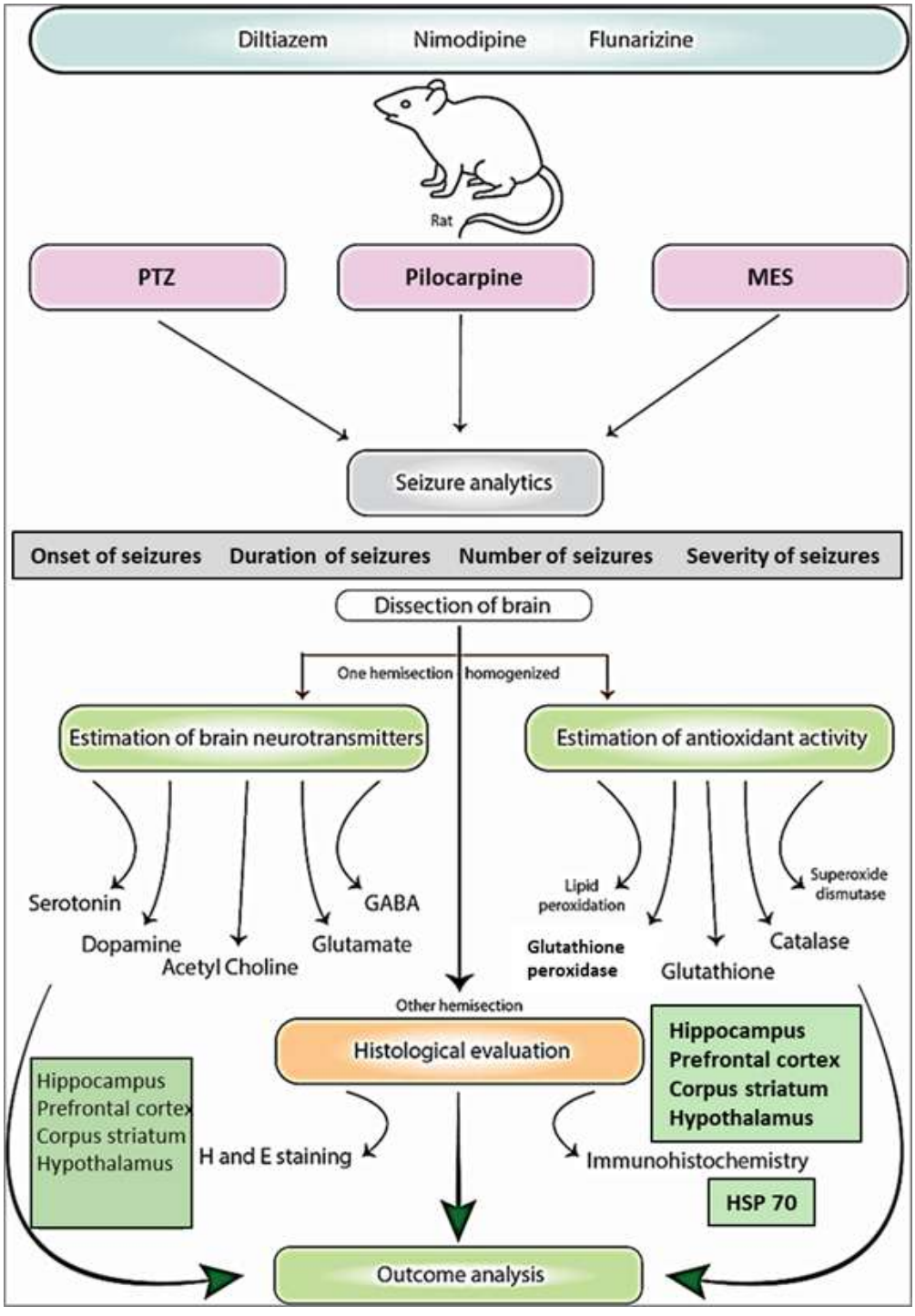

Figure 14: Evaluation plan of the study

Table 4: Detailed description of study groups

Model I: Pentylenetetrazole (PTZ) Model	
Group I	Vehicle control [Equivalent normal saline i.p]**
Group II	Negative control for Pentylenetetrazol model 70 mg/kg BW s.c
Group III	Standard/Positive control for Pentylenetetrazol model [Sodium valproate 150 mg/kg BW i.p]
Group IV	Diltiazem 20 mg/kg BW i.p
Group V	Nimodipine 20 mg/kg BW i.p
Group VI	Flunarizine 10 mg/kg BW i.p
Model II: Pilocarpine Model	
Group I	Vehicle control [Equivalent normal saline i.p]**
Group VII	Negative control for Pilocarpine model 360 mg/kg BW i.p
Group VIII	Standard/Positive control for Pilocarpine model [Phenobarbitone 25 mg/kg BW i.p]
Group IX	Diltiazem 20 mg/kg BW i.p
Group X	Nimodipine 20 mg/kg BW i.p
Group XI	Flunarizine 10 mg/kg BW i.p
Model III: Maximal Electroshock Seizure [MES] Model	
Group I	Vehicle control [Equivalent normal saline i.p]**
Group XII	Negative control for MES model 150 mA for 0.2 seconds. MES stimulation were given using ear-clip electrodes
Group XIII	Standard/Positive control for MES model [Diphenylhydantoin 25 mg/kg BW i.p]
Group XIV	Diltiazem 20 mg/kg BW i.p
Group XV	Nimodipine 20 mg/kg BW i.p
Group XVI	Flunarizine 10 mg/kg BW i.p

*BW - Body weight; **Group I (Vehicle control) was same for all the three models to reduce the number of animals in the study; i.p – Intraperitoneal

Parameters to be studied:

For chemical models [Pentylenetetrazol and Pilocarpine]

i. Seizure Analytics

- Onset of seizures [in seconds]
- Duration of seizures [in seconds]
- Number of seizures [in 6 Hours]
- Severity of seizures [Scores 0-5][39]

The scoring system for evaluating the severity of seizure was as follows:

Table 5: Scoring system for evaluating the severity of seizure (Scores 0-5) [39]

Scores	Operational Definition [In Terms of Test Drug(s) Activity]	Interpretation
0	Excellent	No behavioral changes
1	Good	Isolated myoclonic jerks [Ear and facial twitching]
2	Borderline	Atypical minimal seizures [Convulsive wave through the body]
3	Poor	Fully developed minimal seizures, clonus of head muscles & forelimbs, righting reflex present
4	Very Poor	Major seizures [Generalized without the tonic phase]
5	Not Acceptable	Generalized tonic-clonic seizures beginning with running

ii. Assay of oxidative stress markers

iii. Assay of neurotransmitters

iv. Assay of inflammatory marker

v. Microanatomical changes in different parts of brain of wistar albino rats in all the three models (PTZ, Pilocarpine and MES)

The following areas of the rat brain were considered for the histopathological evaluation.

- Hippocampus
- Prefrontal cortex [Cerebrum]
- Corpus striatum
- Hypothalamus

The scoring system for microanatomical changes in different parts of brain in all the three models (PTZ, Pilocarpine and MES models) were as follows:

Table 6: Scoring system for evaluating microanatomical changes in different parts of the brain (Scores 0 - 4) [23]

Scores [HPE]	**Operational Definition [In Terms of Test Drug(s) Activity]**	**Interpretation**
0	Excellent	Histological section undistinguishable from control group [Number of healthy neurons appeared normal, even if few pyknotic cells found]
1	Good	More than 75% of healthy neuronal cells with others 25% with clear evidence of cell death
2	Borderline	50 - 74% of healthy neuronal cells
3	Poor	25 - 49% of healthy neuronal cells
4	Very Poor	Less than 25% of healthy neuronal cells

vi. Immunohistochemistry study in different parts of wistar albino rats brain in all the three models (PTZ, Pilocarpine and MES)

The following areas of the rat brain were considered for immunohistochemistry evaluation.

- Hippocampus
- Prefrontal cortex [Cerebrum]
- Corpus striatum [Basal nuclei]
- Hypothalamus

The scoring system for immunohistochemistry changes in different parts of brain in all the three models (PTZ, pilocarpine and MES) were as follows:

Table 7: Scoring system for evaluating immunohistochemistry changes in different parts of the brain (Scores 0 - 5) [23]

IHC Hsp70 Expression	Scores	Operational Definition [In Terms of Test Drug(s) Activity]	Interpretation
Nil	0	Nil/No	No Neuroprotection
+	1	Mild	Mild Neuroprotection
++	2	Borderline	Borderline Neuroprotection
+++	3	Good	Good Neuroprotection
++++	4	Excellent	Excellent Neuroprotection
>4(+)	5		

For electrical model [Maximal electroshock seizure model]

The following parameters were evaluated

- Presence or absence of Tonic Hind Limb Extension [THLE] in animals
- Quantification of seizures by score [Score 0 - 4][39]

The scoring system used for the quantification of seizures were as follows:

Table 8: Scoring system for quantification of seizures in MES Model (Scores 0-4) [43]

Scores	Operational Definition [In Terms of Test Drug(s) Activity]	Interpretation
0	Excellent	No seizure
1	Good	Forelimb extension without hind limb extension
2	Borderline	Complete forelimb extension and partial hindlimb extension
3	Poor	Complete tonic hind limb extension [THLE] [Hind limb become parallel to the tail]
4	Very Poor	Post ictal depression

Procedure in detail:

The research proposal was submitted to the Shri B. M. Patil Medical College, Hospital and Research Centre [B.L.D.E deemed to be University] Institutional Animal Ethics Committee [IAEC] and received approval. [CPCSEA] were followed during the research.

A total of 96 wistar albino rats were chosen and divided into 16 groups: one vehicle control group, five experimental groups for PTZ, Pilocarpine, and MES models. During the induction of seizures in different models, four rats perished. Because of insufficient seizure induction in various models, five rats were removed from the study. In both the PTZ and Pilocarpine models, three rats died during the experiment. All of the rats that were left were exposed to the experiment. The analysis included six rats from each group.

Evaluation of anticonvulsant activity

Pentylenetetrazole (PTZ) Model:[41]

The study employed Wistar albino rats weighing between 180 and 250 grammes. Each set of six rats were present in each six groups..

Table 9: Description of groups and drugs administered in Pentylenetetrazol (PTZ) model during the study

Group	Group specification	Drug administered with dosage
Group I	Normal [Vehicle control]	Normal saline equivalent i.p
Group II	PTZ only [Negative control]	PTZ 70 mg/kg BW s.c
Group III	PTZ + Sodium valproate [Positive control]	Sodium valproate 150 mg/kg BW i.p
Group IV	PTZ + Diltiazem group	Diltiazem 20 mg/kg BW i.p
Group V	PTZ + Nimodipine group	Nimodipine 20 mg/kg BW i.p
Group VI	PTZ + Flunarizine group	Flunarizine 10 mg/kg BW i.p

In the vehicle control group [Group I], the animals were given an equivalent volume of normal saline intraperitoneally. PTZ (Pentylenetetrazole) 70 mg/kg body weight was administered subcutaneously to the animals in the negative control group [Group II]. The standard drug Sodium valproate was given intraperitoneally to the animals in Group III [positive control]. Calcium channel blockers Diltiazem (20 mg/kg body weight), Nimodipine (20 mg/kg body weight), and Flunarizine (10 mg/kg body weight) were administered intraperitoneally for seven days in Groups IV to VI. All of the animals were given Pentylenetetrazol [PTZ] 70 mg/kg body weight subcutaneously after 30 minutes of receiving the above test drugs, and the relevant parameters were recorded. The animals were kept under observation for six hours. In each animal, the onset of seizures, duration of seizures, number of seizures, and seizure scores were recorded.

Figure 15: Pentylenetetrazole (PTZ) (Sigma Aldrich, India)

Pilocarpine model:[41]

The study employed Wistar albino rats weighing between 180 and 250 grammes. Each set of six rats were present in each six groups.

Table 10: Description of groups and drugs administered in Pilocarpine model during the sudy

Group	Group specification	Drug administered with dosage
Group I	Normal [Vehicle control]	Normal saline equivalent i.p
Group VII	Pilocarpine only [Negative control]	Pilocarpine 360 mg/kg BW i.p
Group VIII	Pilocarpine + Phenobarbitone [Positive control]	Phenobarbitone 25 mg/kg BW i.p
Group IX	Pilocarpine + Diltiazem group	Diltiazem 20 mg/kg BW i.p
Group X	Pilocarpine + Nimodipine group	Nimodipine 20 mg/kg BW i.p
Group XI	Pilocarpine + Flunarizine group	Flunarizine 10 mg/kg BW i.p

In the vehicle control group [Group I], the animals were given an equivalent volume of normal saline intraperitoneally. To keep the number of rats in the study down, all three models used the identical vehicle control group [Group I]. To reduce the unwanted peripheral cholinergic effects of Pilocarpine, the other groups were given lithium carbonate at 15 mg/kg for 24 hours before being given Pilocarpine. To

produce seizures in rats, the required dose of Pilocarpine [360 mg/kg BW i.p] was given to Group VII [Negative Control Group] rats. The standard drug, Phenobarbitone, at 25 mg/kg body weight, was given intraperitoneally to Group VIII animals for seven days. Similarly, Groups IX to XI, were administered with the calcium channel blockers Diltiazem (20 mg/kg body weight), Nimodipine (20 mg/kg body weight), and Flunarizine (10 mg/kg body weight) intraperitoneally for seven days. Pilocarpine (360 mg/kg body weight intraperitoneally) was then given to the rats. The time it took for the first convulsion to occur was recorded. For the first 90 minutes, latency, fictive scratching, tremors, forelimb clonus, and mortality were monitored every 15 minutes, then every 30 minutes until 180 minutes. Rats that survived for more than 30 minutes were considered acceptable.

Maximum electroshock (MES) model:[41]

The study employed Wistar albino rats weighing between 180 - 250 gms. The rats were placed into six groups, each with six rats.

Table 11: Description of groups and drugs administered in MES model during the study

Groups	Group specifications	Drug administered with dosage
Group I	Normal [Vehicle control]	Normal saline equivalent i.p
Group XII	MES alone [Negative control]	150 mA for 0.2 seconds
Group XIII	MES + Diphenylhydantoin [Positive control]	Diphenylhydantoin 25 mg/kg BW i.p
Group XIV	MES + Diltiazem group	Diltiazem 20 mg/kg BW i.p
Group XV	MES + Nimodipine group	Nimodipine 20 mg/kg BW i.p
Group XVI	MES + Flunarizine group	Flunarizine 10 mg/kg BW i.p

Prior to the drug administration, the rats were assessed for electroshock sensitivity. Electro-convulsiometers were used to cause convulsions (Techno India Ltd).

Figure 16: Electro-convulsiometer used in the study

The equipment's trans-auricular (ear-clip) electrodes were employed to give MES stimulation. The MES intensity was tuned to 150 mA for 0.2 seconds using constant voltage stimulators at 250 V. At this intensity and duration, all of the rats in the control group showed tonic hind limb extension. Only rats who consistently

demonstrated tonic hind limb extension in three trials on three different days were included in the study. The rats were placed into six groups, each with six rats. Except for Group I, all the groups were treated with MES (vehicle control group). In the vehicle control group [Group I], the animals were given an equivalent volume of normal saline intraperitoneally.

For seven days, animals in the Group XIII were given the standard drug Phenytoin sodium (Diphenylhydantoin) 25 mg/kg body weight intraperitoneally. Calcium channel blockers Diltiazem (20 mg/kg body weight), Nimodipine (20 mg/kg body weight), and Flunarizine (10 mg/kg body weight) were administered intraperitoneally for seven days in Groups XIV to XVI. Group XII and other standard and test drug groups were given electroshock using an electroconvulsiometer through the ear electrodes [after moistening the animals' ears with a drop of normal saline] at an intensity of 150 mA, 60Hz for 0.2 seconds after 30 minutes of receiving the above drugs. After that, each animals parameters were recorded.

The duration of flexion, tonic hind limb extension, and clonus (measured in seconds) were measured before and after the MES. The anticonvulsant action of the test drug was defined as the elimination of hind limb extension and the reduction (or absence) of clonus duration following drug administration.

Figure 17: Forelimb extension without hind limb extension

Figure 18: Complete Tonic Hind limb extension [THLE]

Figure 19: Clonus

Dissection of brain

Wistar albino rats were anaesthetized with urethane (1.5 g/kg) by subcutaneous route[42] after passing all of the above said seizure activity screening procedures. In all the three models (PTZ, Pilocarpine, and MES), the animals were sacrificed by cervical decapitation on the second day after administering the test drug. The wistar albino rats were then transcardially administered 4% paraformaldehyde[24]. The brains of wistar albino rats were dissected out of the cranial cavity and placed in a 10% buffered formalin solution for 10 - 15 minutes to harden the brain tissue [Figure 20]. Along the longitudinal fissure, each animal's brain [Figure 21] was hemisectioned into right and left halves. In one homogenised hemisection, antioxidants, neurotransmitters, and inflammatory markers were analyzed. The other hemisection was formalin preserved and used for histopathology and immunohistochemistry analyses.

Figure 20: Dissection of rat to remove the brain from the cranial cavity

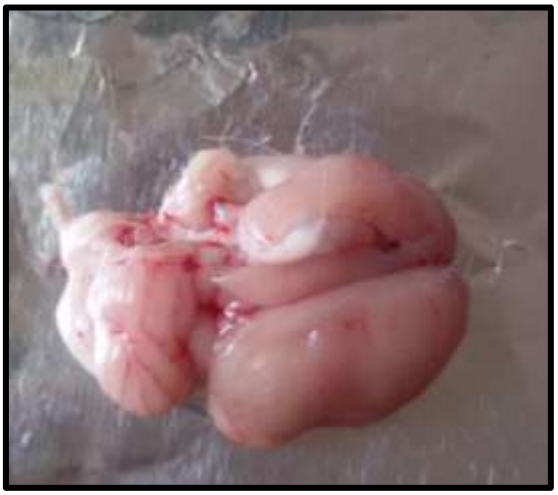

Figure 21: Rat brain dissected out from the cranial cavity

After that, coronal sections of various brain areas with a thickness of 2 mm were taken and fixed in a 10% buffered formalin solution for another 24 hours. It was prepared for paraffin blocks with 5 micrometre sections for Haematoxylin and Eosin [H & E] staining to examine the various microanatomical changes in the various parts of the rat brain.[43-45]

Method of Disposal

The carcass was disposed of in compliance with the [CPCSEA]'s guidelines.

Estimation of brain antioxidants, neurotransmitters and inflammatory markers

One hemisection of the dissected brain tissue was homogenised for 1 minute in 5 mL of HCl-butanol using a homogenizer [Figure 22]. The unbroken cells were removed by centrifugation at 2000 rpm for 10 minutes. A portion of the supernatant phase (1 mL) was taken and mixed with 2.5 mL heptane and 0.31 mL 0.1 M HCL in a centrifuge tube. After 10 minutes of vigorous shaking to separate the two phases, the

tube was centrifuged [Figure 22] under the same circumstances as before, and the overlaying organic phase was discarded. The aqueous phase (0.2 ml) was used to measure antioxidants [SOD, Glutathione peroxidase, Reduced glutathione, Catalase, and Lipid peroxidation], neurotransmitters [Serotonin (5-HT), Dopamine (DA), Gamma-amino butyric acid (GABA), Glutamate, and Acetyl choline (ACh)], and the inflammatory marker [Myeloperoxidase]. At 0°C, all of the processes were completed.

Figure 22: Homogeniser used in the study

Figure 23: Centrifuge used in the study

Figure 24: UV Spectrophotometer used in the study

Estimation of total protein: 0.1 mL homogenate, 0.9 mL water, and 4.5 mL alkaline copper sulphate reagent were added to 0.1 mL homogenate and left to rest at room temperature for 10 minutes. Folin's reagent (0.5 mL) was added to this. The colour obtained after 20 minutes was measured at 640 nm. The amount of protein in each tissue was measured in milligrammes per gramme of tissue.[46]

Estimation of Oxidative Stress Markers[47-50]

- Superoxide Dismutase (SOD)
- Glutathione Peroxidase (GPx)
- Reduced Glutathione (GSH)
- Catalase (CAT)
- Lipid Peroxidase (LPO)

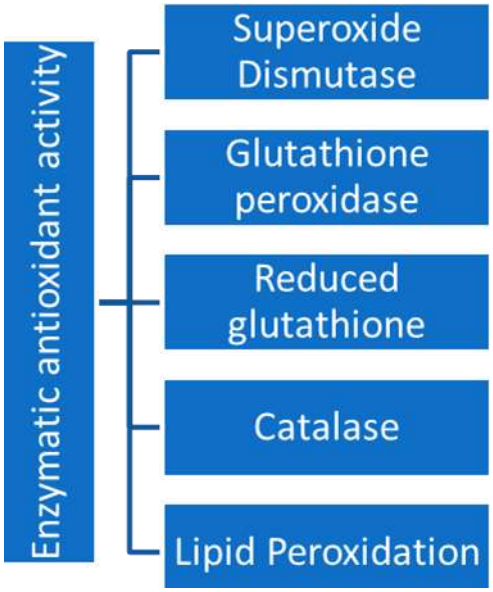

Figure 25: List of oxidative stress markers assayed in the study

Superoxide Dismutase (SOD): 0.1 mL homogenate combination supernatant, 0.1 mL Ethylene-diamine-tetra-acetic acid, EDTA (1 104 M), 0.5 mL carbonate buffer, and 1 mL epinephrine (1 mM) were mixed together. The combination was spectrophotometrically measured for 3 minutes at 480nm. The activity of SOD was measured in units of U/min/mg.[50]

Glutathione Peroxidase: A 3-ml cuvette was filled with 2.0 mL of phosphate buffer (75 mmol/L, PH 7.0), 50µL glutathione reductase solution, 50µL of (0.12 mol/L) NaN3, 0.1 mL of (0.15 mol/L) Na2 EDTA, 100µL of (3.0 mmol/L) NADPH, and 100µL of tissue supernatant. To make a total volume of 2.9 ml, water was added. The

reaction was begun by adding 100L of (7.5 mmol/L) H202, and the conversion of NADPH to NADP was measured by using a UV spectrophotometer to continuously record the change in absorbance at 340 nm at one minute intervals for five minutes. The enzyme activity of GPx was measured in milligrammes of proteins..[50]

Reduced Glutathione: 1 mL of 5 percent TCA was added to 250 L of tissue homogenate in a 2 mL eppendroff tube, and the mixture was centrifuged at 3000 g for ten minutes at room temperature. 1.5 ml of 0.2 M phosphate buffer was added to 250 mL of the aforesaid supernatant and thoroughly mixed. Within ten minutes, 250 µL of 0.6 mM Ellman's reagent (DTNB solution) was added to the aforesaid mixture, and the absorbance was measured at 412 nm. The glutathione reduction solution (1 mg/mL) was used to create a standard graph, and the GSH concentration in the tissue homogenates was estimated using interpolation. Glutathione concentration given as g/mg protein.[47]

Catalase: 1.95 mL of 50 nM phosphate buffer and 1 mL of 30 mM hydrogen peroxide were added to the homogenate mixture. At fifteen-second intervals, the catalase activity was measured at 240 nm. The catalase activity was calculated using the change in catalase absorbance/minute as a function of the hydrogen peroxide extinction coefficient (0.071 mmol cm-1). Catalase activity was measured in micromoles of H_2O_2 oxidised per milligramme of protein per minute.[50]

Lipid Peroxidation: 100 mL tissue homogenate was mixed with 2 mL (1:1:1) thiobarbituric acid reagent (thiobarbituric acid 0.37 percent, 0.25 N hydrochloric acid, and 15% trichloroacetic acid). The material was heated in a boiling water bath for fifteen minutes, then cooled and centrifuged at 3500 rpm for ten minutes at room temperature. The pink colour developed was quantified in a spectrophotometer at 535 nm against a reagent. LPO was calculated as nmol MDA/mg protein.[48]

Estimation of Neurotransmitter Levels[51-56]

- Serotonin (5-HT)
- Dopamine (DA)
- GABA
- Glutamate
- Acetylcholine (ACh)

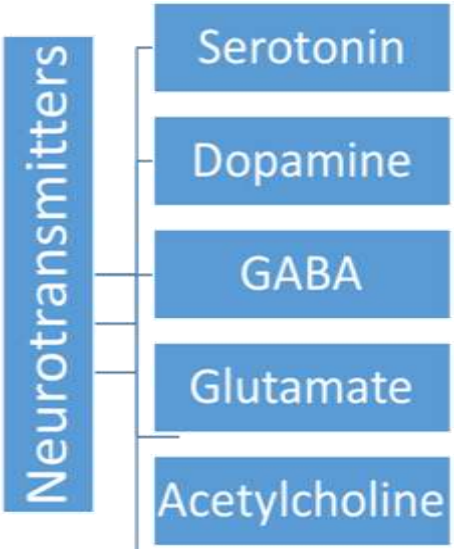

Figure 26: List of neurotransmitters assayed in the study

Serotonin: To 0.2 ml aqueous phase, 0.25 ml of OPT reagent was added. The fluorophore was developed by heating to 100ºC for ten minutes. After the samples reached equilibrium with the ambient temperature, readings were taken at 360- 470 nm in the spectrophotometer.[53]

Dopamine: To the 0.2 ml of aqueous phase, 0.05 ml 0.4 M HCL and 0.1 ml of sodium acetate buffer (pH 6.9) were added, followed by 0.1 ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stopped after two minutes by addition of 0.1 ml Na_2SO_3 solution. 0.1 ml acetic acid is added after one and half minutes. The solution was then heated to 100ºC for six minutes when the sample again reached room temperature; excitation and emission spectra were read from the spectrophotometer. The readings were taken at 330-375 nm for dopamine and 395- 485 nm for nor adrenaline.[52]

GABA: A 0.1 ml sample of tissue extract was mixed with 0.2 ml of 0.14 M ninhydrin solution in 0.5 M carbonate - bicarbonate buffer (pH 9.95) and kept at 60^{o}C in a water bath for thirty minutes. The samples were then treated with 5 mL of copper tartarate reagent after cooling (0.16 percent disodium carbonate, 0.03 percent copper sulphate and 0.0329 percent tartaric acid). After 10 minutes, the fluorescence at 377/455 nm was measured in a spectrophotometer.[54]

Glutamate: To a boiling and ice-cooled supernatant extract of ninhydrin mixed brain homogenate, 0.4 mL guanidine carbonate was added. 1 ml of 100 mM lead acetate, 0.5 ml of 1 N NaOH, and 6 ml of dH2O were added to this mixture. Under ice-cold conditions, 0.1 percent 2,4-dinitrophenyl hydrazine dissolved in 0.01N HCl was added to this mixture and incubated for thirty minutes. A spectrophotometer was used to measure the colour intensity of this combination at 420 nm. The glutamate levels were measured in grammes of monoamine per gramme of moist tissue weight.[56]

Acetylcholine: To activate acetyl cholinesterase, the brain homogenate tissues were boiled. The bound acetyl choline is then released. The addition of ferric chloride solution resulted in the formation of a brown-colored solution. At 540 nm, it was

measured. The acetylcholine content was measured in moles of acetylcholine per gramme of wet tissue weight.[55]

Estimation of Inflammatory marker

Myeloperoxidase (MPO): The tetra-methyl-benzidine technique was used to measure MPO. 10µl sample was mixed to 80µl 0.75 mM H_2O_2 and 110 µl TMB solution. For 5 minutes, the mixture was incubated at 370°C. The reaction was halted with 50µl 2 M H_2SO_4 and absorbance was measured at 450 nm to determine MPO activity.[49]

Histopathological (Microanatomical) evaluation

Histopathology is the examination of tissues under a microscope for pathological changes. This entails collecting morbid tissues, fixing them, preparing sections, staining them, and examining them under a microscope.

Collection of materials

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

Fixation:

Fixation is the first step towards the preparation of a histological section from a dead biological specimen. The substances used for fixation are called as fixatives.

Common Fixatives: Formalin, Zenker's fluids, Bouin's fluid

10% Formalin was used as a fixative in this study and the tissue was kept in fixative for 24-48 hours at room temperature.

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein

b) Prevents autolysis

c) Preserves the structure of the tissue

d) Prevents shrinkage

Washing: After fixation tissue is washed under running tap water one to two hours and it removed the fixative from the tissue.

Dehydration: It was done for the tissue as follows –

One dip in 30% Alcohol

One dip in 50% Alcohol

One dip in 80% Alcohol

One dip in 80% Alcohol

One dip in 90% Alcohol

One dip in 100% Alcohol

One dip in 100% Alcohol

One dip in 100% Alcohol

Two dips in Xylene were used to clear the surface.

Preparation of sections

Infiltration: Tissues were infiltrated with paraffin by dipping three times consecutively in paraffin at 50-56ºC

Embedding: Was done by using L-blocks. The tissue was put in it over which the melted paraffin is poured which solidifies slowly

Section cutting: The tissues were sectioned into thin slices by using microtome

Haematoxylin & Eosin Method of Staining:

Rehydration – Tissues were dipped serially as follows

- Xylene 2 minutes
- Xylene 2 minutes
- Absolute alcohol 1 minute
- Absolute alcohol 1 minute
- 90% Alcohol 2 minutes
- 70% alcohol 2 minutes
- 50% alcohol 2 minutes
- Distilled Water 5 minutes
- Haematoxyllin 2 to 5 minutes with Harris Haematoxyllin
- Washed well in running tap water for 2-3 minutes
- Removed excess stain by differentiating in acid alcohol (1% HCL in 70% alcohol) for a few seconds. Blue staining of haematoxylin stained section is changed to red by the action of the acid
- Then immediately washed in alkaline tap water for at least 5 minutes to regain the blue colour
- Dipped in 1% aqueous Eosin 1 to 3 minutes and washed of surplus eosin in water

- Dipped in 90% alcohol for 10 to 15 seconds
- Dipped in Absolute alcohol I for 10 to15 seconds
- Dipped in Absolute alcohol II for 30 seconds
- Dipped in Xylene I for 1 to 2 minutes
- Dipped in Xylene II for 1 to 2 minutes
- Mounted on D.P.X and kept the slide for drying

Staining resulted as follows:

Nuclei – Blue to blue-black

Nucleolus – Dark blue

Cytoplasm – Pink

Collagen fibers - Lighter pink

Erythrocytes and eosinophil granules - Bright orange red

H and E stained slides showing regional changes in the hippocampus, pre frontal cortex [cerebrum], corpus striatum [basal nuclei] and hypothalamus were evaluated for histoarchitectural changes. Scoring were given on the basis of scoring system.

Scores:

- **0** = Histological section undistinguishable from the control group [Number of healthy neurons appeared normal, even if few pyknotic cells found]
- 1 = More than 75% of healthy neuronal cells with others 25% with clear evidence of cell death
- **2** = 50-74% of healthy neuronal cells
- **3** = 25-49% of healthy neuronal cells
- **4** = Less than 25% of healthy neuronal cells

Immunohistochemistry evaluation with Hsp70 marker

3µ thick sections were obtained from formalin fixed and paraffin embedded brain tissues.

Immunohistochemistry procedure: [46,57-61]

- Paraffin sections were coated with poly L lysine.
- The sections were placed along with antigen retrival pretreatment and buffer.
- Slides were covered with peroxidase blocker and incubated for 10 minutes
- Later slides were washed with deionised water and buffer solution periodically.
- Then covered with power blocking solution and incubated for 10 minutes. It was followed by washing with buffer solution in three changes.
- The pretreated sections were covered with primary antibody against HSP-70 kD (Bio SB, Bio Sciences For the World, CA 93117, USA) diluted in 1:100 phosphate-buffered saline. Incubated for 30 minutes, washed with buffer solutions in three changes.
- Then secondary antibody was added, incubated for 30 minutes in the room temperature; washed with buffer solutions in three changes
- Slides were placed in DAB solution for 10 minutes; washed with buffer in three changes.
- Then the slides were counter stained with haematoxylin solution in 3 dips
- Slides were dried and mounted. Observed under light microscope. All the slides were observed under microscope and evaluated for HSP-70 immunohistochemistry by the pathology expert and the scoring was done as per the scoring system.[57]

On the basis of staining intensity[57]

0 = No color

(1+) = Weak brown

(2+) = Moderate brown

(3+ & above) = Dense brown, strong expression

Scores:

- 0 = Nil/No neuroprotection,
- 1 (Plus$^{+)}$ = Mild Neuroprotection
- 2 (Plus$^{++)}$ = Borderline Neuroprotection
- 3 (Plus$^{+++)}$ = Good Neuroprotection
- 4 Plus$^{++++)}$ = Excellent Neuroprotection/ Normal
- 5 (>Plus$^{++++)}$ = Excellent Neuroprotection/ Normal

Statistical analysis

All the data obtained from each model were tabulated separately by entering into Microsoft Office Excel 2007 and subjected to statistical analysis. The data was expressed in Median±SE [For the scores] and Mean±SE [For all other parameters].

- The statistical significance among the groups of each model were carried by using one way ANOVA followed by

a. Bonferroni's post hoc test for the data with Gaussian or normal distribution, and

b. Kruskal-Wallis test followed by Dunn's post hoc test for the data with non-Gaussian or non-normal distribution.

All the calculations were done with software SPSS V 20 32 bit and the 'P' value less than 0.05 was taken as significant.

RESULTS

RESULTS

The results (observations) are presented serially as follows:

- Evaluation of anticonvulsive activity in all the three models [PTZ, Pilocarpine & MES]
- Evaluation of oxidative stress markers in all the three models [PTZ, Pilocarpine & MES]
- Evaluation of neurotransmitters in all the three models [PTZ, Pilocarpine & MES]
- Evaluation of inflammatory marker in all the three models [PTZ, Pilocarpine & MES]
- Microanatomy [Histopathological] study in all the three models [PTZ, Pilocarpine & MES]
- Immunohistochemistry in all the three models [PTZ, Pilocarpine & MES]

EVALUATION OF ANTI-CONVULSIVE ACTIVITY IN ALL THE THREE MODELS

PENTYLENETETRAZOLE [PTZ] MODEL

Anticonvulsant activity in PTZ Model:

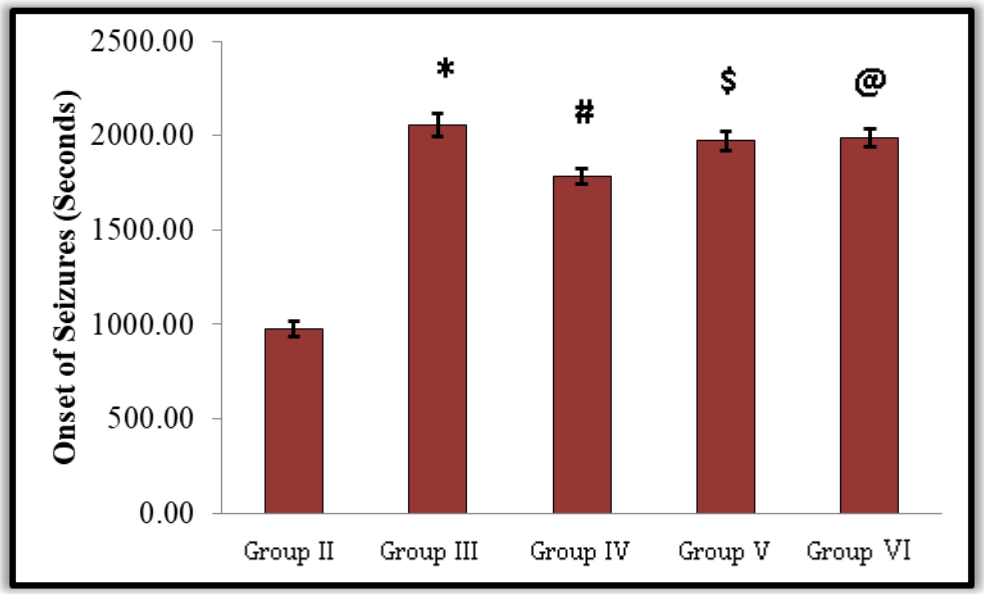

Figure 27: Bar diagram depicting the onset of seizures in seconds in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Results showed that Sodium valproate (standard drug) caused a significant increase in the onset of seizures compared to the negative control group [Group II]. When compared to the negative control group [Group II], all the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] exhibited statistically significant increase in the onset of seizures (P<0.05).

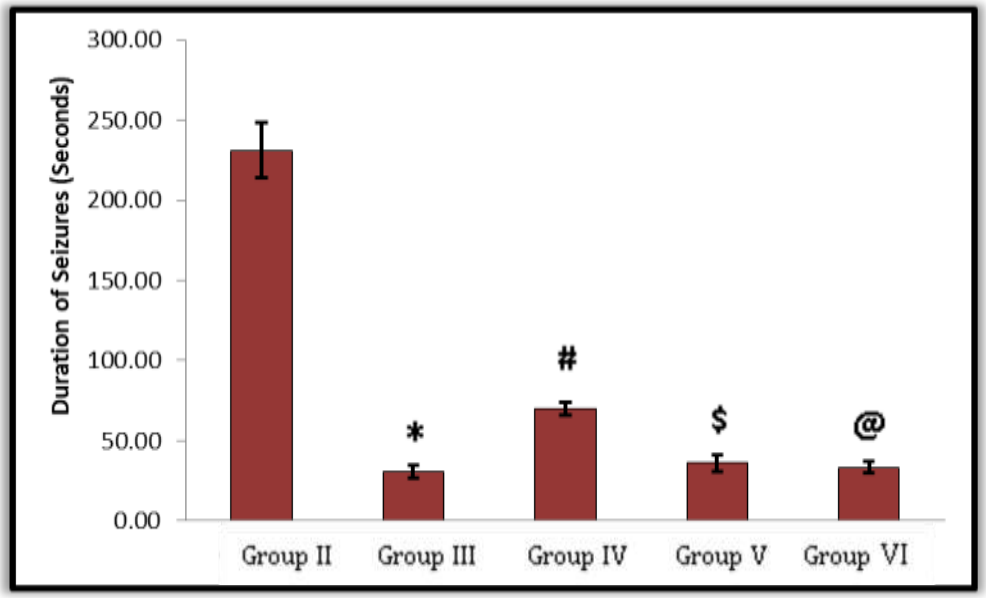

Figure 28: Bar diagram depicting the duration of seizures in seconds in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

It was observed that the Sodium valproate group [Group III] (standard drug group) had a shorter duration of seizures than the negative control group [Group II] (P<0.05). Similarly, all the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] reported a reduction in seizure duration when compared to the negative control group [Group II], which was statistically significant (P<0.05).

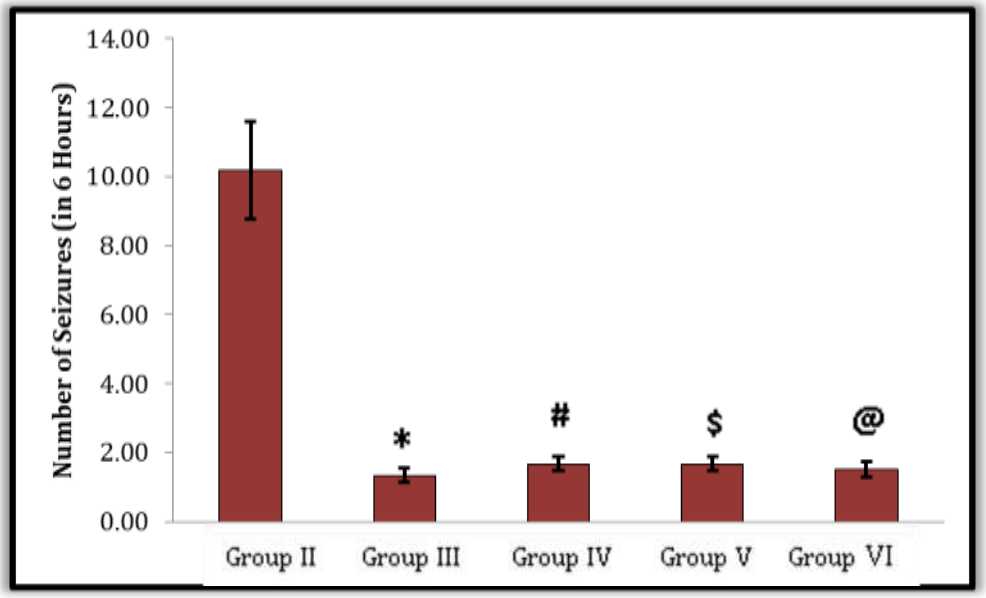

Figure 29: Bar diagram depicting the number of seizures in six hours in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When the parameter (the number of seizures in six hours) was compared to the negative control group [Group II], it was observed that Group III [Sodium valproate group (standard drug group)] had a substantial reduction ($P<0.05$). Additionally, when compared to the negative control group [Group II], the experimental groups (IV, V and VI, i.e. Diltiazem, Nimodipine and Flunarizine) revealed a statistically significant ($P<0.05$) reduction in the number of seizures in six hours.

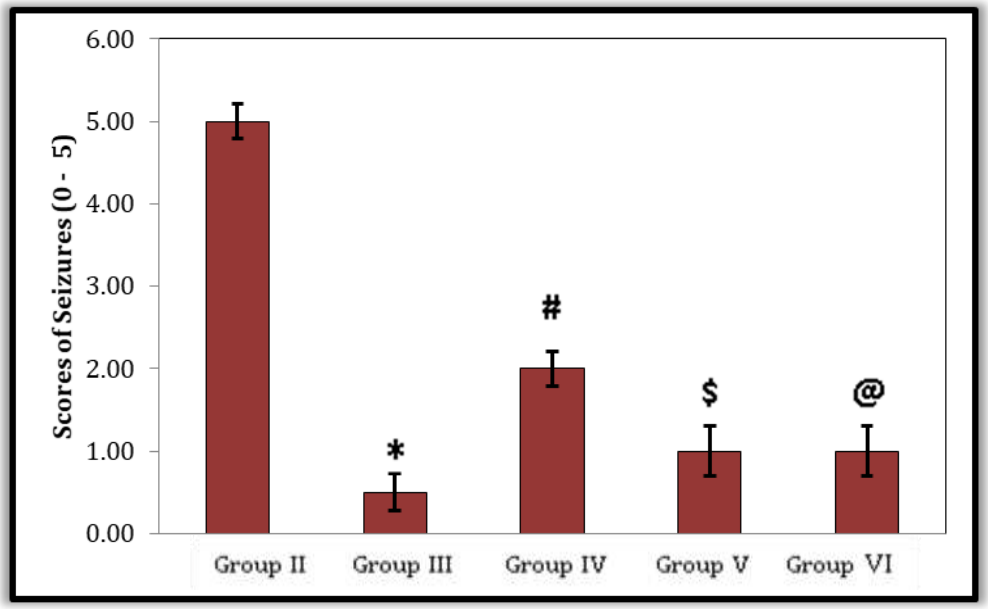

Figure 30: Bar diagram depicting the scores of seizures in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant decrease in the scores of seizures in Group III [Sodium valproate group (standard drug group)] when compared to Group II [Negative control group] (P<0.05). Additionally, it was observed that when compared to Group II [Negative control group], the experimental groups IV, V and VI (Diltiazem, Nimodipine and Flunarizine) indicated a statistically significant (P<0.05) decrease in the seizure scores.

Table 12: Seizure parameters in PTZ model screening test in wistar albino rats

Sl. No.	Parameters	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Onset of seizures (Seconds)	976.33±39.89	2055.67±58.76	1787.17±40.41	1973.5±52.42	1985.83±47.53
2	Duration of seizures (Seconds)	231.17±16.90	30.67±4.01	69.83±3.86	36.17±5.29	33.5±3.70
3	Number of seizures (6 h)	10.17±1.42	1.33±0.21	1.67±0.21	1.67±0.21	1.5±0.22
4	Scores of seizures (0 - 5)	5.00±0.21	0.50±0.22	2.00±0.21	1.00±0.31	1.00±0.31

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the negative control group [Group II], behavioural parameters (onset of seizures) were considerably higher in Group III (Sodium valproate group - standard drug group) and the experimental groups (Diltiazem, Nimodipine, and Flunarizine). Other behavioural parameters (seizure duration, seizure count in six hours, and seizure scores) were significantly reduced in the sodium valproate group [Group III] (standard drug group) and the experimental groups IV, V and VI (Diltiazem, Nimodipine and Flunarizine) when compared to Group II [Negative control group] ($P<0.05$).

PILOCARPINE MODEL

Anticonvulsant activity in Pilocarpine Model

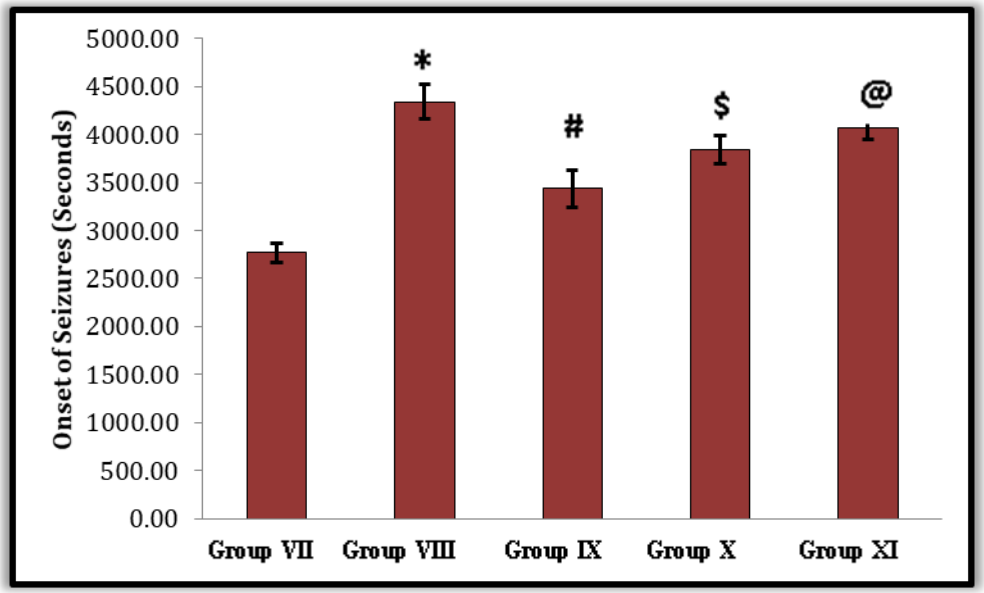

Figure 31: Bar diagram depicting the Onset of seizures in seconds in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group VII.

The results indicated that Group VIII [Phenobarbitone group - Standard drug group] reported a significant increase in the onset of seizures as compared to Group VII [Negative control group] ($P<0.05$). Similarly, all the experimental groups [IX, X and XI - Diltiazem, Nimodipine and Flunarizine] displayed an increase in the seizure onset when compared to Group VII [Negative control group], which was found to be statistically significant ($P<0.05$).

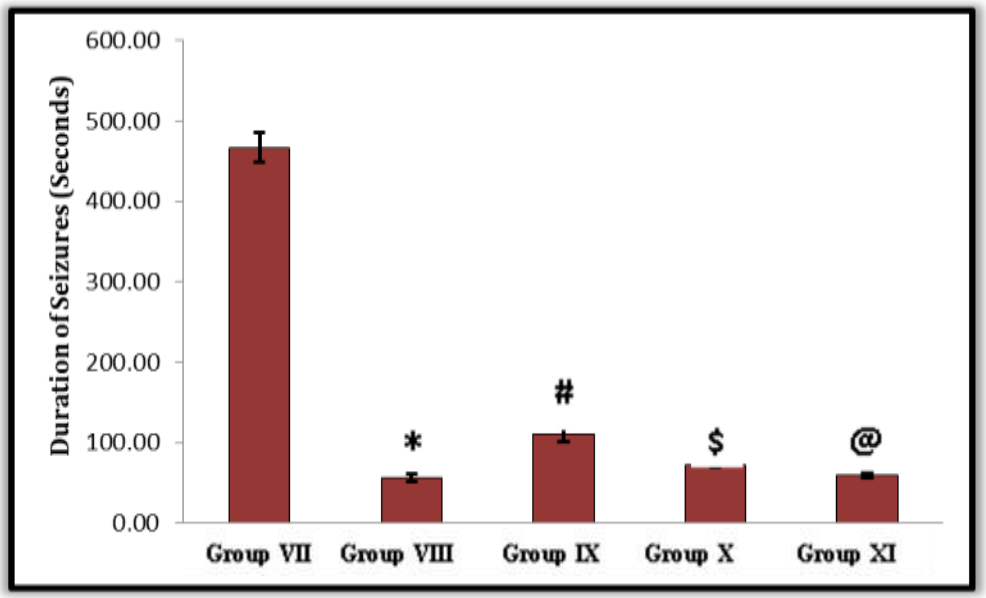

Figure 32: Bar diagram depicting the Duration of seizures in seconds in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the negative control group [Group VII], there was a decrease in the duration of seizures in the Phenobarbitone group - Standard drug group [Group VIII] (P<0.05). Similarly, all the experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine] reported a decrease in the seizure duration when compared to the negative control group [Group VII], which was statistically significant (P<0.05).

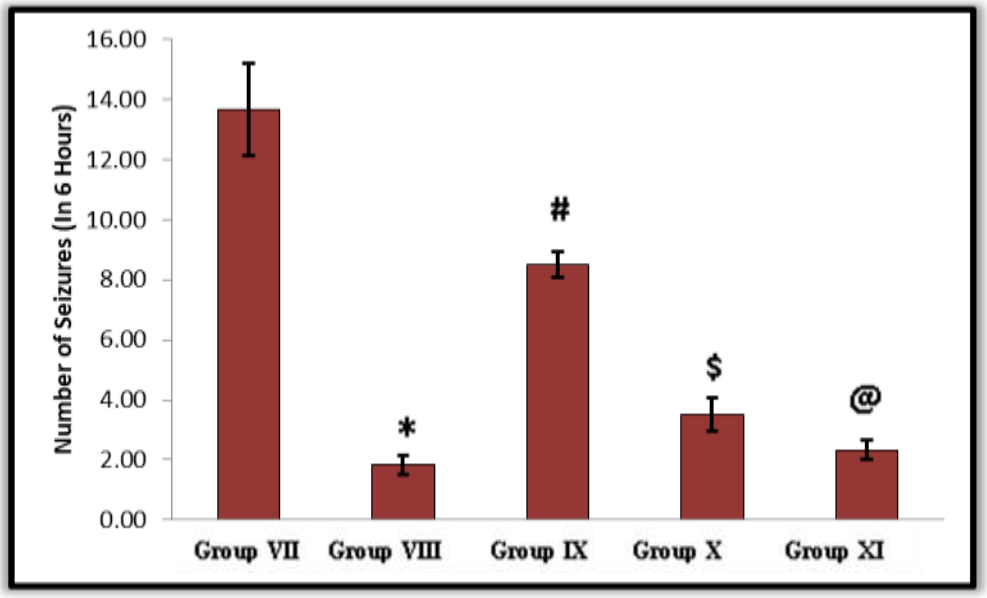

Figure 33: Bar diagram depicting the Number of seizures in six hours in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When the Phenobarbitone group - Standard drug group [Group VIII] was compared to the negative control group [Group VII], it was seen that there was a significant reduction in the parameter (number of seizures in six hours) (P<0.05). Additionally, it was determined that when the experimental groups (Diltiazem, Nimodipine and Flunarizine) were compared to the negative control group [Group VII], the experimental groups demonstrated a statistically significant (P<0.05) reduction in the number of seizures in six hours

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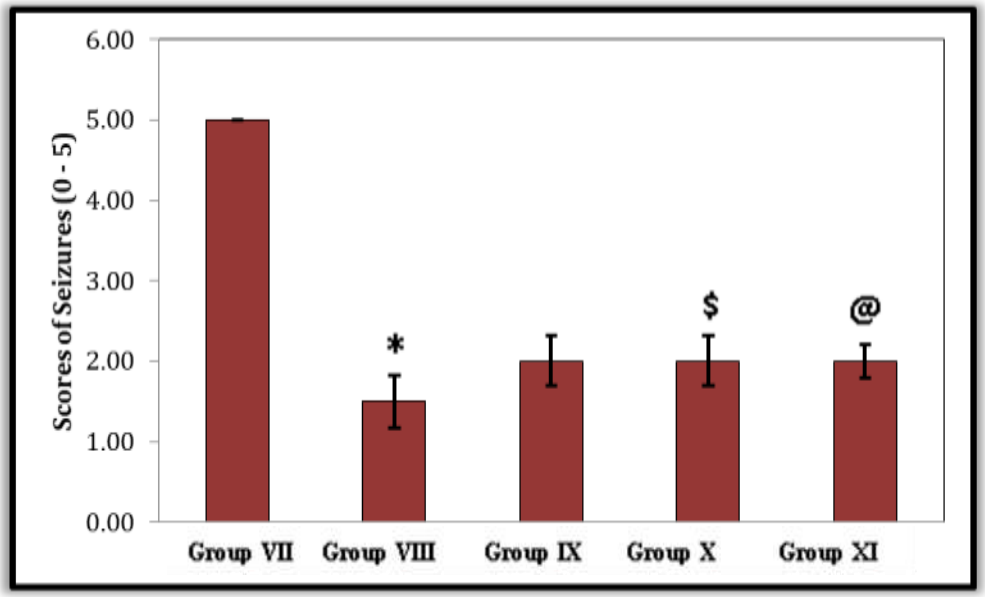


Figure 34: Bar diagram depicting the Scores of seizures in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When comparing Group VIII [Phenobarbitone group - Standard drug group] to Group VII [Negative control group], it was observed that the seizure scores were considerably lower in Group VIII (P<0.05). When the experimental groups [Group IX – Diltiazem, Group X – Nimodipine and Group XI – Flunarizine] were compared to the negative control group [Group VII], the experimental groups showed a statistically significant (P<0.05) decrease in the score.

Table 13: Seizure parameters in Pilocarpine model screening test in wistar albino rats

Sl. No.	Parameters	Group VII	Group VIII*	Group IX#	Group X$	Group XI@
1	Onset of seizures (Seconds)	2771.33±102.61	4341.17±180.49	3436.50±193.42	3843.67±143.55	4069.83±113.68
2	Duration of seizures (Seconds)	466.83±18.23	56.17±4.97	109.67±9.22	71.67±3.25	59.50±3.63
3	Number of seizures (6 h)	13.67±1.54	1.83±0.31	8.50±0.43	3.50±0.56	2.33±0.33
4	Scores of seizures (0 - 5)	5.00±0.00	1.50±0.33	2.00±0.31	2.00±0.31	2.00±0.21

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to Group VII [Negative control group], behavioural parameters (onset of seizures) were significantly enhanced in Group VIII [Phenobarbitone group-standard drug group] and in experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine] (P<0.05). Other behavioural parameters (seizure duration, seizure count in six hours and seizure scores) were significantly decreased in Group VII [Phenobarbitone group - Standard drug group] and in Groups IX, X and XI [Experimental groups - Diltiazem, Nimodipine and Flunarizine] when compared to Group VII [Negative control group] (P<0.05).

MAXIMAL ELECTROSHOCK SEIZURE [MES] MODEL

Anticonvulsant activity in MES Model

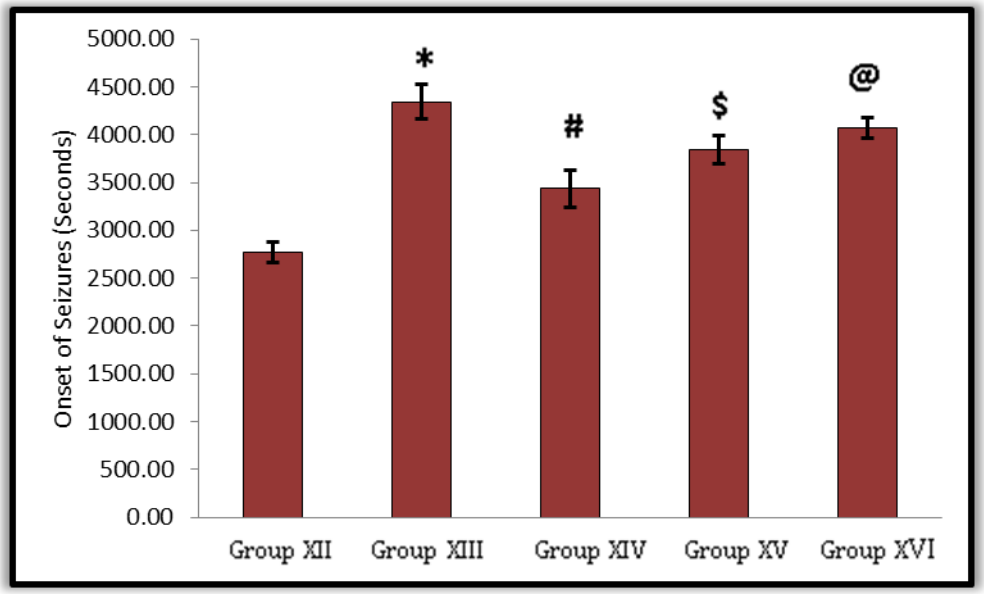


Figure 35: Bar diagram depicting the percentage of protection against Tonic Hind Limb Extension [THLE] in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The results indicated that when the Diphenylhydantoin group (Standard drug group) was compared to the negative control group [Group XII], the percentage of protection against Tonic Hind Limb Extension increased significantly (P<0.05). Similarly, as compared to the negative control group [Group XII], all the experimental groups (IV, V and VI - Diltiazem, Nimodipine and Flunarizine) presented an increase in the percentage of protection against Tonic Hind Limb Extension, which was shown to be statistically significant (P< 0.05).

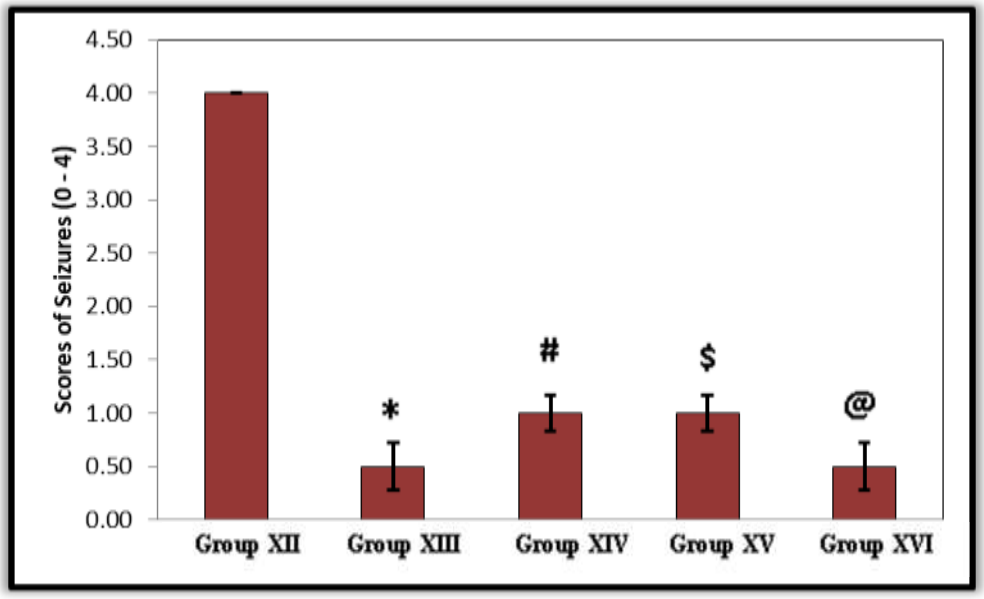


Figure 36: Bar diagram depicting the scores of seizures in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When the Diphenylhydantoin group [Group XIII] (Standard drug group) was compared to the negative control group [Group XII], it was seen that there was a significant decrease in the seizure score (P<0.05). Additionally, it was observed that when compared to the negative control group [Group XII], both the positive control group - standard drug group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) proved a statistically significant (P< 0.05) decrease in the seizure scores.

Table 14: Seizure parameters in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Sl. No.	Parameters	Group XII	Group XIII*	Group XIV#	Group XV$	Group XVI@
1	% Protection against THLE	0	100	50	66.67	83.33
2	Scores of seizures (0 - 4)	4.00±0.00	0.50±0.22	1.00±0.17	1.00±0.17	0.50±0.22

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When compared to the negative control group [Group XII], behavioural parameters (percentage of protection against THLE) were significantly increased in the Diphenylhydantoin group - standard drug group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI). Other behavioural parameter, seizure scores were significantly reduced in the Diphenylhydantoin group - Standard drug group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) when compared to the negative control group [Group XII] (P<0.05).

EVALUATION OF OXIDATIVE STRESS ENZYMES IN ALL THE THREE MODELS [PTZ, PILOCARPINE & MES]

ASSAY OF ANTIOXIDANTS IN PTZ MODEL

Estimation of Superoxide Dismutase (SOD)

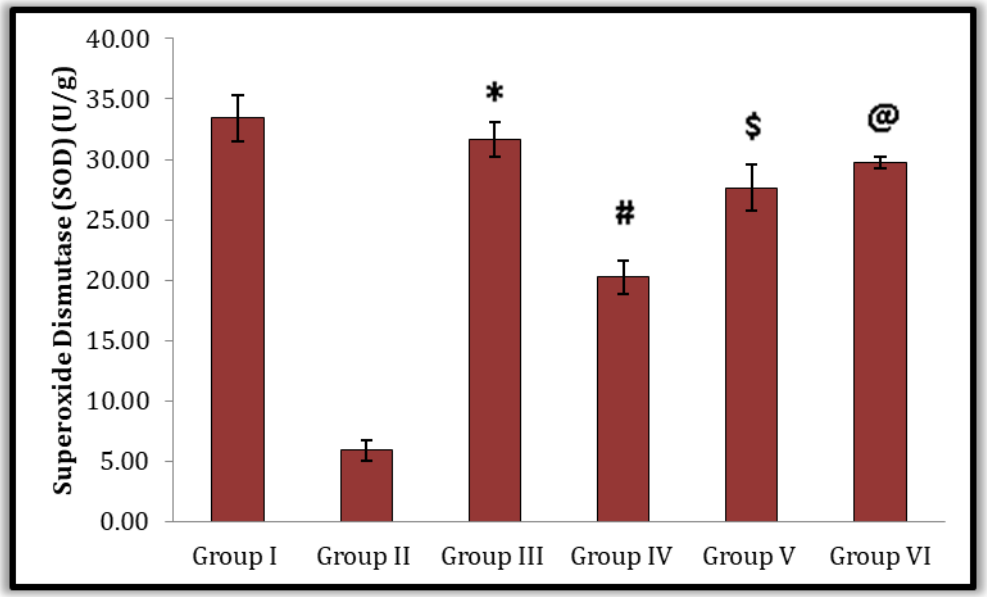


Figure 37: Bar diagram depicting the anti-oxidant enzyme (Superoxide dismutase) level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The level of an anti-oxidant enzyme (Superoxide dismutase [SOD]) was significantly lower in Group II [Negative control group] than in Group I [Vehicle control group] (P<0.05). The study findings indicated that there was an increase in the anti-oxidant enzyme (Superoxide dismutase) level in Group III [Standard drug – sodium valproate] and in the experimental groups IV, V and VI (Diltiazem, Nimodipine and Flunarizine), which was statistically significant when compared to Group II [negative control group] (P<0.05).

Estimation of Glutathione Peroxidase (GPx)

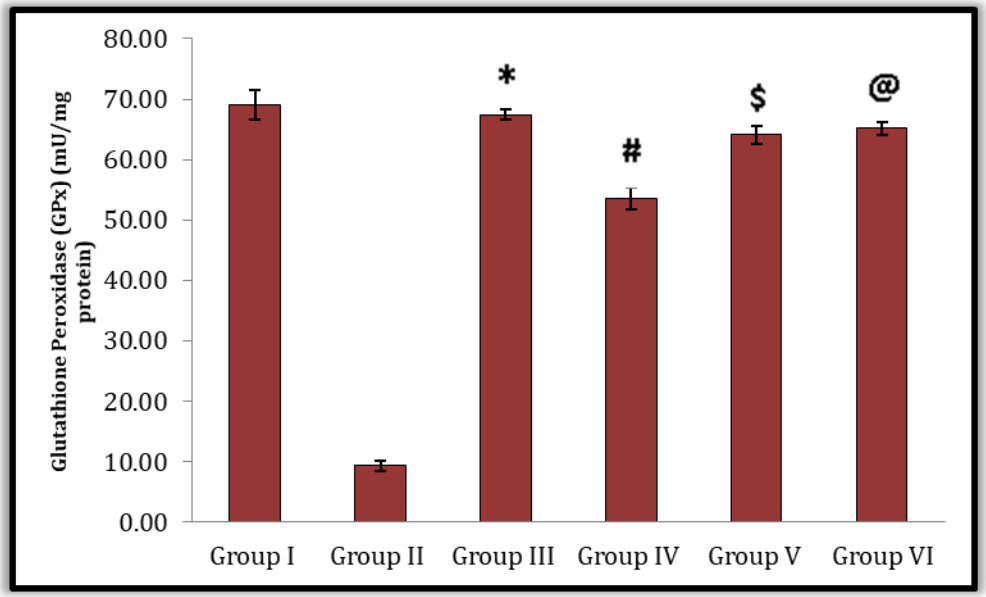


Figure 38: Bar diagram depicting the anti-oxidant enzyme (Glutathione peroxidase) level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When the negative control group [Group II] compared to the vehicle control group [Group I], the anti-oxidant enzyme, Glutathione peroxidase level was decreased ($P<0.05$). Additionally, the study results indicated that there was a rise in the anti-oxidant enzyme (Glutathione peroxidase) level in the standard drug (Sodium valproate) group [Group III] and in the experimental groups [IV - Diltiazem, V - Nimodipine and VI - Flunarizine] ($P<0.05$).

Estimation of Reduced Glutathione (GSH)

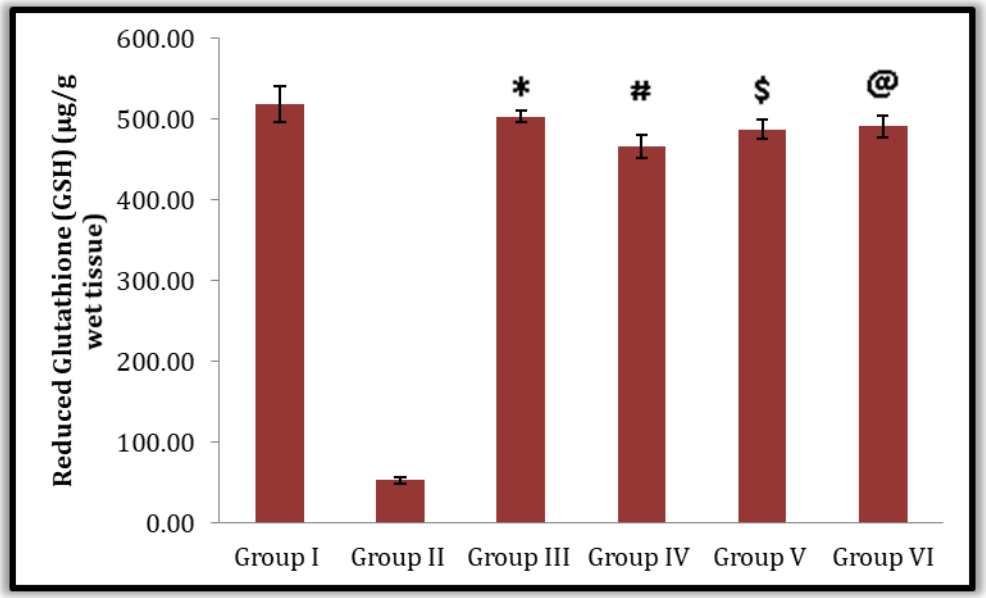


Figure 39: Bar diagram depicting the anti-oxidant enzyme (Reduced glutathione) level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Reduced glutathione) level was decreased in the negative control group [Group II] ($P<0.05$). Additionally, the findings revealed that there was an increase in the anti-oxidant enzyme (Reduced glutathione) level in the standard drug (Sodium valproate) group [Group III] and the experimental groups [IV - Diltiazem, V - Nimodipine and VI - Flunarizine] that was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

Estimation of Catalase

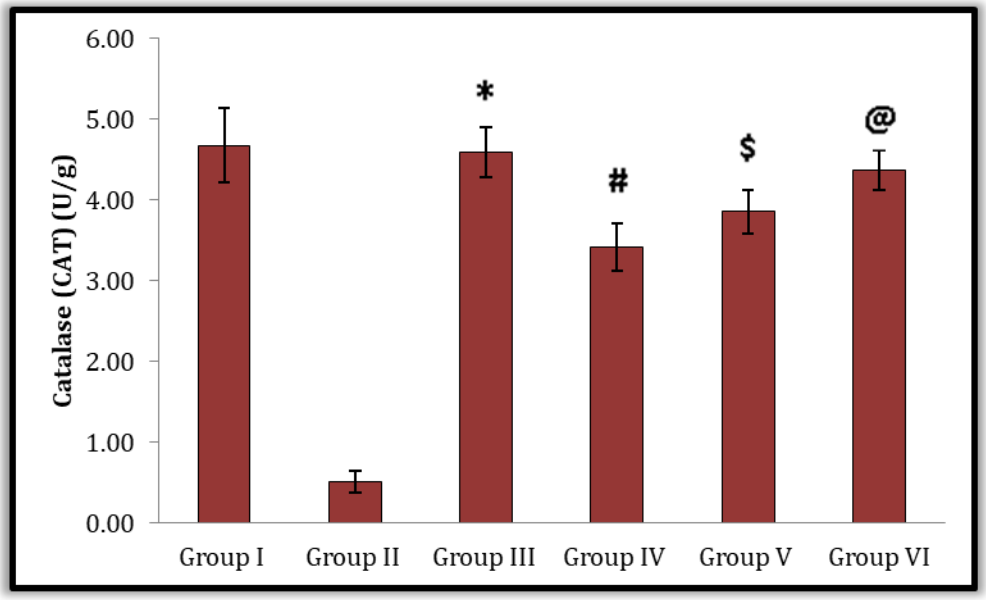


Figure 40: Bar diagram depicting the anti-oxidant enzyme (Catalase) level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Catalase) level was decreased in the negative control group [Group II] ($P<0.05$). The assay of the standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] revealed a statistically significant increase in the level of Catalase when compared to the negative control group [Group II] ($P<0.05$).

Estimation of Lipid peroxidation (LPO)

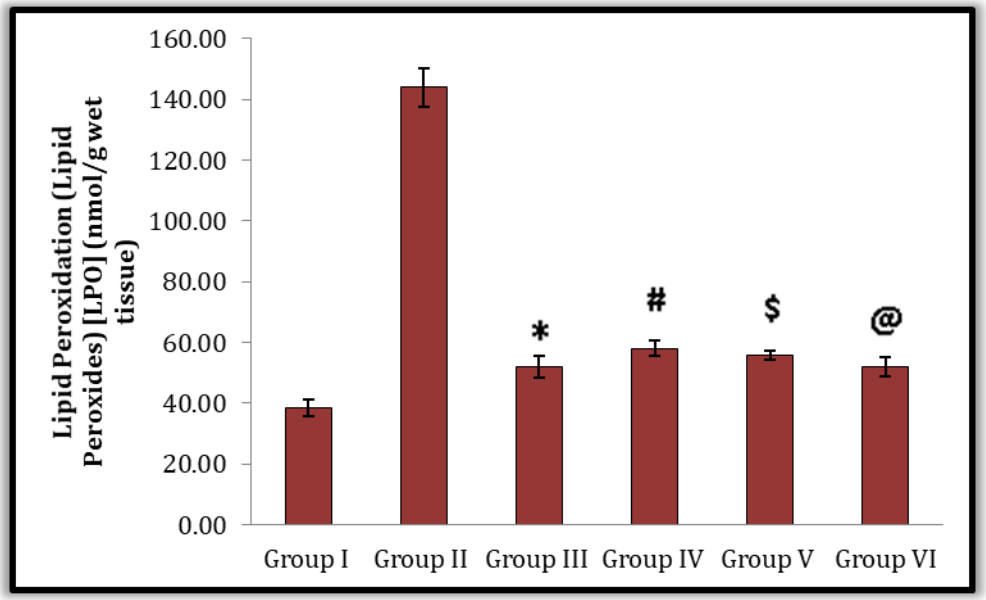


Figure 41: Bar diagram depicting the lipid peroxidation in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When compared to the vehicle control group [Group I], lipid peroxidation was considerably higher in the negative control group [Group II] ($P<0.05$). We detected a decrease in Lipid peroxidation in the standard drug group [Group III] and the experimental drug groups [IV, V, and VI - Diltiazem, Nimodipine and Flunarizine], which was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

Table 15: Anti-oxidant enzymes in PTZ model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Superoxide Dismutase (SOD) (*U/g*)	33.42±1.92	5.90±0.82	31.62±1.42	20.23±1.35	27.64±1.93	29.76±0.50
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	69.07±2.42	9.39±0.87	67.46±0.75	53.55±1.75	64.10±1.50	65.16±1.12
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	518.59±22.33	52.91±4.33	503.52±7.21	466.07±14.61	487.67±11.42	491.09±13.96
4	Catalase (CAT) (*U/g*)	4.68±0.46	0.51±0.14	4.59±0.31	3.42±0.29	3.86±0.27	4.37±0.24
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	38.40±2.65	144.09±6.34	51.95±3.50	58.09±2.62	55.75±1.46	52.09±3.31

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When the negative control group [Group II] was compared to the vehicle control group, the levels of anti-oxidant enzymes Superoxide dismutase, Glutathione peroxidase, Reduced glutathion and Catalase decreased and the lipid peroxidation increased (P<0.05). The standard drug group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] evidenced an increase in anti-oxidant enzymes - Superoxide dismutase, Glutathione peroxidase, Reduced glutathione and Catalase and a decrease in lipid peroxidation levels which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

ASSAY OF ANTIOXIDANTS IN PILOCARPINE MODEL

Estimation of Superoxide Dismutase [SOD]

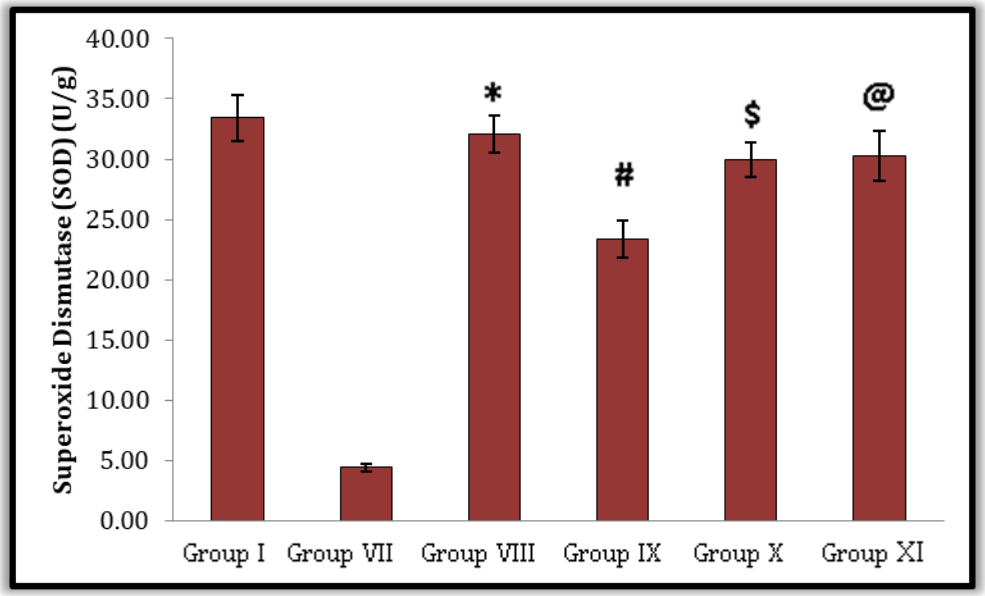


Figure 42: Bar diagram depicting the anti-oxidant enzyme (Superoxide dismutase) level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Superoxide dismutase) level was decreased in the negative control group [Group VII] (P<0.05). The study findings indicated that both the standard drug group - Phenobarbitone [Group VIII] and the experimental groups [Group IX – Diltiazem, Group X – Nimodipine and Group XI – Flunarizine] demonstrated an increased level of anti-oxidant enzyme (Superoxide dismutase) that was statistically significant when compared to the negative control group [Group VII] (P<0.05).

Estimation of Glutathione Peroxidase [GPx]

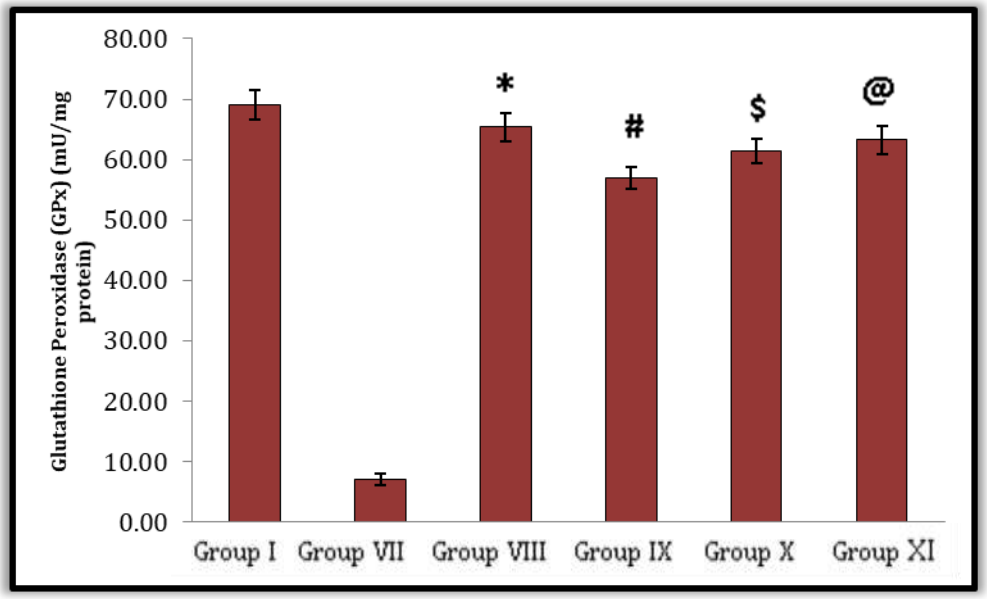


Figure 43: Bar diagram depicting the anti-oxidant enzyme (Glutathione peroxidase) level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group VII.

Antioxidant enzyme (Glutathione peroxidase) levels were significantly lower in the negative control group [Group VII] than in the vehicle control group [Group I] ($P<0.05$). Additionally, the study results indicated that there was an increase in the level of an anti-oxidant enzyme (Glutathione peroxidase) in the standard drug group, Phenobarbitone [Group VII], and the experimental groups [Group IX – Diltiazem, Group X – Nimodipine and Group XI – Flunarizine] which was statistically significant when compared to the negative control group [Group VII] ($P<0.05$).

Estimation of Reduced Glutathione [GSH]

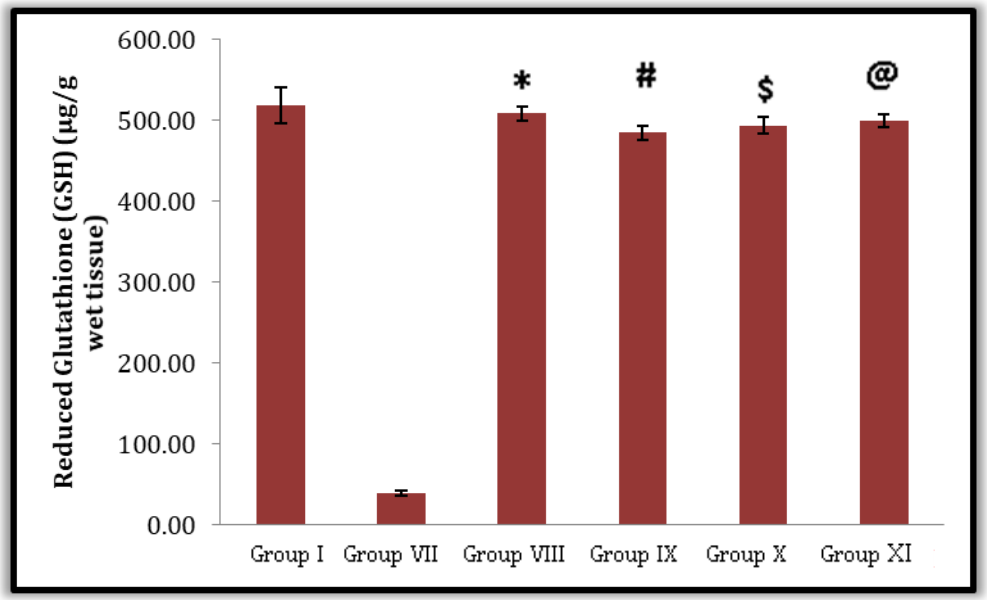


Figure 44: Bar diagram depicting the anti-oxidant enzyme (Reduced glutathione) level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to Group I [vehicle control group], the anti-oxidant enzyme (reduced glutathione) level was decreased in Group VII [Negative control group] (P<0.05). Additionally, the study discovered that when Group VIII [standard drug group – Phenobarbitone] and Groups IX, X and XI [experimental drug groups – Diltiazem, Nimodipine and Flunarizine] were compared to Group VII [negative control group], the anti-oxidant enzyme (Reduced glutathione) level was increased and found to be statistically significant (P<0.05).

Estimation of Catalase

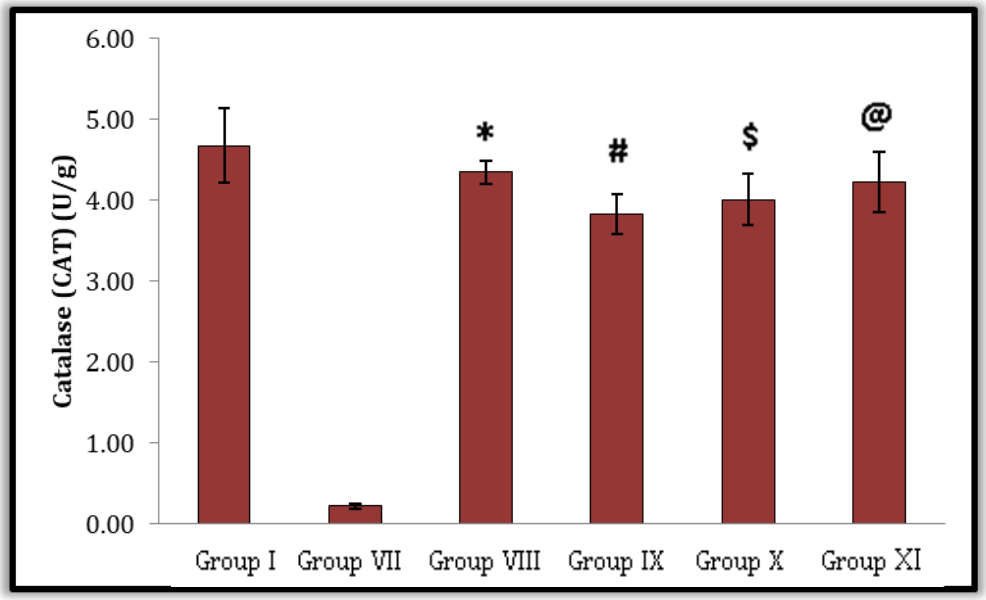


Figure 45: Bar diagram depicting the anti-oxidant enzyme (Catalase) level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

The negative control group [Group VII] had a lower amount of anti-oxidant enzyme (Catalase) than the vehicle control group [Group I] (P<0.05). Additionally, the study also noticed that the anti-oxidant enzyme (Catalase) level was increased in the standard drug (Phenobarbitone) group [Group VIII] and in the experimental groups (Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine) and was found to be statistically significant when compared to the negative control group [Group VII] (P<0.05).

Estimation of Lipid peroxidation [LPO]

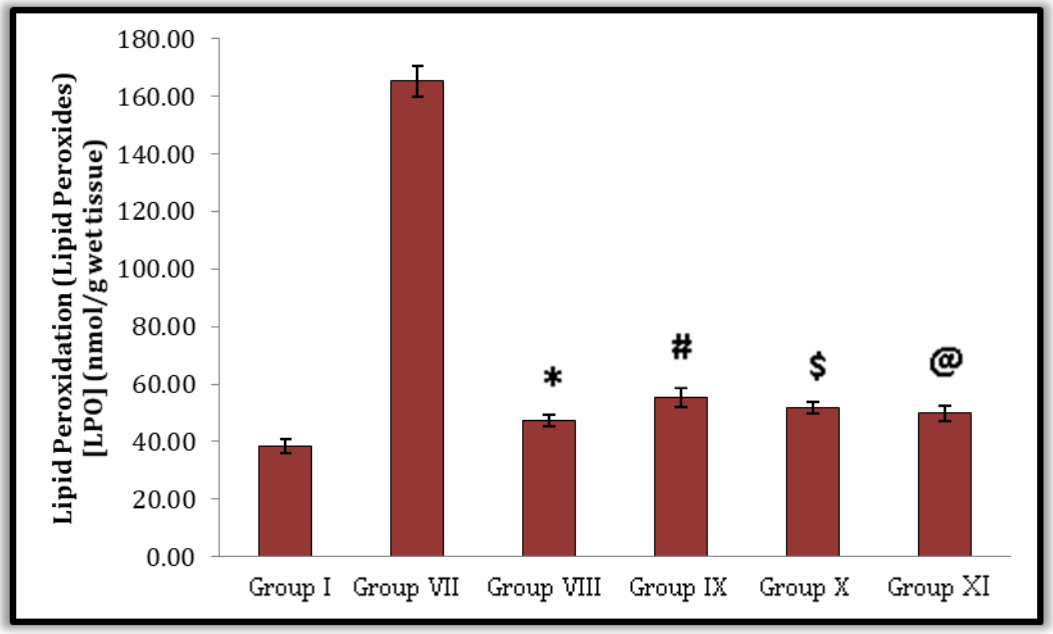


Figure 46: Bar diagram depicting the lipid peroxidation level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to Group I [Vehicle control group], lipid peroxidation was significantly higher in Group VII [Negative control group] (P<0.05). When compared to Group VII [Negative control group], there was a significant decrease in lipid peroxidation in Group VIII [Standard drug (Phenobarbitone) group] and Groups IX, X and XI [Experimental groups - Diltiazem, Nimodipine and Flunarizine].

Table 16: Anti-oxidant enzymes in Pilocarpine model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group VII	Group VIII*	Group IX#	Group X$	Group XI@
1	Superoxide Dismutase (SOD) (*U/g*)	33.42±1.92	4.41±0.35	32.08±1.57	23.41±1.54	29.95±1.43	30.28±2.11
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	69.07±2.42	7.03±0.94	65.38±2.28	56.91±1.84	61.39±2.02	63.26±2.29
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	518.59±22.33	38.79±3.25	508.99±8.97	485.23±8.51	493.93±9.98	499.31±8.00
4	Catalase (CAT) (*U/g*)	4.68±0.46	0.22±0.03	4.35±0.14	3.83±0.25	4.01±0.32	4.23±0.37
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	38.40±2.65	165.31±5.42	47.35±1.93	55.25±3.38	51.63±2.04	49.79±2.84

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group VII.

The anti-oxidant enzymes Superoxide dismutase, Glutathione peroxidase, Reduced glutathione, and Catalase were significantly decreased in the negative control group [Group VII] compared to the vehicle control group [Group I] ($P<0.05$). However, there was a considerable rise in the degree of Lipid peroxidation. When the data were analyzed, it was discovered that the standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX – Diltiazem, Group X – Nimodipine and Group XI – Flunarizine] all had a statistically significant increase in the anti-oxidant enzyme when compared to the negative control group [Group VII] ($P<0.05$). However, there was a statistically significant decrease in Lipid peroxidation in the standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX – Diltiazem, Group X – Nimodipine, and Group XI – Flunarizine] as compared to the negative control group [Group VII] ($P<0.05$).

ASSAY OF ANTIOXIDANTS IN MES MODEL

Estimation of Superoxide Dismutase [SOD] in Brain

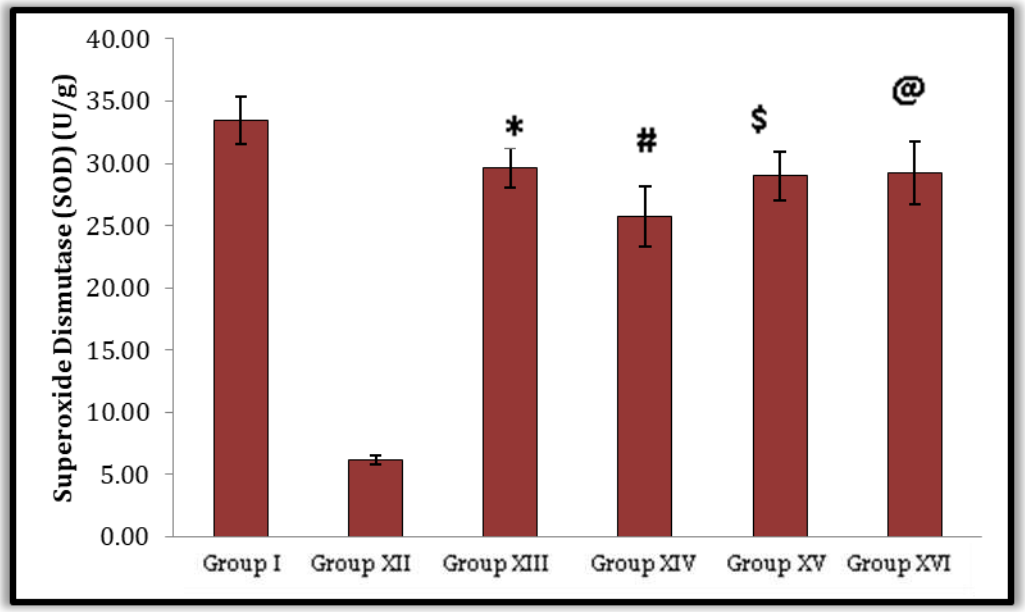


Figure 47: Bar diagram depicting the anti-oxidant enzyme (Superoxide dismutase) level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Superoxide dismutase) level was significantly decreased in the negative control group [Group XII]. The study's findings indicated that both the standard drug Diphenylhydantoin [Group - XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) increased the level of an anti-oxidant enzyme (Superoxide dismutase), which was statistically significant when compared to the negative control group [Group-XII] ($P<0.05$).

Estimation of Glutathione Peroxidase [GPx]

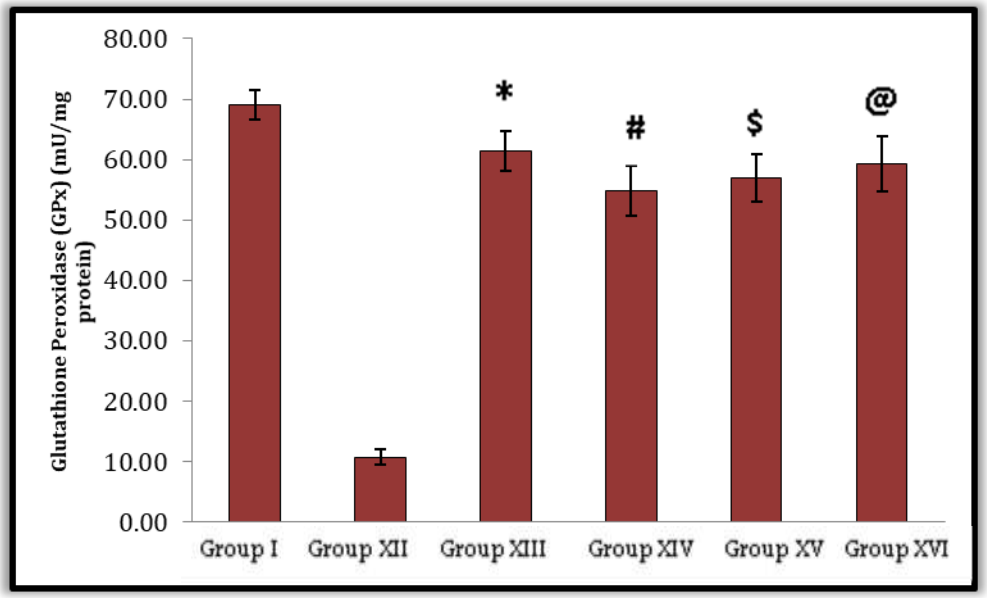


Figure 48: Bar diagram depicting the anti-oxidant enzyme (Glutathione peroxidase) level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group XII.

This study results indicated a considerable ($P<0.05$) decrease in the anti-oxidant enzyme (Glutathione peroxidase) level in the negative control group [Group XII] compared to the vehicle control group [Group I]. The findings confirmed that both the Standard drug (Diphenylhydantoin) group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) increased the anti-oxidant enzyme (Glutathione peroxidase) level, which was statistically significant when compared to the negative control group [Group XII].

Estimation of Reduced glutathione [GSH]

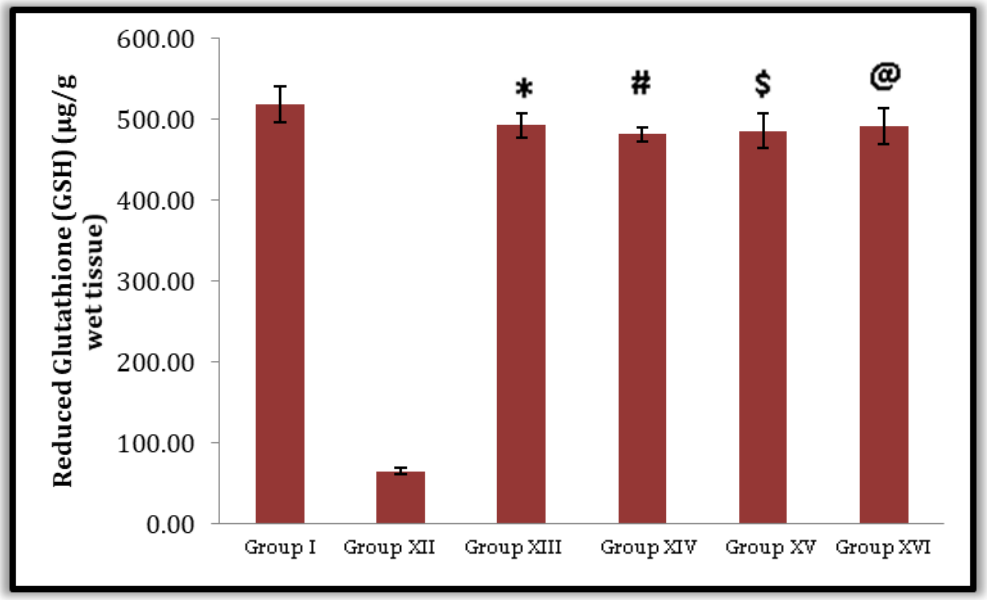


Figure 49: Bar diagram depicting the anti-oxidant enzyme (Reduced glutathione) level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group XII.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Reduced glutathione) level was decreased in the negative control group [Group XII] ($P<0.05$). Additionally, the research found that both the standard drug group (Diphenylhydantoin [Group XIII]) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) had a statistically significant increase in the anti-oxidant enzyme (Reduced glutathione) when compared to the negative control group [Group XII] ($P<0.05$).

Estimation of Catalase

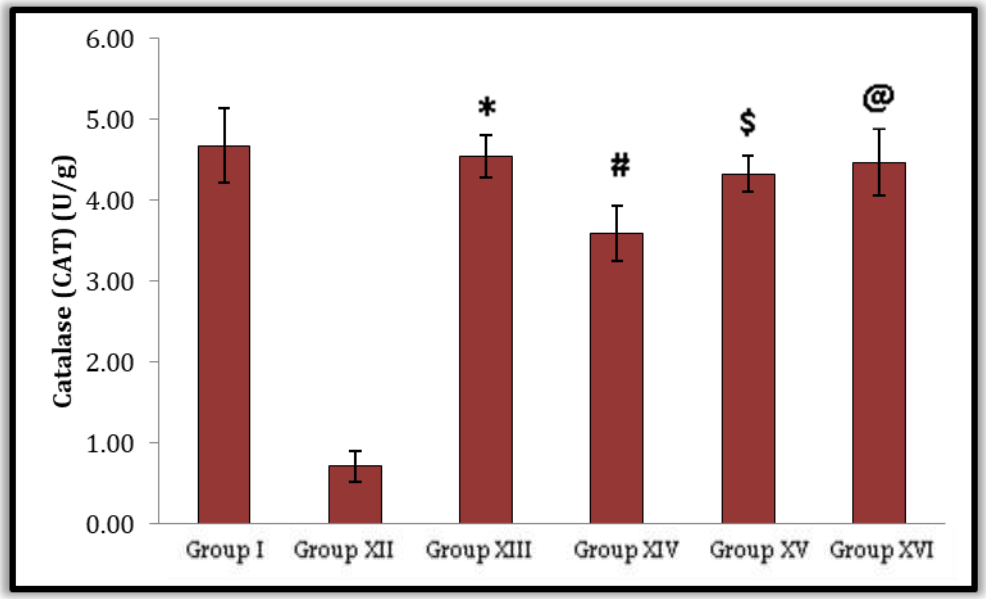


Figure 50: Bar diagram depicting the anti-oxidant enzyme (Catalase) level in Maximal Electroshock Seizure [MES] model screening test in wistar Albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group XII.

When the negative control group [Group XII] was compared to the vehicle control group [Group I], the level of the anti-oxidant enzyme (Catalase) decreased significantly ($P<0.05$). Additionally, the findings revealed that both the standard drug (Diphenyl hydantoin) group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) increased the level of the anti-oxidant enzyme (Catalase), which was statistically significant when compared to the negative control group [Group XII] ($P<0.05$).

Estimation of Lipid Peroxidation [LPO]

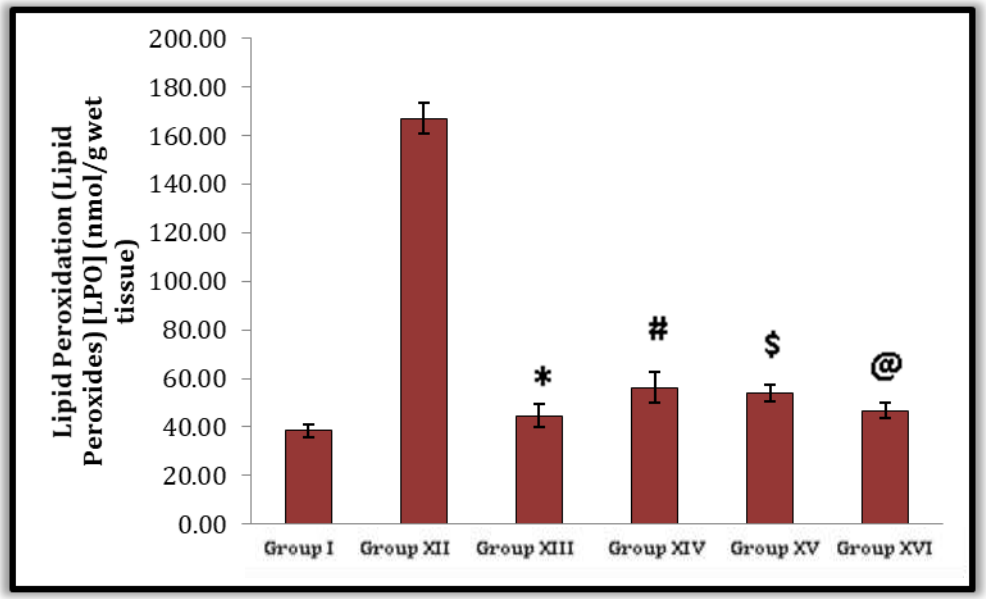


Figure 51: Bar diagram depicting the lipid peroxidation in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], Lipid peroxidation in the negative control group [Group XII] was significantly (P <0.05) higher. Furthermore, when compared to the negative control group [Group XII], both the Standard drug group - Diphenylhydantoin [Group XIII] - and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) showed a statistically significant decrease in lipid peroxidation (P<0.05).

Table 17: Anti-oxidant enzymes in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group XII	Group XIII*	Group XIV#	Group XV$	Group XVI@
1	Superoxide Dismutase (SOD) (*U/g*)	33.42±1.92	6.19±0.38	29.63±1.58	25.69±2.43	28.99±1.95	29.23±2.48
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	69.07±2.42	10.72±1.29	61.33±3.31	54.79±4.09	56.89±3.93	59.38±4.57
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	518.59±22.33	65.05±4.22	493.12±14.95	481.61±8.47	485.73±21.61	491.49±22.03
4	Catalase (CAT) (*U/g*)	4.68±0.46	0.71±0.19	4.55±0.26	3.59±0.34	4.33±0.22	4.47±0.41
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	38.40±2.65	167.13±6.33	44.65±4.66	56.13±6.35	53.81±3.55	46.67±3.11

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group XII.

The results of this study indicated that while the levels of all anti-oxidant enzymes (Superoxide Dismutase, Glutathione Peroxidase, Reduced Glutathione, and Catalase) decreased in the negative control group [Group XII], lipid peroxidation increased significantly ($P<0.05$) when compared to the vehicle control group [Group I]. Additionally, the study observed that both the standard drug group (Diphenylhydantoin) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) proved an increase in anti-oxidant enzymes (Superoxide Dismutase, Glutathione Peroxidase, Reduced Glutathione, and Catalase) and a decrease in the level of Lipid Peroxidation which was found to be statistically significant when compared to the negative control group [Group XII] ($P<0.05$).

EVALUATION OF NEUROTRANSMITTERS IN ALL THE THREE MODELS [PTZ, PILOCARPINE & MES]

ASSAY OF NEUROTRANSMITTERS IN PENTYLENETETRAZOL [PTZ] MODEL

Estimation of Serotonin level

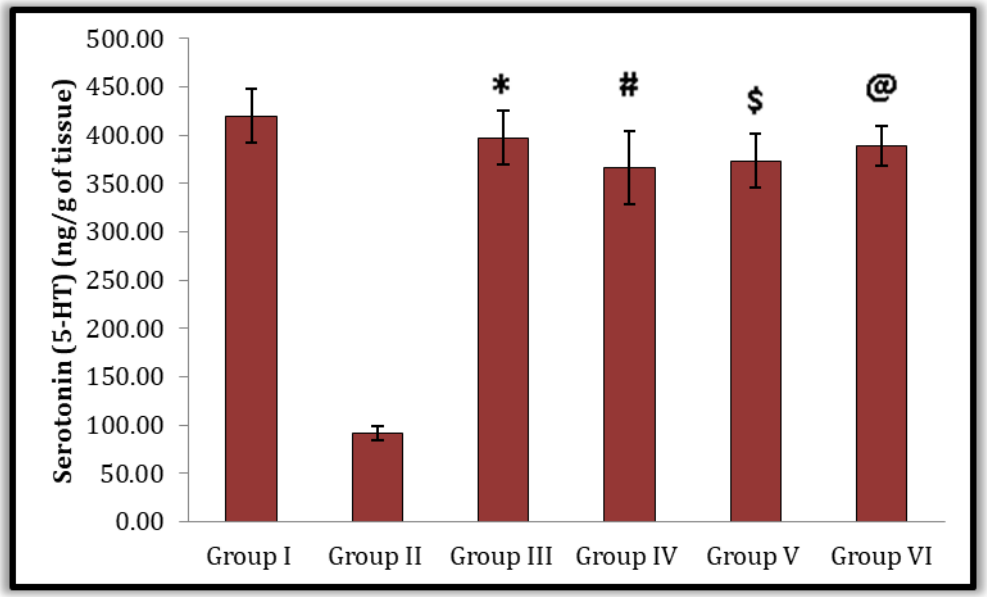


Figure 52: Bar diagram depicting the Serotonin level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

The neurotransmitter (Serotonin) level was significantly lower in the negative control group [Group II] than in the vehicle control group [Group I]. It was also observed that both the standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] proved a statistically significant increase in the level of neurotransmitter (Serotonin) when compared to the negative control group [Group II] ($P<0.05$).

Estimation of Dopamine level:

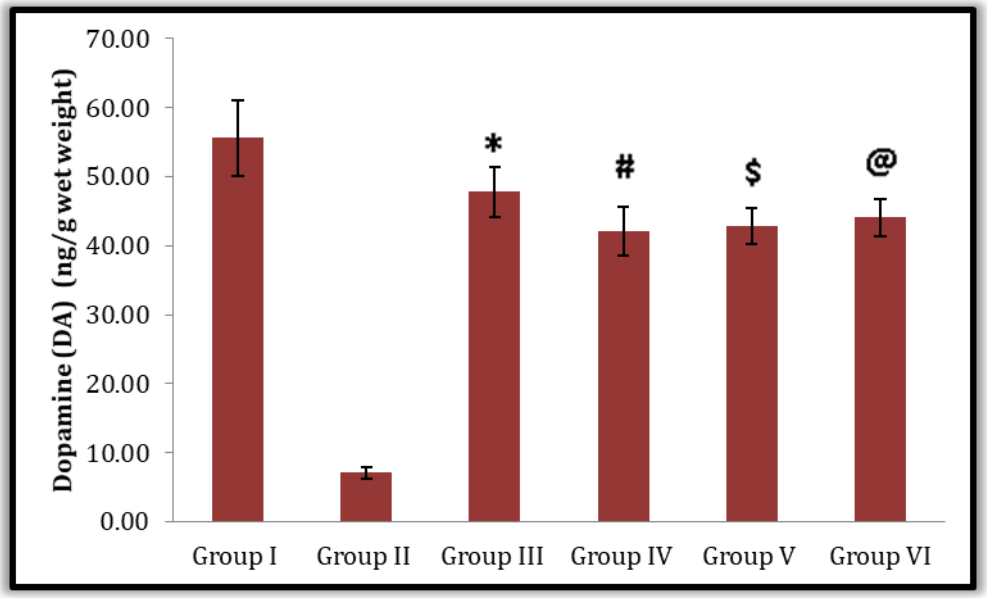


Figure 53: Bar diagram depicting the Dopamine level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When the negative control group [Group II] was compared to the vehicle control group [Group I], the neurotransmitter (Dopamine) level was found to be significantly lower (P<0.05). Additionally, it was observed that both the standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] increased the level of neurotransmitter (Dopamine), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Estimation of GABA level:

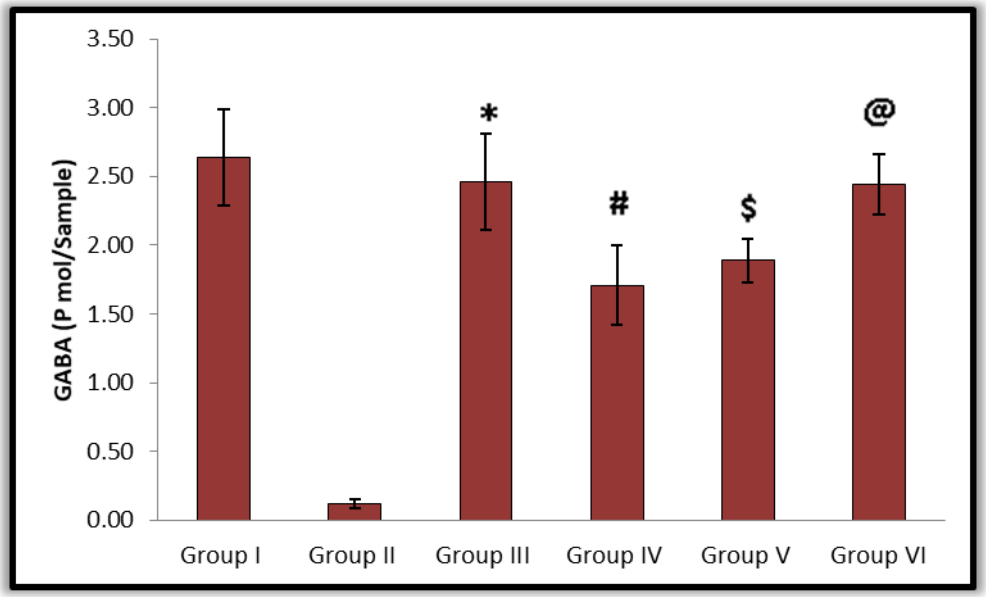


Figure 54: Bar diagram depicting the GABA level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (GABA) level was significantly lower in the negative control group [Group II] than in the vehicle control group [Group I]. Additionally, it was observed that the Standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI] (Diltiazem, Nimodipine and Flunarizine) had an increased level of neurotransmitter [GABA], which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Estimation of Glutamate level:

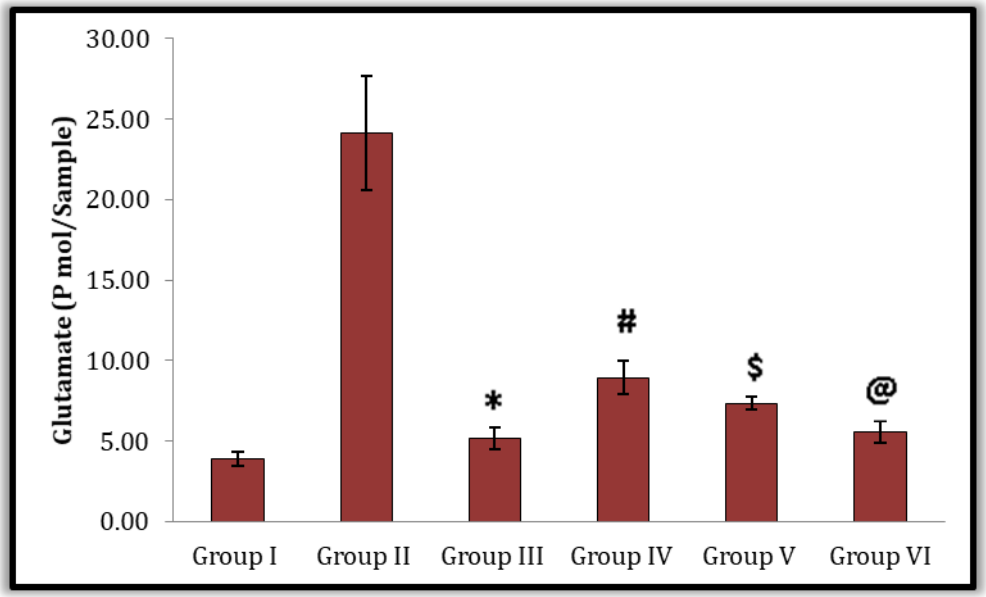


Figure 55: Bar diagram depicting the Glutamate level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

It was reported that the negative control group [Group II] had a statistically significant (P<0.05) rise in the neurotransmitter (Glutamate) level when compared to the vehicle control group [Group I]. However, there was a statistically significant reduction in the Glutamate level in the Standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI] (Diltiazem, Nimodipine and Flunarizine) as compared to the negative control group [Group II] (P<0.05).

Estimation of Acetylcholine level:

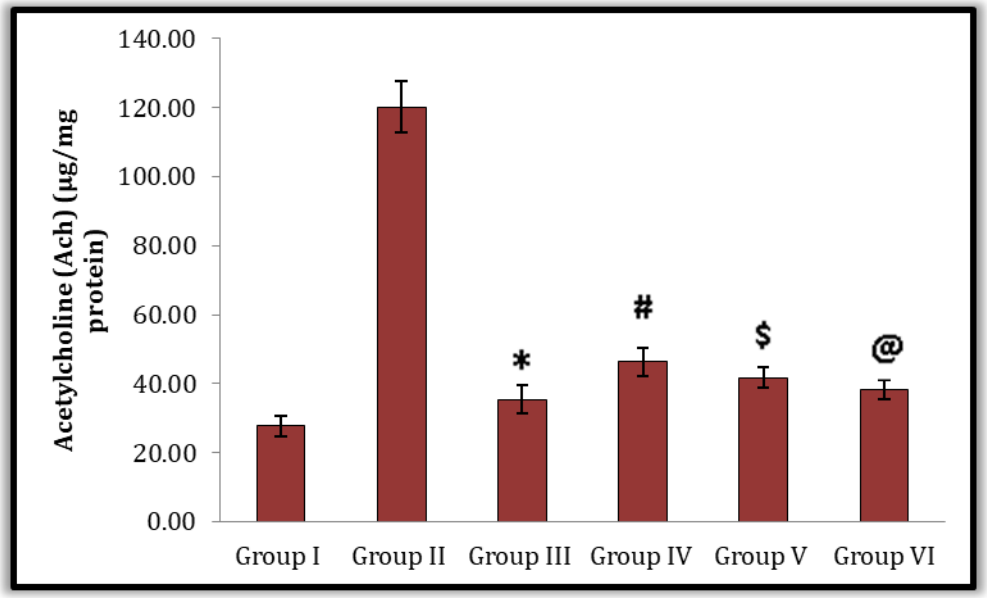


Figure 56: Bar diagram depicting the Acetylcholine level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The level of the neurotransmitter (Acetyl choline - ACh) was significantly improved in Group II [Negative control group] compared to Group I [Vehicle control group] (P<0.05). Similarly, it was observed that the level of neurotransmitter (Acetyl choline - ACh) was decreased in Group III [Standard drug (Sodium valproate) group] and in the experimental groups IV, V and VI [Diltiazem, Nimodipine and Flunarizine] which was statistically significant when compared to Group II [Negative control group] (P<0.05).

Table 18: Neurotransmitters in PTZ model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Serotonin (*ng/g of wet tissue*)	419.78±28.12	91.35±7.02	397.37±27.90	366.09±37.79	373.59±28.10	388.57±20.59
2	Dopamine (*ng/g wet weight*)	55.59±5.41	7.08±0.85	47.80±3.58	42.07±3.47	42.81±2.60	44.05±2.62
3	GABA (*P mol/Sample*)	2.64±0.35	0.12±0.03	2.46±0.35	1.71±0.29	1.89±0.16	2.44±0.22
4	Glutamate (*P mol/Sample*)	3.89±0.42	24.13±3.57	5.18±0.69	3.93±1.04	7.35±0.38	5.57±0.68
5	Acetylcholine (*µg/mg protein*)	27.68±3.02	120.21±7.42	35.34±4.04	46.29±4.20	41.77±3.10	38.09±2.78

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

The levels of neurotransmitters (serotonin, DA and GABA) were significantly lower in the negative control group [Group II] than in the vehicle control group [Group I]. Similarly, there was an increase in the levels of these neurotransmitters (Serotonin, Dopamine, and GABA) in the standard drug group (Sodium valproate) and in the experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine] which was statistically significant when compared to the negative control group [Group II] ($P<0.05$)

The levels of neurotransmitters (Glutamate and ACh) were significantly elevated in the negative control group [Group II] compared to the vehicle control group [Group I]. Additionally, it was found that the standard drug group (Sodium valproate) and the experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine] had a decreased level of these neurotransmitters (Glutamate and ACh) which was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

ASSAY OF NEUROTRANSMITTERS IN PILOCARPINE MODEL

Estimation of Serotonin level

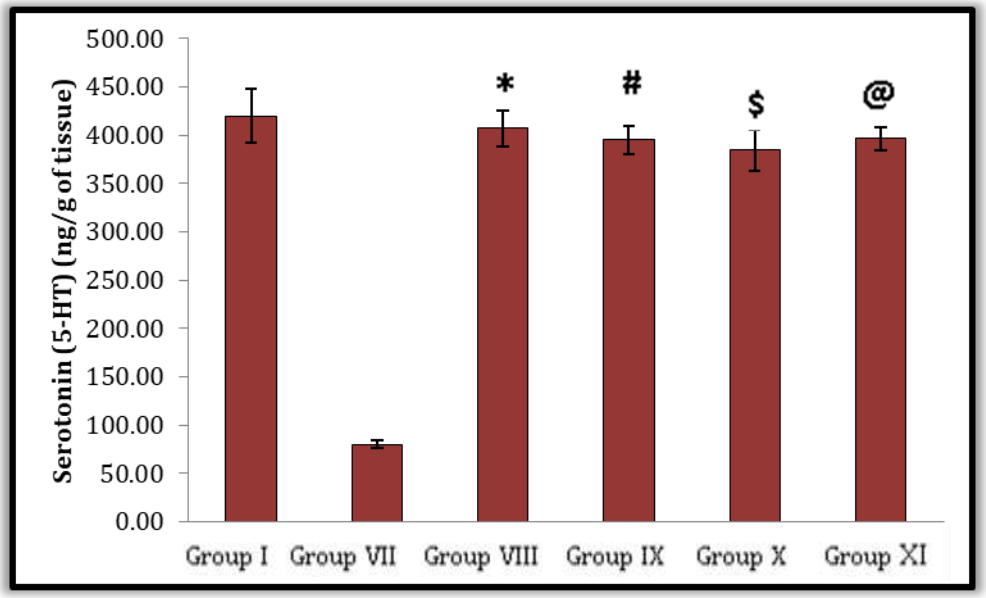


Figure 57: Bar diagram depicting the Serotonin level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group VII.

When the negative control group [Group VII] was compared to the vehicle control group [Group I], the neurotransmitter (Serotonin) level was found to be significantly lower ($P<0.05$). It was also observed that when compared to the negative control group [Group VII], the serotonin level was significantly increased ($P <0.05$) in the standard drug (Phenobarbitone) group [Group VIII] and in the experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine].

Estimation of Dopamine level:

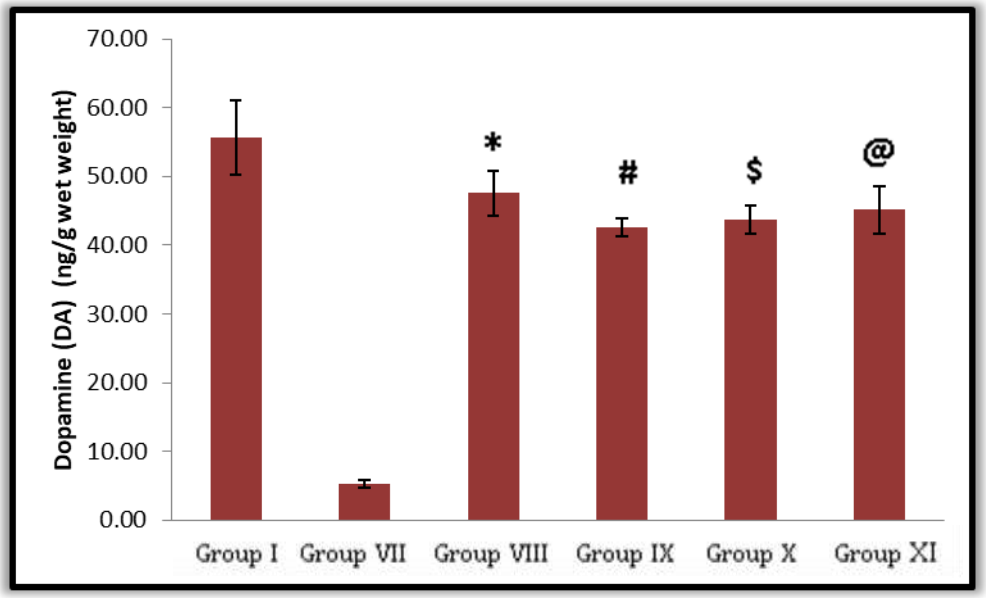


Figure 58: Bar diagram depicting the Dopamine level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When the negative control group [Group VII] was compared to the vehicle control group [Group I], the neurotransmitter (Dopamine) level was found to be significantly lower (P<0.05). Additionally, it was observed that the standard drug group – Phenobarbitone [Group VIII] – and the experimental groups [Group IX – Diltiazem, Group X – Nimodipine and Group XI – Flunarizine] all had an increase in Dopamine levels that was statistically significant when compared to the negative control group [Group VII] (P<0.05).

Estimation of GABA level:

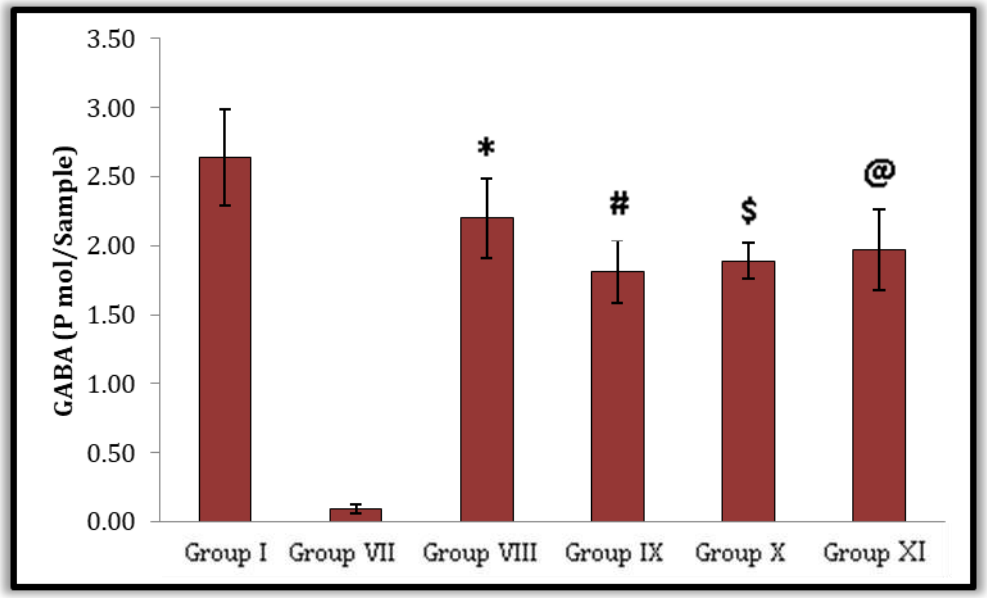


Figure 59: Bar diagram depicting the GABA level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to Group I [Vehicle control group] the level of neurotransmitter (GABA) in Group VII [Negative control group] was significantly lower (P<0.05). When compared to Group VII [negative control group], there was a substantial (P<0.05) rise in the GABA level in Group VIII [standard drug group – Phenobarbitone] and Groups IX, X and XI [experimental drug groups – Diltiazem, Nimodipine and Flunarizine].

Estimation of Glutamate [GA] level

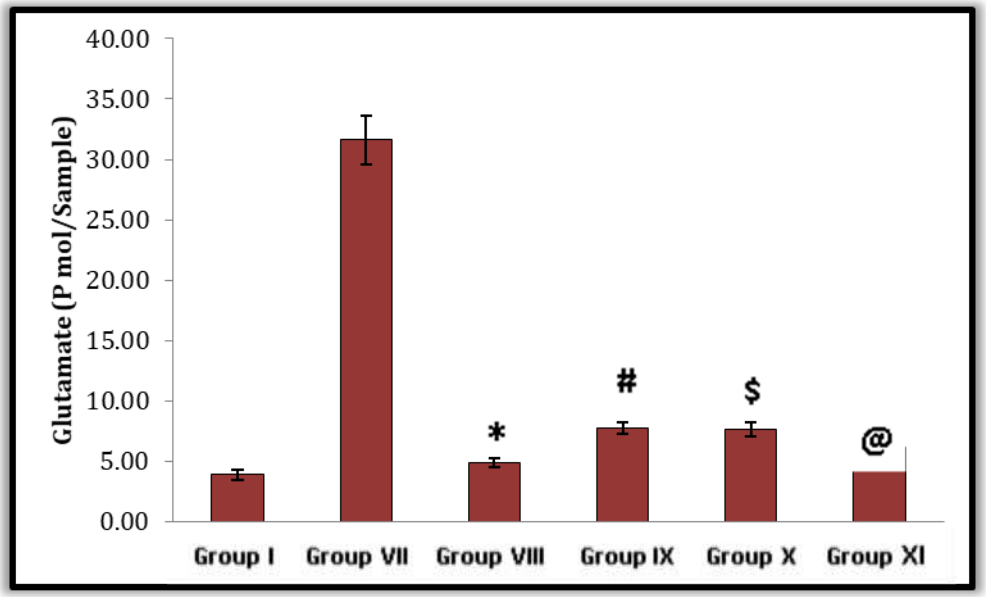


Figure 60: Bar diagram depicting the Glutamate level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When the negative control group [Group VII] was compared to the vehicle control group [Group I] the neurotransmitter (Glutamate) levels increased significantly (P<0.05).Additionally, it was observed that both the standard drug group - Phenobarbitone [Group VIII] and the experimental groups [Group IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine] proved a decreased level of neurotransmitter (Glutamate), which was statistically significant when compared to the negative control group [Group VII] (P<0.05).

Estimation of Acetyl choline [ACh] level

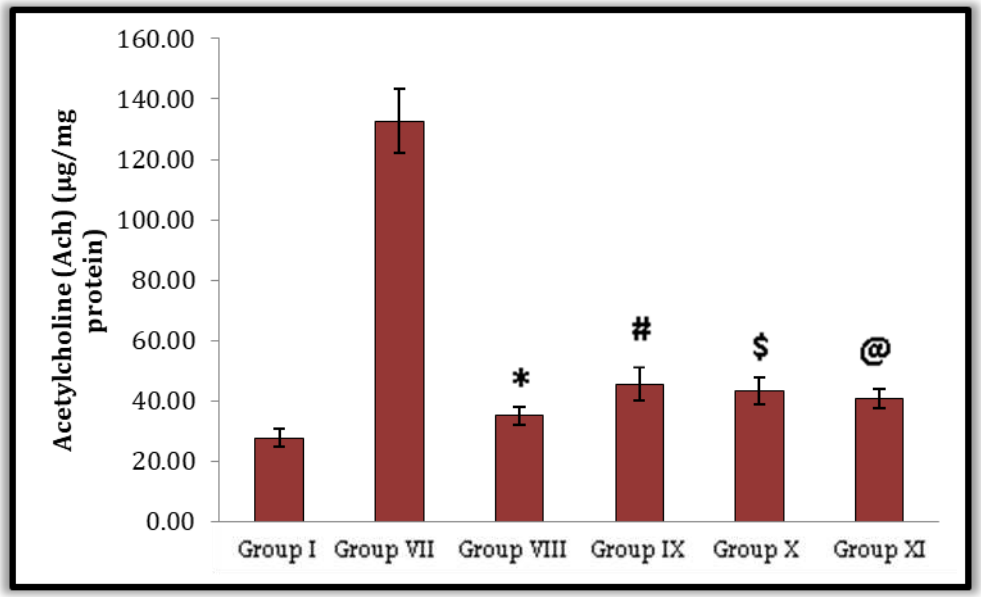


Figure 61: Bar diagram depicting the Acetylcholine level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

The neurotransmitter (ACh) level was significantly elevated in the negative control group [Group VII] when compared to the vehicle control group [Group I]. Additionally, it was revealed that when the standard drug group, Phenobarbitone [Group VIII], was compared to the experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine], there was a significant (P<0.05) decrease in the neurotransmitter (ACh) level.

Table 19: Neurotransmitters in Pilocarpine model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group VII	Group VIII*	Group IX#	Group X$	Group XI@
1	Serotonin (*ng/g of tissue*)	419.78±28.12	80.08±3.80	407.09±18.60	395.27±14.62	384.46±21.18	396.43±11.95
2	Dopamine (*ng/g wet weight*)	55.59±5.41	5.31±0.52	47.60±3.29	42.62±1.30	43.73±2.04	45.12±3.41
3	GABA (*P mol/Sample*)	2.64±0.35	0.09±0.03	2.20±0.29	1.81±0.23	1.89±0.13	1.97±0.29
4	Glutamate (*P mol/Sample*)	3.89±0.42	31.60±2.00	4.89±0.37	7.71±0.48	7.60±0.60	6.16±1.02
5	Acetylcholine (*µg/mg protein*)	27.68±3.02	132.75±10.46	35.16±3.01	45.62±5.56	43.37±4.33	40.90±3.23

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the levels of neurotransmitters (Serotonin, Dopamine, and GABA) were considerably (P <0.05) lowered in the negative control group [Group VII]. It was also observed that, the levels of these neurotransmitters increased in the Standard drug group [Group VIII] and in the experimental groups [Groups IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine] which was statistically significant when compared to the negative control group [Group VII] (P<0.05).

The levels of neurotransmitters (Glutamate and ACh) were significantly higher in the negative control group [Group VII] when compared to the vehicle control group [Group I]. But, the Standard drug group - Phenobarbitone [Group VIII] and the experimental groups [Group IX -Diltiazem, Groups X - Nimodipine, and Groups XI - Flunarizine] exhibited a significant (P<0.05) decrease in neurotransmitter levels (Glutamate and ACh) when compared to the negative control group [Group VII].

ASSAY OF NEUROTRANSMITTERS IN MES MODEL

Estimation of Serotonin level

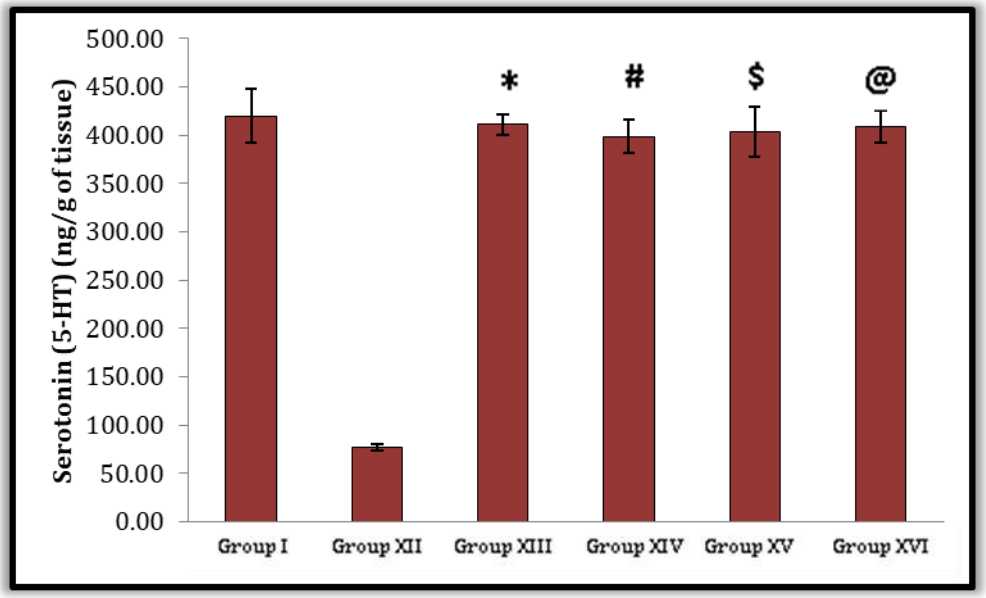


Figure 62: Bar diagram depicting the Serotonin level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The neurotransmitter (Serotonin) level was significantly lower in the negative control group [Group XII] than in the vehicle control group [Group I]. Additionally, it was observed that both the Standard drug (Diphenylhydantoin) group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) caused a statistically significant increase in the neurotransmitter (Serotonin) level when compared to the negative control group [Group XII] (P<0.05).

Estimation of Dopamine level

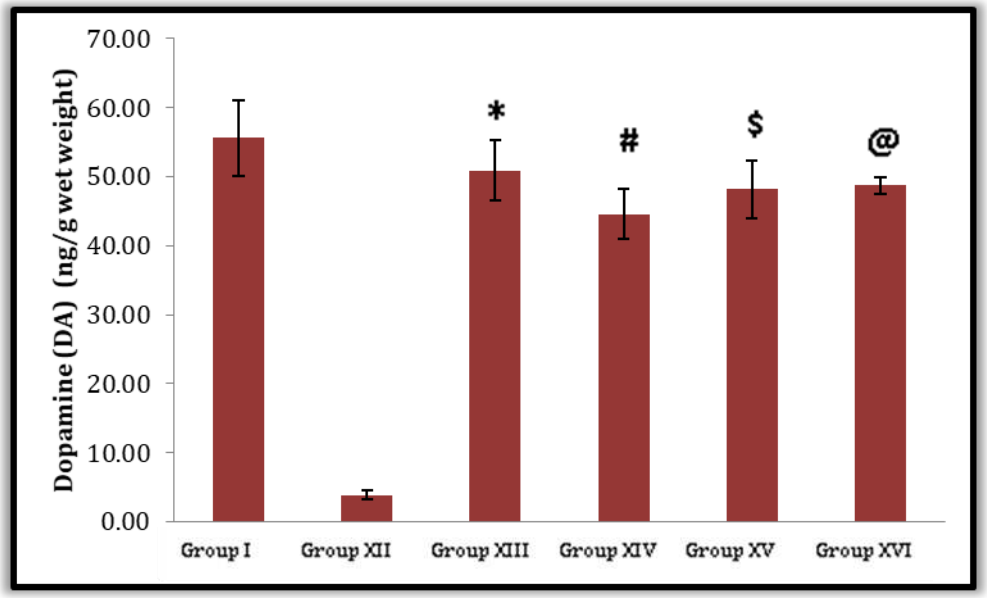


Figure 63: Bar diagram depicting the Dopamine level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When the negative control group [Group XII] was compared to the vehicle control group [Group I], the neurotransmitter (Dopamine) level was found to be significantly lower (P<0.05) in Group XII. Furthermore, both the standard drug group - Diphenylhydantoin [Group XIII] - and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) demonstrated a statistically significant increase in the neurotransmitter (Dopamine) level when compared to the negative control group [Group XII] (P<0.05).

Estimation of GABA level:

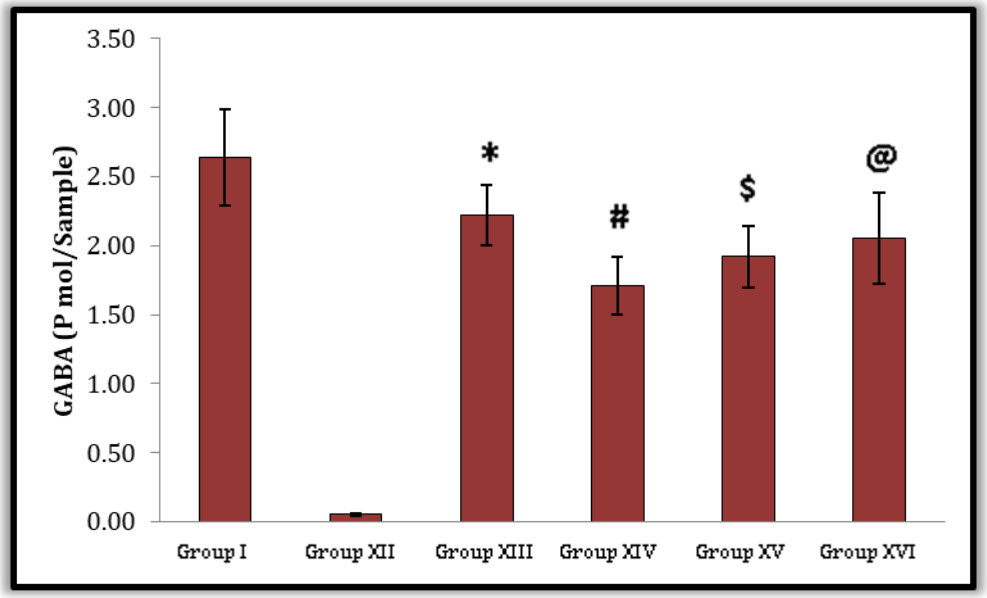


Figure 64: Bar diagram depicting the GABA level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The neurotransmitter (GABA) level was observed to be significantly lower in the negative control group [Group XII] than in the vehicle control group [Group I]. Additionally, it was observed that both the standard drug, Diphenylhydantoin [Group XIII], and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) increased the level of neurotransmitter (GABA) which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

Estimation of Glutamate level:

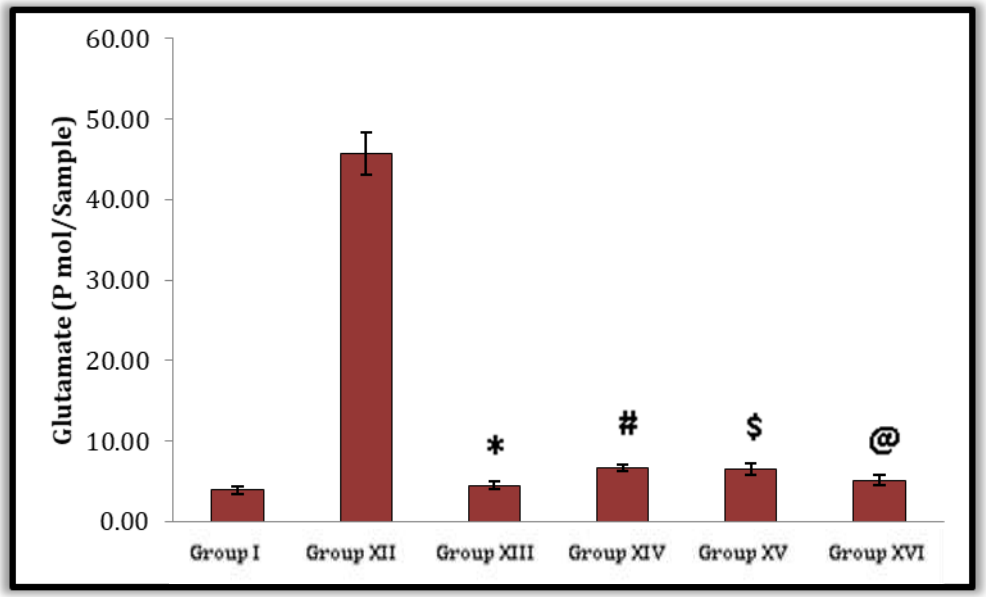


Figure 65: Bar diagram depicting the Glutamate level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The results of this study indicated that, as compared to the vehicle control group [Group I], the neurotransmitter (Glutamate) level was substantially elevated in the negative control group [Group XII]. There was also a decrease in the neurotransmitter (Glutamate) level in the standard drug group [Diphenyl hydantoin] and in the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI), which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

Estimation of Acetylcholine [ACh] level:

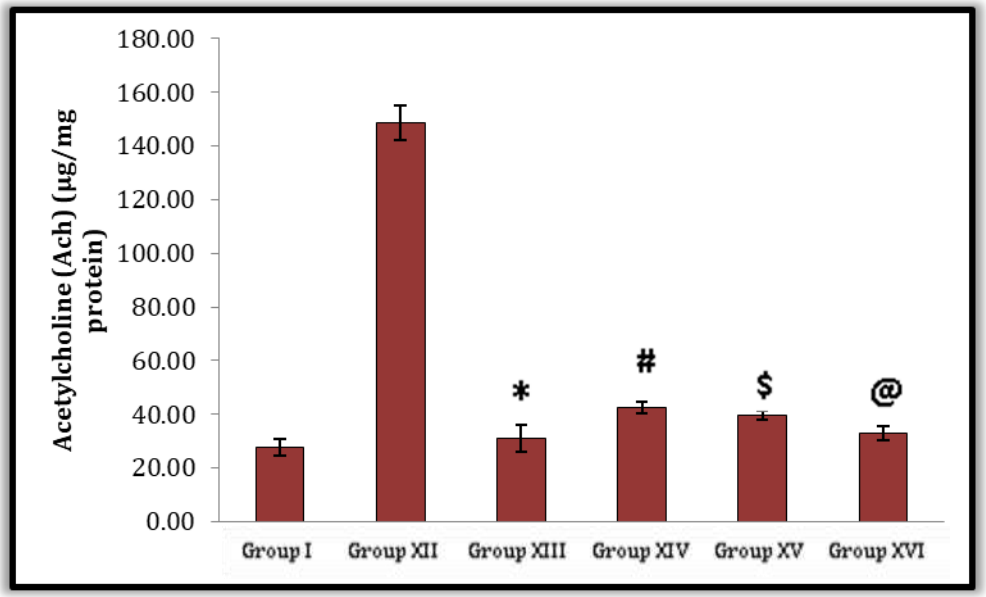


Figure 66: Bar diagram depicting the Acetylcholine level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group XII.

The neurotransmitter (ACh) level was significantly elevated in the negative control group [Group XII] when compared to the vehicle control group [Group I]. Additionally, it was observed that both the standard drug group (Diphenyl hydantoin) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) evidenced a decreased level of neurotransmitter (ACh), which was statistically significant when compared to the negative control group [Group XII] ($P<0.05$).

Table 20: Neurotransmitters in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group XII	Group XIII*	Group XIV#	Group XV$	Group XVI@
1	Serotonin (*ng/g of tissue*)	419.78±28.12	76.63±2.97	411.22±10.39	398.91±16.87	403.91±25.93	408.71±17.06
2	Dopamine (*ng/g wet weight*)	55.59±5.41	3.84±0.60	50.89±4.38	44.60±3.60	48.15±4.18	48.70±1.18
3	GABA (*P mol/Sample*)	2.64±0.35	0.05±0.01	2.22±0.22	1.71±0.21	1.92±0.22	2.05±0.33
4	Glutamate (*P mol/Sample*)	3.89±0.42	45.74±2.65	4.49±0.44	6.70±0.42	6.51±0.70	5.15±0.62
5	Acetylcholine (*µg/mg protein*)	27.68±3.02	148.48±6.42	30.94±4.87	46.66±2.13	39.74±1.57	33.05±2.65

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The neurotransmitter (Serotonin, Dopamine, and GABA) assay revealed a statistically significant (P<0.05) decline in the negative control group [Group XII] as compared to the vehicle control group [Group I]. It was also noted that both the Standard drug (Diphenyl hydantoin) group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) proved statistically significant increases in neurotransmitters (Serotonin, DA and GABA) when compared to the negative control group [Group XII] (P<0.05).

The levels of neurotransmitters (Glutamate and ACh) were significantly higher in the negative control group [Group XII] when compared to the vehicle control group [Group I]. Additionally, it was observed that both the Standard drug group (Diphenyl hydantoin) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) revealed a decrease in neurotransmitter levels (Glutamate and ACh), which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

ESTIMATION OF INFLAMMATORY MARKER [MYELOPEROXIDASE (MPO)] IN ALL THE THREE MODELS

Estimation of Myeloperoxidase (MPO) in PTZ model

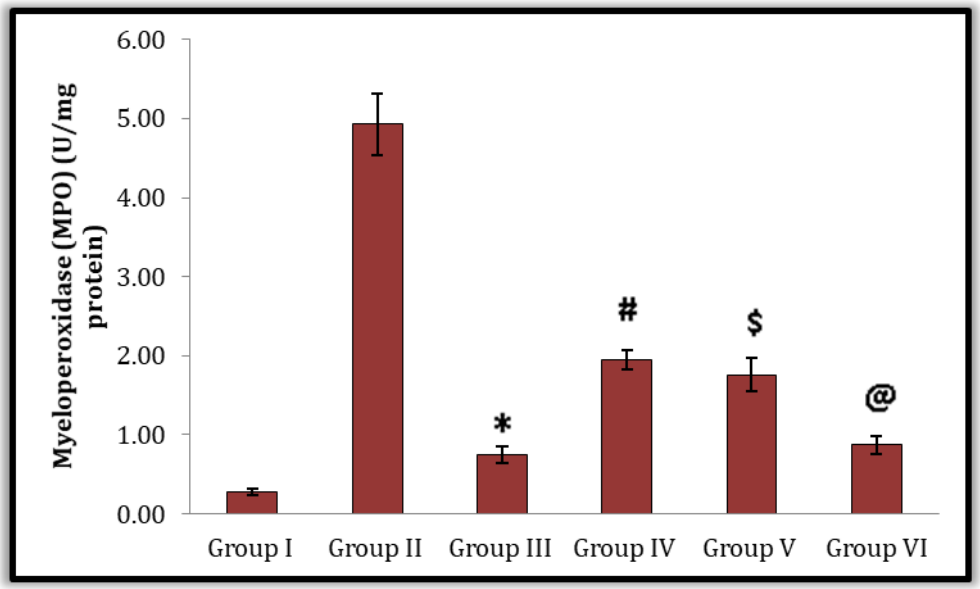


Figure 67: Bar diagram depicting the inflammatory marker (Myeloperoxidase) level in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a significantly higher level of Myeloperoxidase than the vehicle control group [Group I], which was statistically significant (P<0.05). It was also observed that the Standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI] (Diltiazem, Nimodipine and Flunarizine) had a significantly lower level of Myeloperoxidase when compared to the negative control group [Group II] (P<0.05).

Estimation of Myeloperoxidase (MPO) in Pilocarpine model

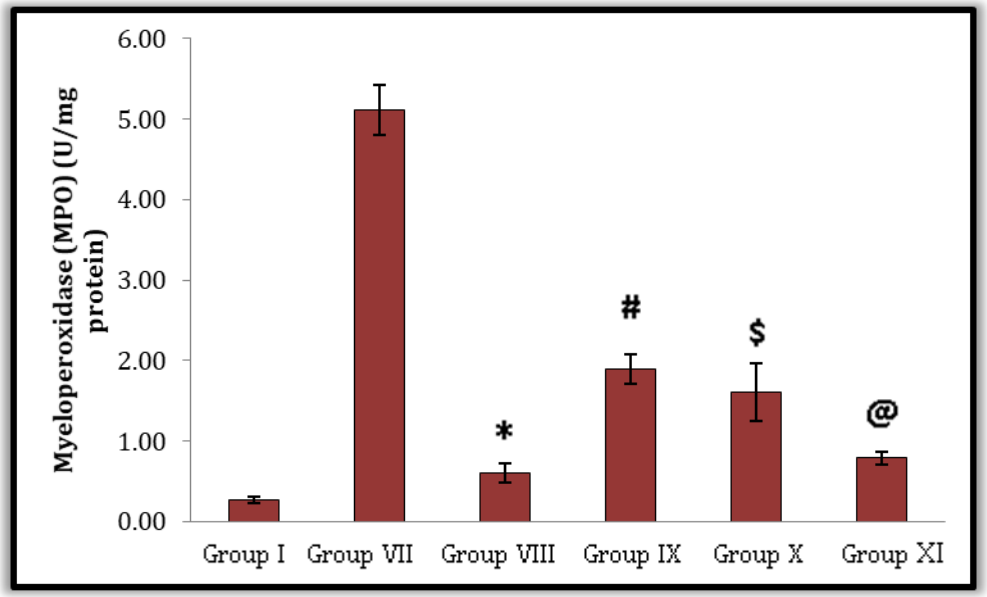


Figure 68: Bar diagram depicting the inflammatory marker (Myeloperoxidase) level in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

Although the level of Myeloperoxidase was significantly (P<0.05) increased in the negative control group [Group VII] when compared to the vehicle control group [Group I], it was observed that the standard drug group [Group VIII] and the experimental groups [Groups IX - Diltiazem, Groups X -Nimodipine and Groups XI - Flunarizine] all had a decreased level of Myeloperoxidase which was found to be statistically significant when compared to the negative control group [Group VII] (P<0.05).

Estimation of Myeloperoxidase (MPO) in MES model

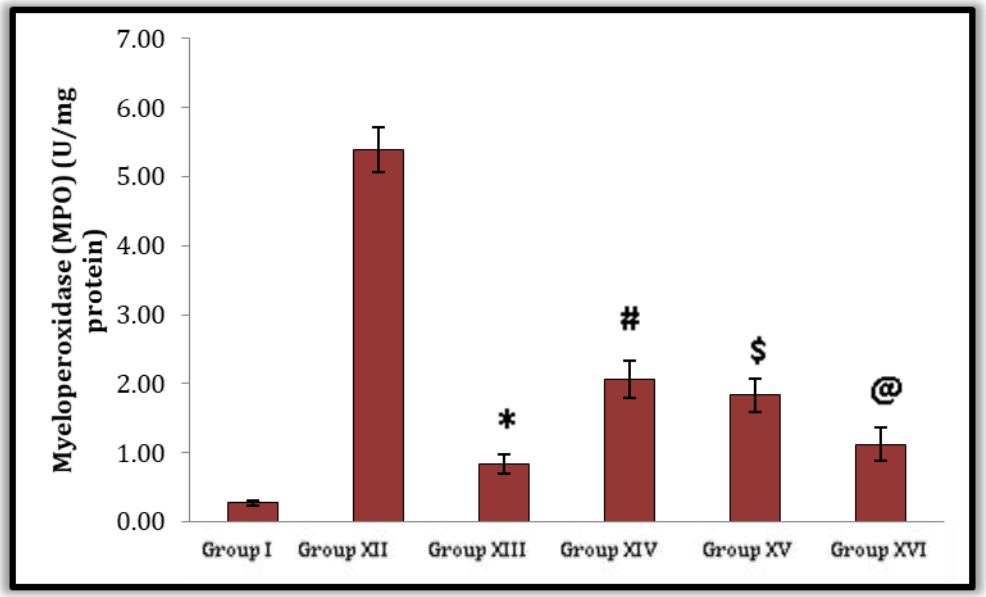


Figure 69: Bar diagram depicting the inflammatory marker (Myeloperoxidase) level in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The negative control group [Group XII] had a significantly higher level of Myeloperoxidase than the vehicle control group [Group I], which was statistically significant (P<0.05). Furthermore, when compared to the negative control group [Group XII], the Standard drug group - Diphenyl hydantoin [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) all had a statistically significant decrease in Myeloperoxidase level (P<0.05).

Table 21: Inflammatory marke parameter [MPO] in PTZ, Pilocarpine and MES model screening tests in wistar albino rats

Parameter	Vehicle control	Negative control	Positive/ Standard control	Diltiazem	Nimodipine	Flunarizine
Myeloperoxidase (MPO) *(U/mg protein)*	**PTZ Model**					
	0.27±0.04	4.93±0.39	0.75±0.11	1.95±0.12	1.76±0.21	0.87±0.12
	Pilocarpine Model					
	0.27±0.04	5.11±0.31	0.61±0.12	1.89±0.18	1.61±0.36	0.79±0.08
	MES Model					
	0.27±0.04	5.39±0.33	0.84±0.14	2.07±0.27	1.83±0.24	1.12±0.24

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

Myeloperoxidase levels were significantly enhanced in the negative control group when compared to the vehicle control group in all the three models (PTZ, Pilocarpine, and MES). It was also observed that both the standard drug group and the experimental groups [Diltiazem, Nimodipine and Flunarizine] revealed a decreased Myeloperoxidase level that was statistically significant when compared to the negative control group in all the three models (PTZ, Pilocarpine and MES) with a P<0.05 value.

MICROANATOMY [HISTOPATHOLOGICAL] STUDY

Scoring system for microanatomical changes in different parts of brain in all the three models (PTZ, Pilocarpine and MES) [23]

Table 22: Scoring system for microanatomical changes in different parts of brain

Scores [HPE]	Operational Definition [In Terms of Test Drug(s) Activity]	Interpretation
0	Excellent	Histological section undistinguishable from control group [Number of healthy neurons appeared normal, even if few pyknotic cells found]
1	Good	More than 75% of healthy neuronal cells with others 25% with clear evidence of cell death
2	Borderline	50 - 74% of healthy neuronal cells
3	Poor	25 - 49% of healthy neuronal cells
4	Very Poor	Less than 25% of healthy neuronal cells

Histology of normal Hippocampus

Three layered architecture was appreciated. The layers are:

- Layer 1 – Molecular layer
- Layer 2 – Granular cell layer
- Layer 3 – Polymorphic layer

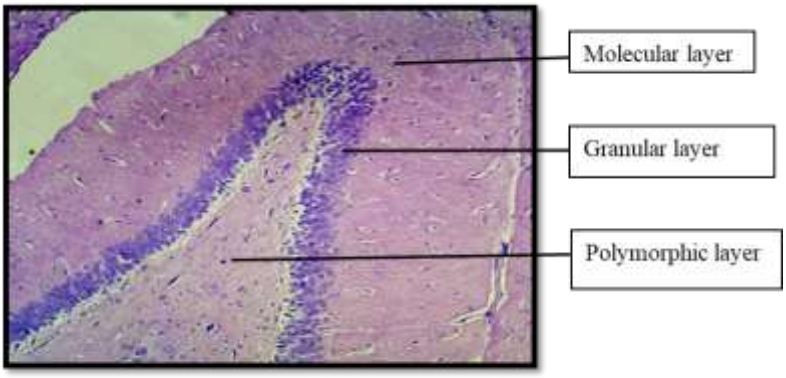
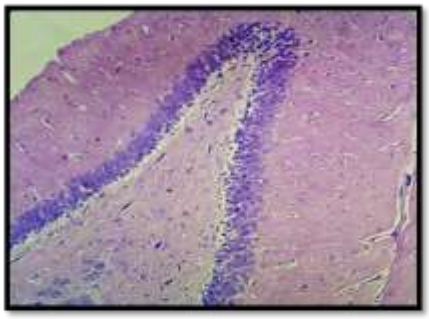


Figure 70: Section of rat brain showing normal **Hippocampus** (10x; H & E stained)

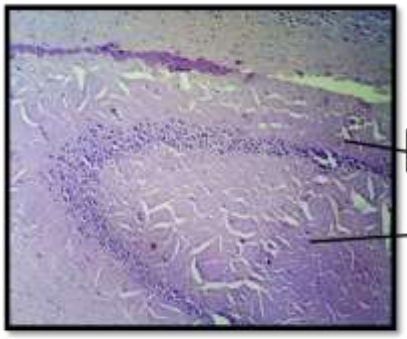
Histopathology of Hippocampus in various groups of PTZ Model



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

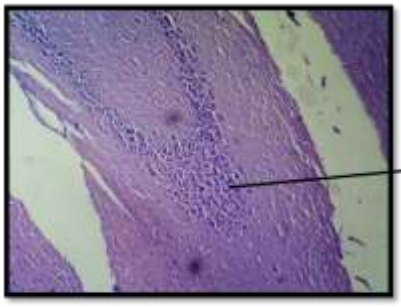
Figure 71: Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of PTZ alone group [Group II] showing severe sclerosis and degeneration amounting to the histopathological score 3.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

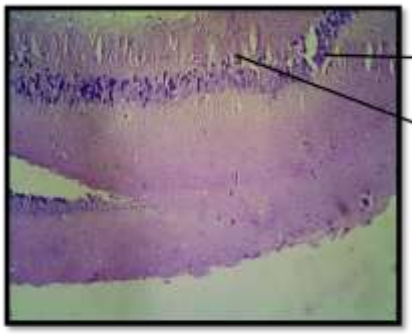
Figure 72: Section of rat brain [**Hippocampus**] from **PTZ alone group [Group II]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of PTZ + Positive control group [Group III] showing mild sclerosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

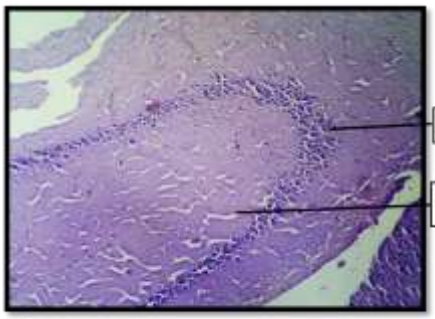
Figure 73: Section of rat brain [**Hippocampus**] from **PTZ+ Sodium valproate [Group III]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of PTZ + Diltiazem group [Group IV] showing moderate degeneration & edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

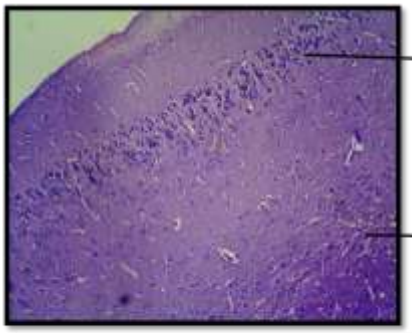
Figure 74: Section of rat brain [**Hippocampus**] from **PTZ+ Diltiazem [Group IV]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of PTZ + Nimodipine group [Group V] showing moderate sclerosis and degeneration amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 75: Section of rat brain [**Hippocampus**] from **PTZ+ Nimodipine [Group V]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of PTZ + Flunarizine group [Group VI] showing mild sclerosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 76 : Section of rat brain [**Hippocampus**] from **PTZ+ Flunarizine [Group VI]** in **PTZ model** (10x; H & E stained)

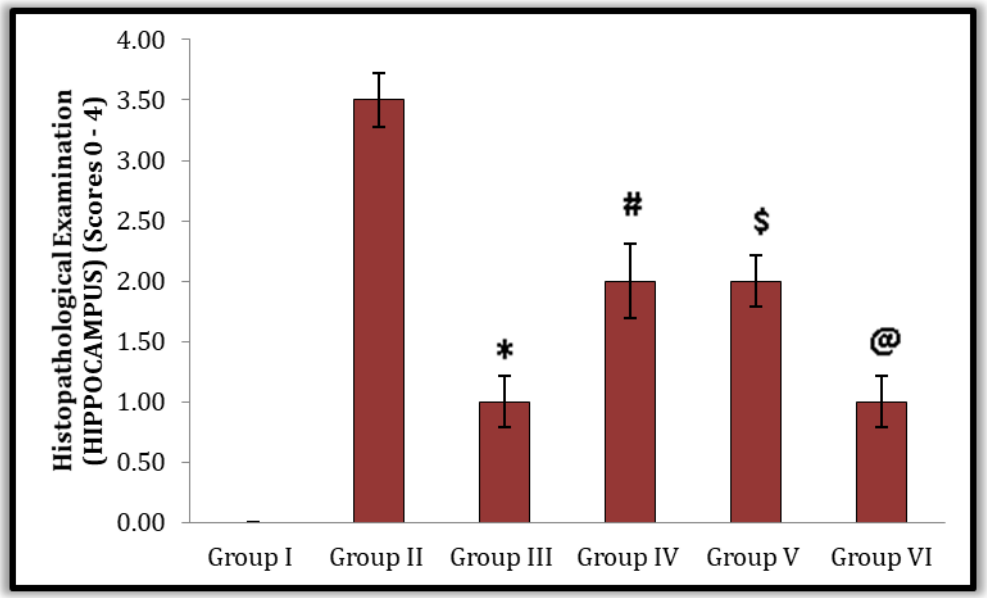


Figure 77: Bar diagram depicting the Histopathological scores of Hippocampus in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a statistically significant increase in histopathological scores for the hippocampus when compared to the vehicle control group [Group I]. Additionally, it was observed that the standard drug group [Group III] and the experimental groups [IV, V and VI] (Diltiazem, Nimodipine and Flunarizine) had decreased histopathological scores in the hippocampus, which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Histology of normal Prefrontal cortex [Cerebrum]

Cerebrum: Showed normal six layers. The layers are:

- Layer I – Molecular layer
- Layer II – External granular layer
- Layer III – External pyramidal cell layer
- Layer IV – Internal granular layer
- Layer V – Internal pyramidal layer
- Layer VI – Multiform layer

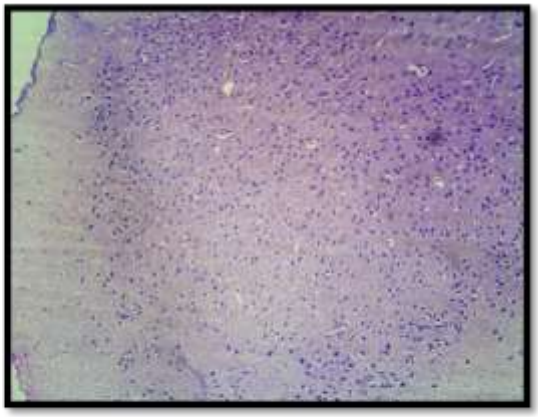
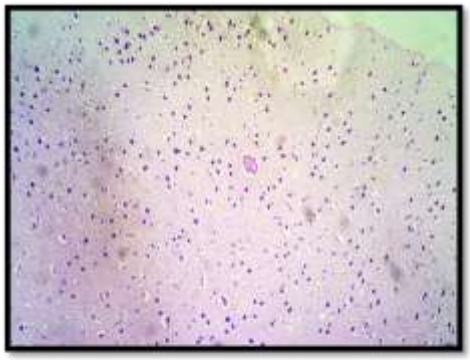


Figure 78 : Section of rat brain showing Prefrontal cortex [Cerebrum] (10x; H & E stained)

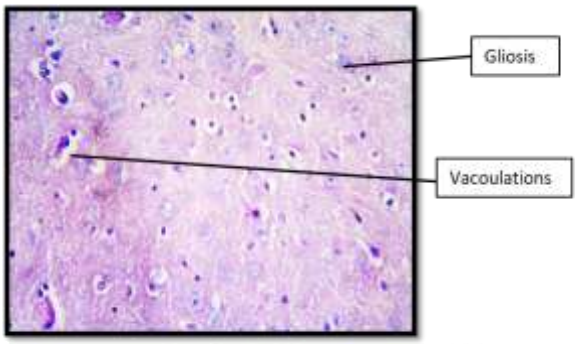
Histopathology of Prefrontal cortex in various groups of PTZ Model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

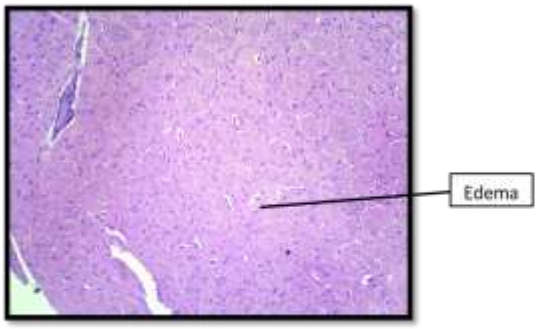
Figure 79: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of PTZ alone group [Group II] showing dense gliosis and vacoulations amounting to the histopathological score 4*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

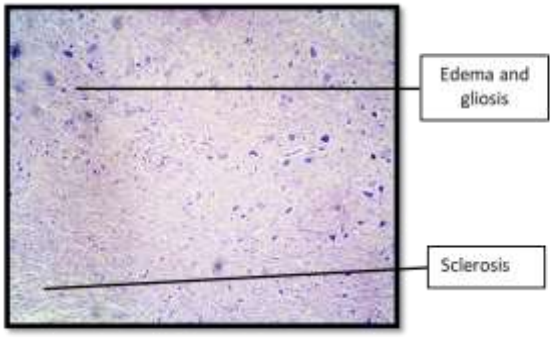
Figure 80: Section of rat brain [**Prefrontal cortex**] from **PTZ alone group [Group II]** in **PTZ model** (40x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Positive control group [Group III] showing mild edema amounting to the histopathological score 1*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

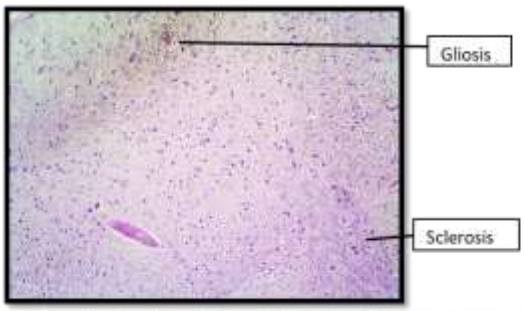
Figure 81: Section of rat brain [**Prefrontal cortex**] from **PTZ+ Sodium valproate [Group III]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Diltiazem group [Group IV] showing moderate sclerosis, edema and gliosis amounting to the histopathological score 2*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

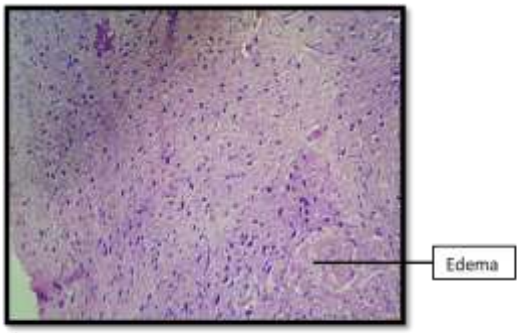
Figure 82: Section of rat brain [**Prefrontal cortex**] from **PTZ+ Diltiazem [Group IV]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Nimodipine group [Group V] showing moderate sclerosis and gliosis amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 83: Section of rat brain [**Prefrontal cortex**] from **PTZ+ Nimodipine [Group V]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Flunarizine group [Group VI] showing mild vacoulations and neuronal loss amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 84: Section of rat brain [**Prefrontal cortex**] from **PTZ+ Flunarizine [Group VI]** in **PTZ model** (10x; H & E stained)

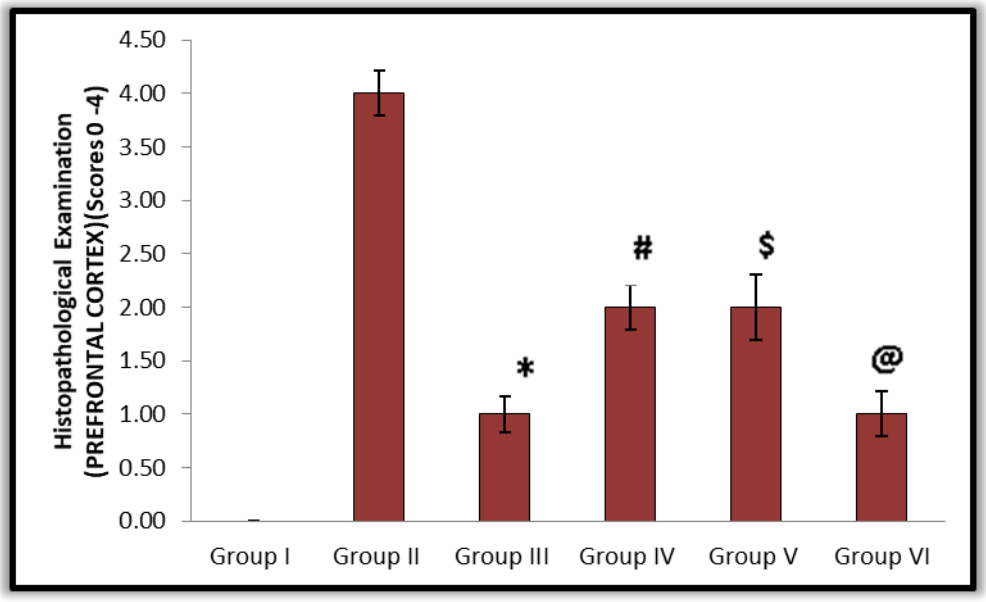


Figure 85: Bar diagram depicting the Histopathological scores of Prefrontal cortex in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a rise in prefrontal cortex histopathology scores as compared to the vehicle control group [Group I], which was statistically significant (P<0.05). Additionally, it was observed that when the standard drug group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] were compared to the negative control group [Group II], histopathological scores in the prefrontal cortex were decreased and found to be statistically significant (P<0.05).

Histology of normal Corpus striatum [Basal ganglia]

Corpus striatum: Heterogeneous mixture of neuronal cell bodies and fibres appreciated

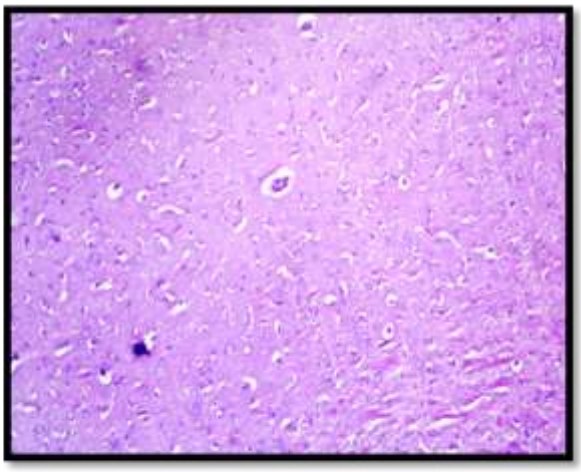
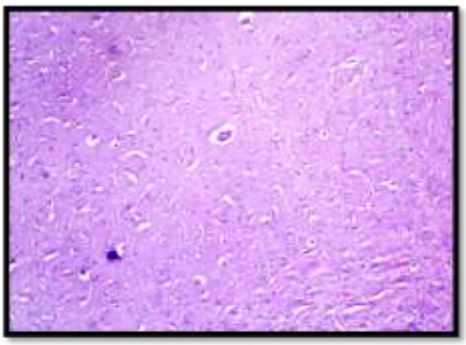


Figure 86: Section of rat brain showing normal **Corpus striatum** (10x; H & E stained)

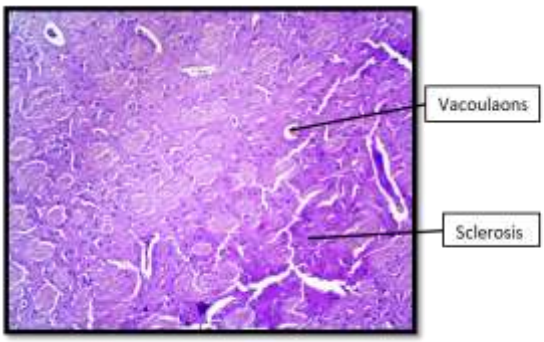
Histopathology of Corpus striatum in various groups of PTZ Model



Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

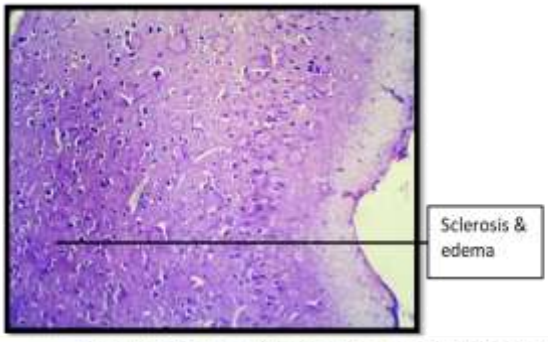
Figure 87: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of PTZ alone group [Group II] showing severe sclerosis and vacoulations amounting to the histopathological score 4*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

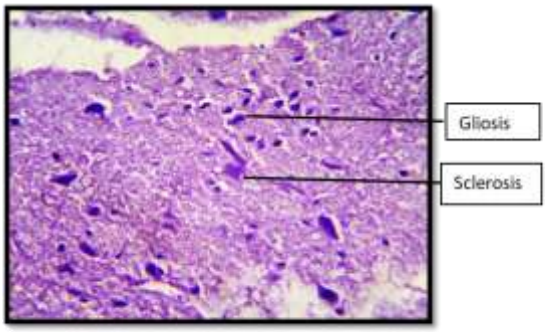
Figure 88: Section of rat brain [**Corpus striatum**] from **PTZ alone group [Group II]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of PTZ + Positive control group [Group III] showing mild sclerosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

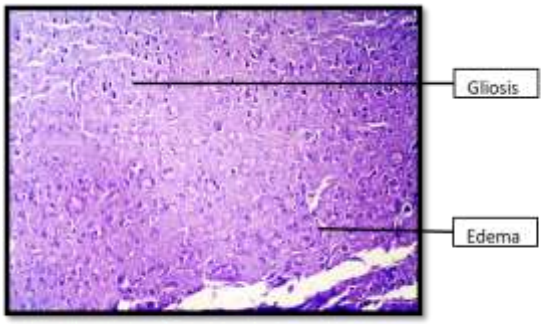
Figure 89: Section of rat brain [**Corpus striatum**] from **PTZ+ Sodium valproate [Group III]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of PTZ + Diltiazem group [Group IV] showing Sclerosis & gliosis amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

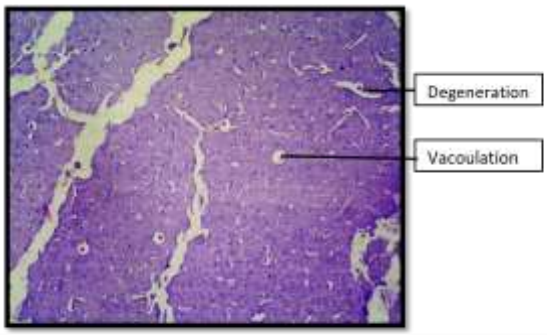
Figure 90: Section of rat brain [**Corpus striatum**] from **PTZ+ Diltiazem [Group IV]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of PTZ + Nimodipine group [Group V] showing moderate gliosis and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 91: Section of rat brain [**Corpus striatum**] from **PTZ+ Nimodipine [Group V]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of PTZ + Flunarizine group [Group VI] showing mild degeneration and vacoulations amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 92: Section of rat brain [**Corpus striatum**] from **PTZ+ Flunarizine [Group VI]** in **PTZ model** (10x; H & E stained)

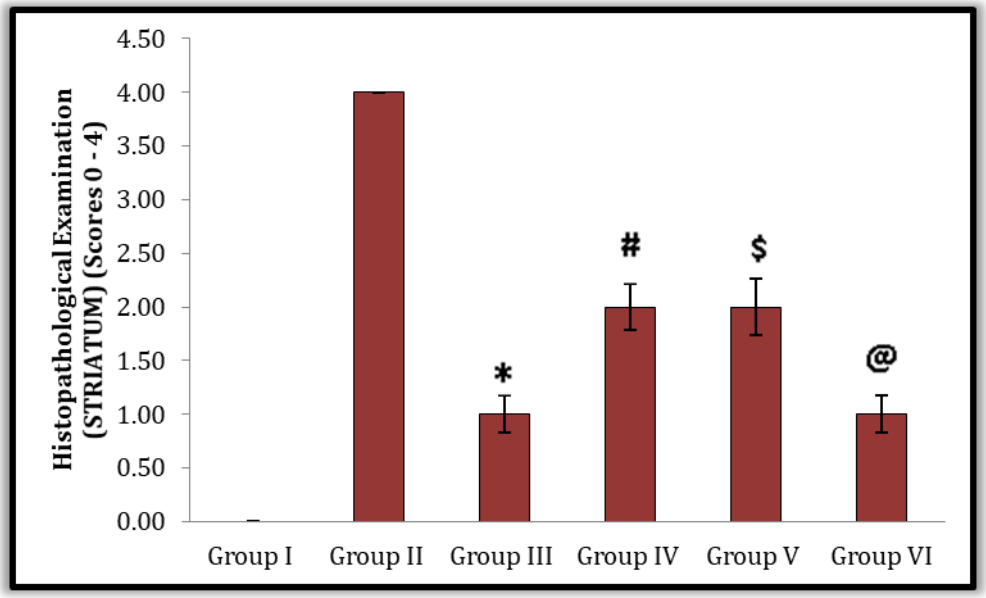


Figure 93: Bar diagram depicting the Histopathological scores of Corpus striatum in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the corpus striatum, which was statistically significant (P<0.05). When compared to the negative control group [Group II], the corpus striatum of the standard drug group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] revealed a decline in histopathological scores that was statistically significant (P<0.05).

Histology of normal Hypothalamus

Hypothalamus: Heterogeneous mixture of neuronal cell bodies are seen in the hypothalamus

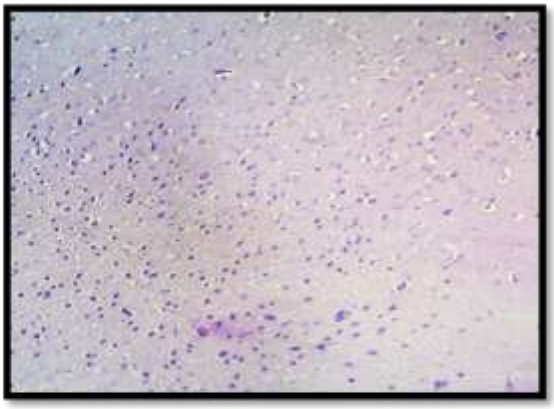
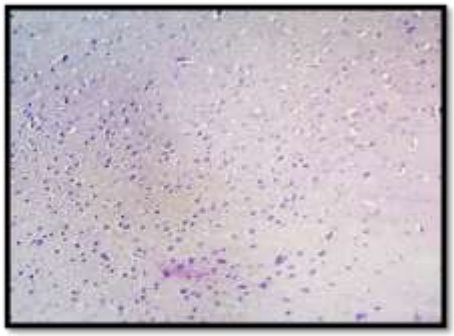


Figure 94: Section of rat brain showing normal Hypothalamus (10x; H & E stained)

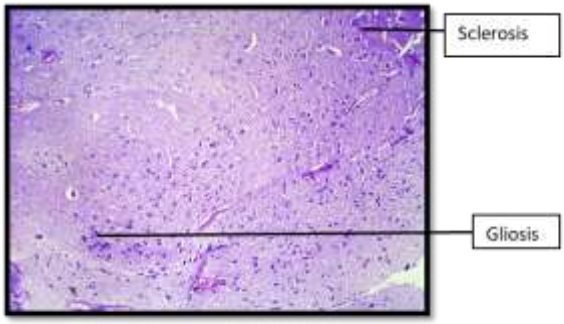
Histopathology of Hypothalamus in various groups of PTZ Model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

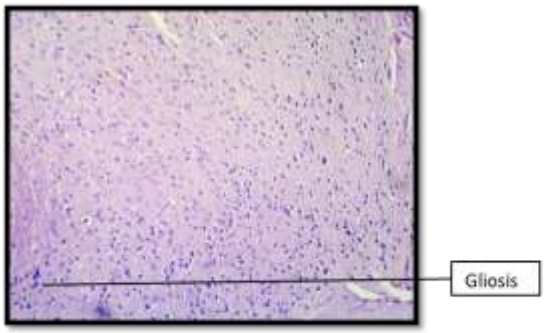
Figure 95: Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of PTZ alone group [Group II] showing severe gliosis and sclerosis amounting to the histopathological score 4*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

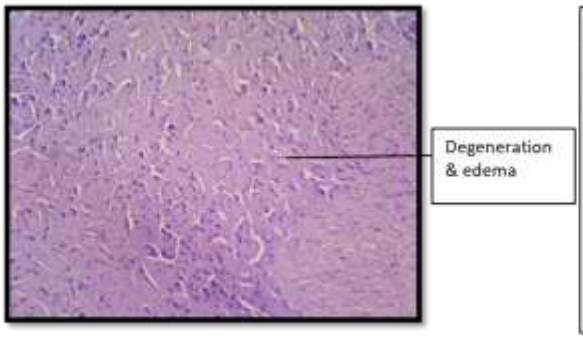
Figure 96: Section of rat brain [**Hypothalamus**] from **PTZ alone group [Group II]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of PTZ + Positive control group [Group III] showing mild gliosis amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

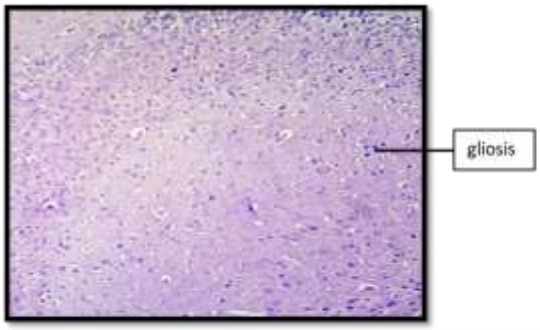
Figure 97: Section of rat brain [**Hypothalamus**] from **PTZ+ Sodium valproate [Group III]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of PTZ + Diltiazem group [Group IV] showing moderate degeneration & edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

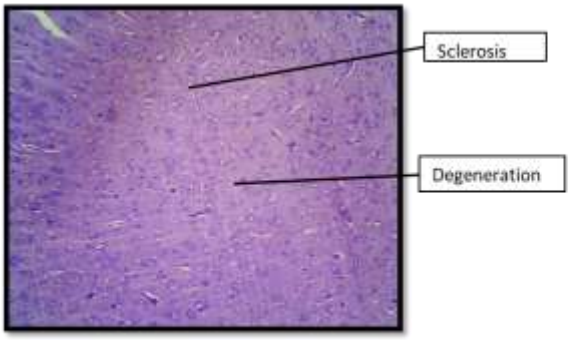
Figure 98: Section of rat brain [**Hypothalamus**] from **PTZ+ Diltiazem [Group IV]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of PTZ + Nimodipine group [Group V] showing moderate gliosis and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 99: Section of rat brain [**Hypothalamus**] from **PTZ+ Nimodipine [Group V]** in **PTZ model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of PTZ + Flunarizine group [Group VI] showing mild sclerosis and degeneration amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 100: Section of rat brain [**Hypothalamus**] from **PTZ+ Flunarizine [Group VI]** in **PTZ model** (10x; H & E stained)

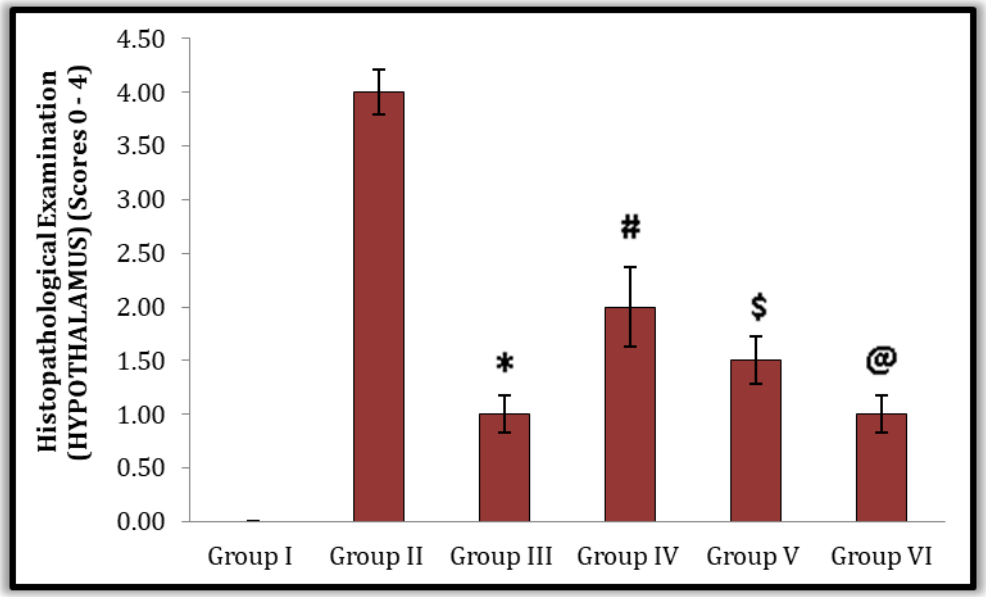


Figure 101: Bar diagram depicting the Histopathological scores in Hypothalamus in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the histopathological scores of the hypothalamus were found to be higher in the negative control group [Group II], which was statistically significant (P<0.05). Similarly, the hypothalamus of the standard drug group [Group III] and the experimental groups [IV, V and VI -Diltiazem, Nimodipine and Flunarizine] had lower histopathological scores than the negative control group [Group II], which was statistically significant (P<0.05).

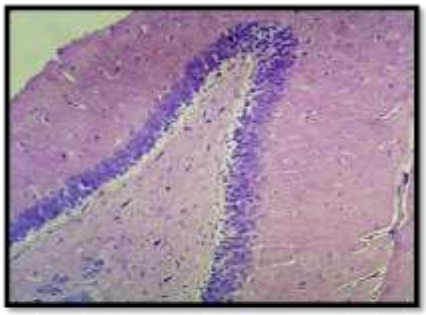
Table 23: Histopathological examination scores for different parts of the brain in PTZ model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
1	Hippocampus (Scores 0 - 4)	0.00±0.00	3.50±0.22	1.00±0.21	2.00±0.31	2.00±0.21	1.00±0.21
2	Prefrontal cortex (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.17	2.00±0.31	1.00±0.21	1.18±0.21
3	Corpus striatum (Scores 0 - 4)	0.00±0.00	4.00±0.00	1.00±0.17	2.00±0.21	2.00±0.26	1.00±0.17
4	Hypothalamus (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.17	2.00±0.37	1.50±0.22	1.00±0.17

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When comparing Group II [Negative control group] to Group I [Vehicle control group], it was observed that the histopathological scores of the hippocampus, prefrontal cortex, corpus striatum and hypothalamus were higher in Group II [Negative control group], which was statistically significant ($P<0.05$). Similarly, there was a decrease in the histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus in the standard drug group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine), which was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

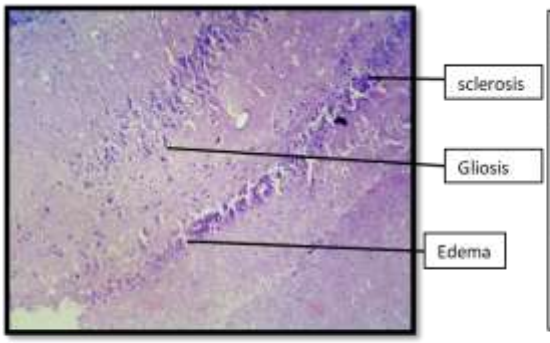
Histopathology of Hippocampus in Pilocarpine Model



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

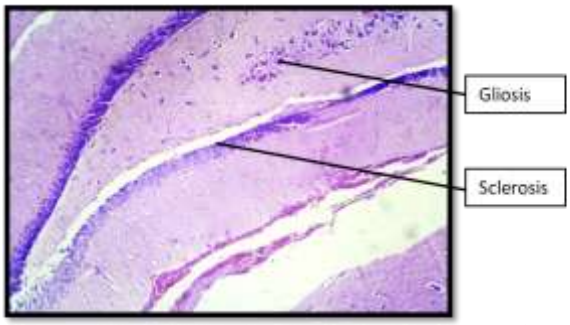
Figure 102: Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of Pilocarpine alone group [Group VII] showing severe sclerosis, gliosis and edema amounting to the histopathological score 3.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 103: Section of rat brain [**Hippocampus**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of Pilocarpine + Positive control group [Group VIII] showing mild gliosis and sclerosis amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 104: Section of rat brain [**Hippocampus**] from **Pilocarpine + Diphenyl hydantoin [Group VIII]** in **Pilocarpine model** (10x; H & E stained)

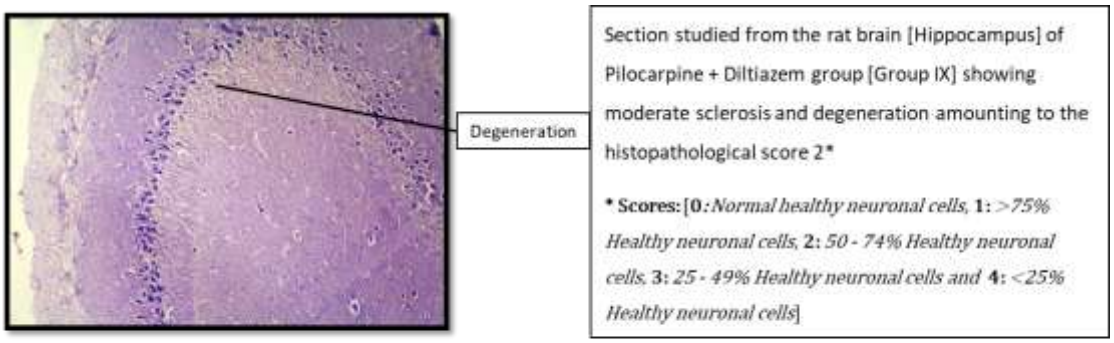


Figure 105: Section of rat brain [**Hippocampus**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model** (10x; H & E stained)

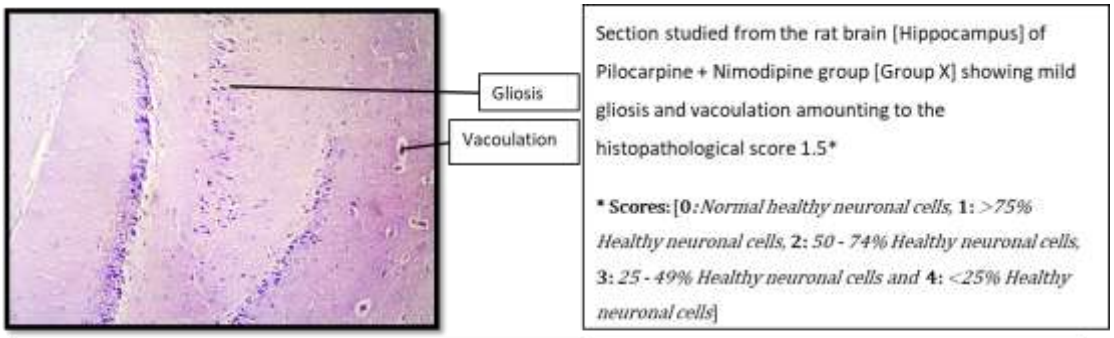


Figure 106: Section of rat brain [**Hippocampus**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine model** (10x; H & E stained)

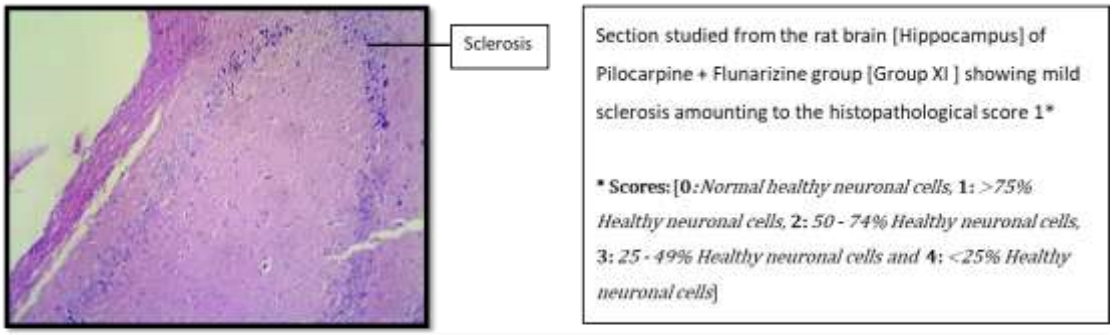


Figure 107: Section of rat brain [**Hippocampus**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model** (10x; H & E stained)

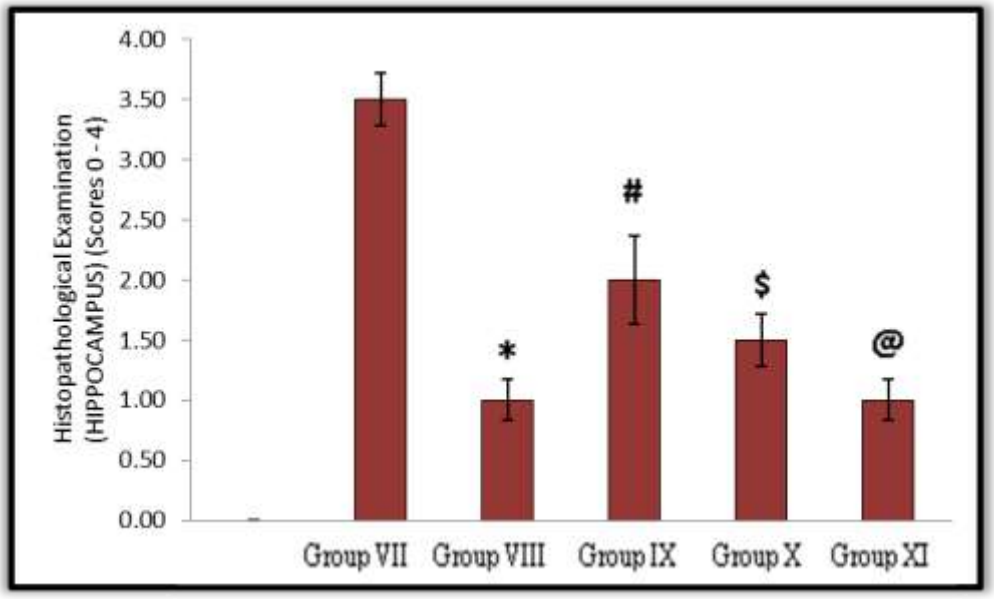
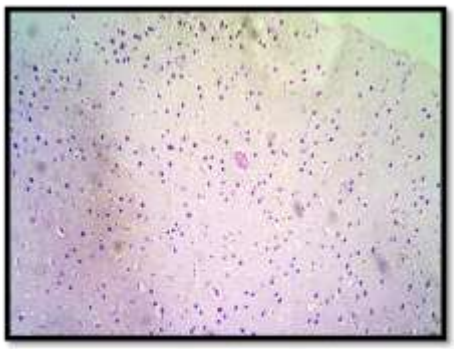


Figure 108: Bar diagram depicting the Histopathological scores in Hippocampus in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the negative control group [Group VII] had higher histopathological hippocampus scores, which was statistically significant (P<0.05). When compared to the negative control group [Group VII], the Standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX – Diltiazem, Groups X – Nimodipine and Groups XI – Flunarizine] showed a decrease in the histopathological scores of the hippocampus, which was statistically significant (P<0.05).

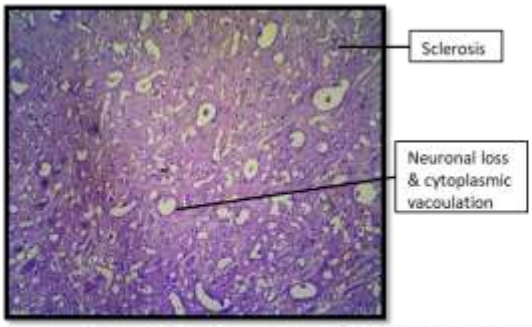
Histopathology of Prefrontal cortex in Pilocarpine Model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

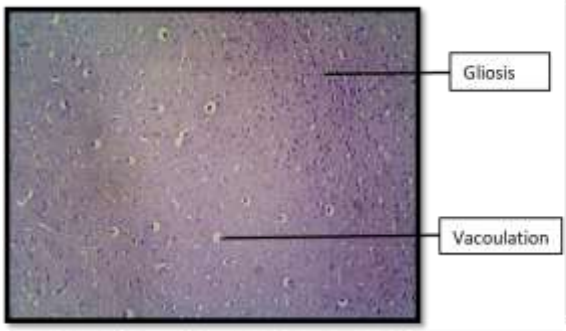
Figure 109: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine alone group [Group VII] showing severe sclerosis, neuronal loss, edema & cytoplasmic vacoulations amounting to the histopathological score 3.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

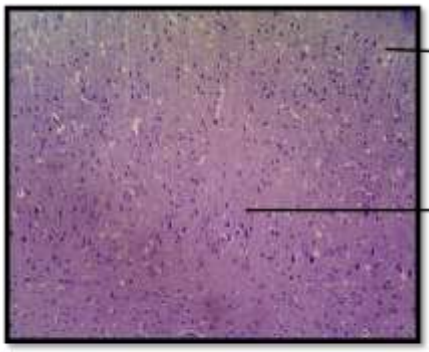
Figure 110: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (40x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Positive control group [Group VIII] showing mild gliosis and vacoulation amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 111: Section of rat brain [Prefrontal cortex] from **Pilocarpine + Diphenyl hydantoin [Group VIII]** in **Pilocarpine model** (10x; H & E stained)

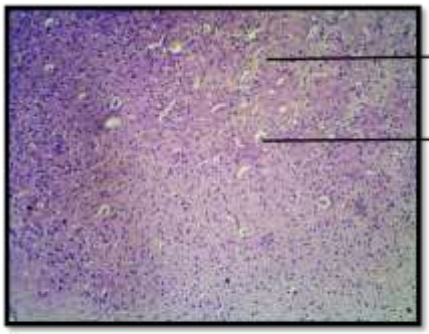


Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Diltiazem group [Group IX] showing moderate degeneration amounting to the histopathological score 2*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 112: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model**

(10x; H & E stained)

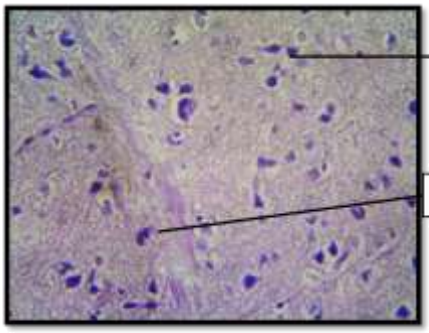


Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Nimodipine group [Group X] showing edema and vacoulations amounting to the histopathological score 1.5*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 113: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine model**

(10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Flunarizine group [Group XI] showing sclerosis and degeneration amounting to the histopathological score 1*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 114: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model**

(40x; H & E stained)

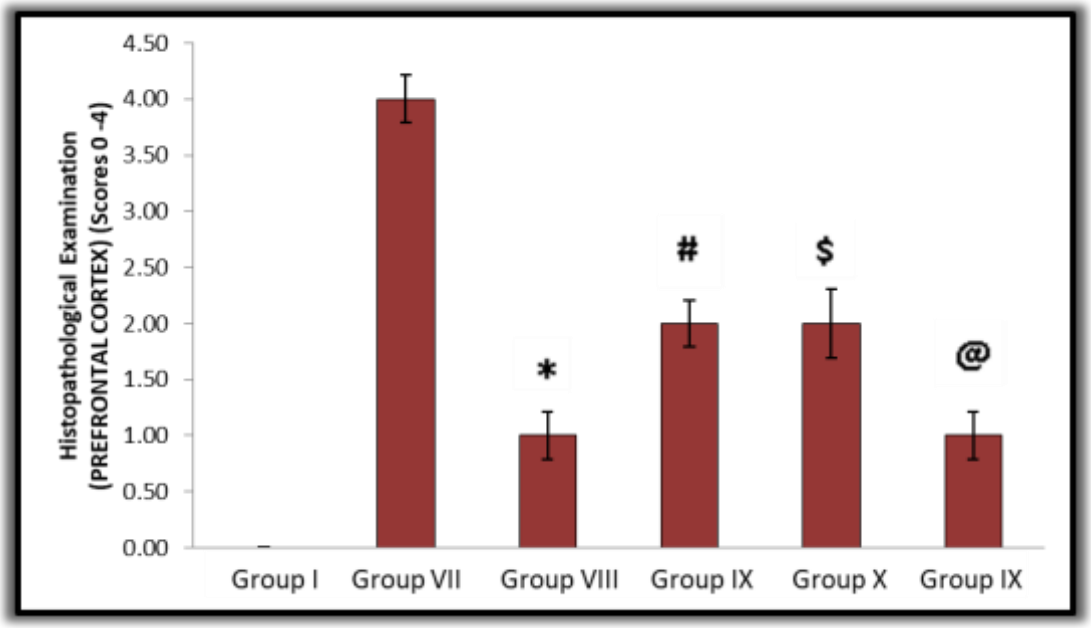
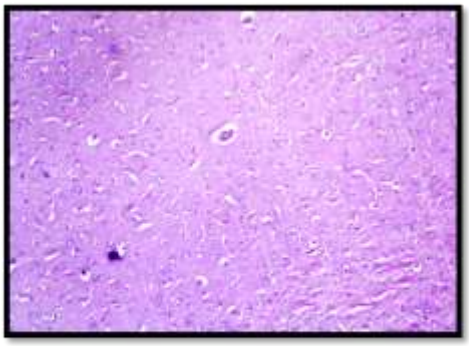


Figure 115: Bar diagram depicting the Histopathological scores of Prefrontal cortex [Cerebrum] in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the negative control group [Group VII] had higher histopathological scores in the prefrontal cortex, which was statistically significant (P<0.05). When compared to the negative control group [Group VII], the histopathological scores in the prefrontal cortex of the Standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX – Diltiazem, Groups X – Nimodipine and Groups XI – Flunarizine] were significantly lower (P<0.05).

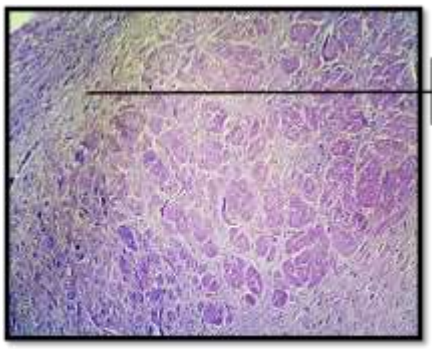
Histopathology of Corpus striatum in Pilocarpine Model



Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

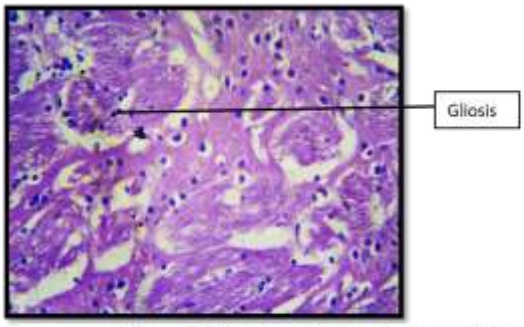
Figure 116: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **Pilocarpine model**(10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of Pilocarpine alone group [Group VII] showing severe sclerosis and degeneration amounting to the histopathological score 3.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

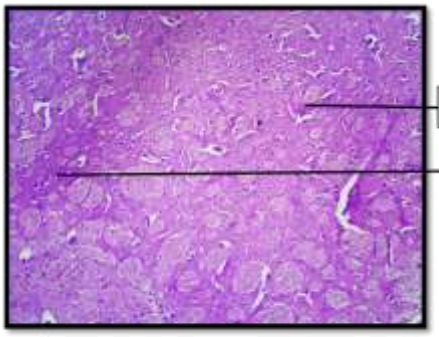
Figure 117: Section of rat brain [**Corpus striatum**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of Pilocarpine + Positive control group [Group VIII] showing mild degeneration and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 118: Section of rat brain [**Corpus striatum**] from **Pilocarpine + Diphenyl hydantoin [Group VIII]** in **Pilocarpine model** (10x; H & E stained)

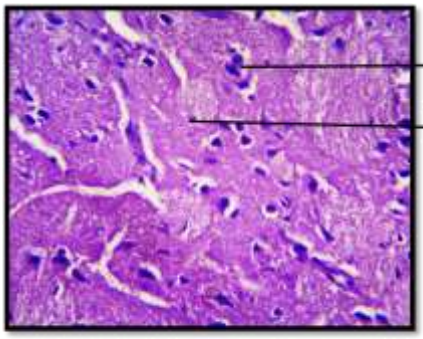


Section studied from the rat brain [Corpus striatum] of Pilocarpine + Diltiazem group [Group IX] showing moderate sclerosis and degeneration amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 119: Section of rat brain [Corpus striatum] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model**

(10x; H & E stained)

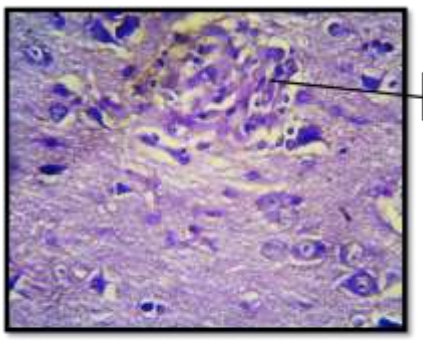


Section studied from the rat brain [Corpus striatum] of Pilocarpine + Nimodipine group [Group X] showing mild gliosis and neuronal loss amounting to the histopathological score 1.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 120: Section of rat brain [**Corpus striatum**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine model**

(40x; H & E stained)



Section studied from the rat brain [Corpus striatum] of Pilocarpine + Flunarizine group [Group XI] showing sclerosis and degeneration amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 121 : Section of rat brain [**Corpus striatum**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model**

(40x; H & E stained)

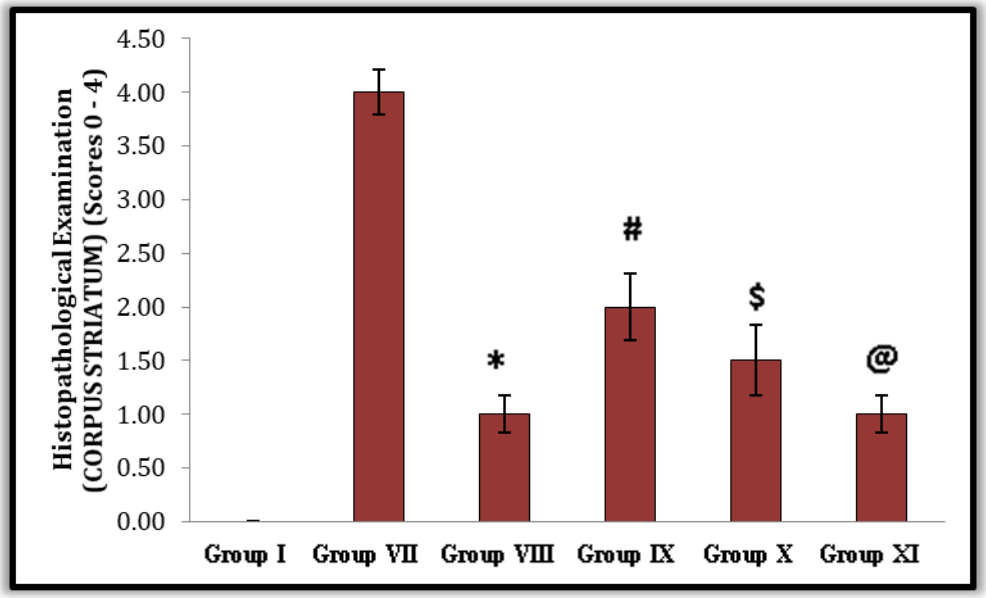
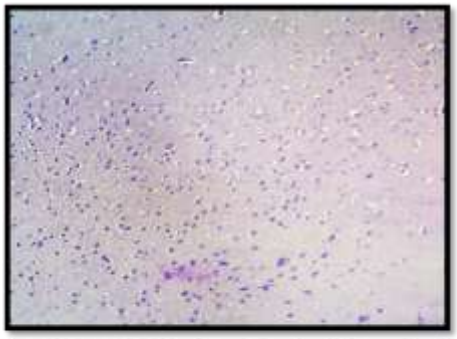


Figure 122: Bar diagram depicting the Histopathological scores of Corpus striatum in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the negative control group [Group VII] had significantly higher histopathological scores in the corpus striatum (P<0.05). Similarly, there was a decrease in the histopathological scores in the corpus striatum in the standard drug group [Group VII] and the experimental groups [Group IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine], which was statistically significant when compared to the negative control group [Group VII] (P<0.05).

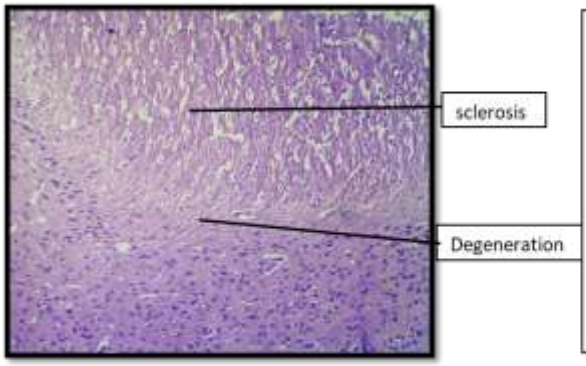
Histopathology of Hypothalamus in Pilocarpine Model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

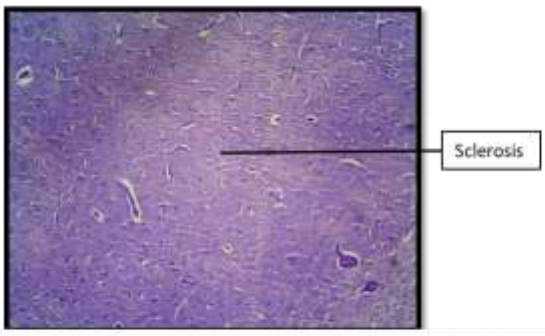
Figure 123: Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Pilocarpine alone group [Group VII] showing severe sclerosis and degeneration amounting to the histopathological score 3.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

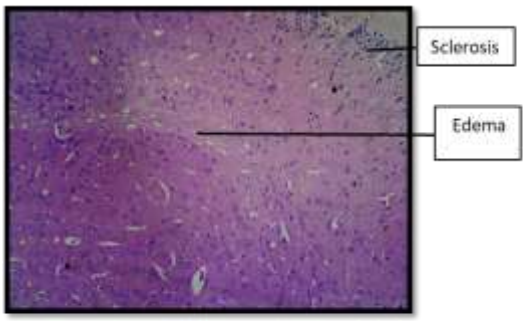
Figure 124: Section of rat brain [**Hypothalamus**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Positive control group [Group VIII] showing mild sclerosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

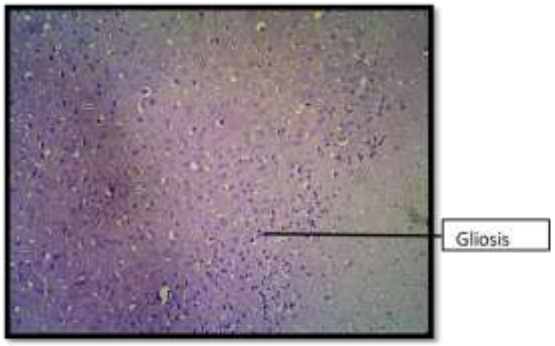
Figure 125: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Phenobarbitone [Group VIII]** in **Pilocarpine model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Diltiazem group [Group IX] showing moderate sclerosis and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

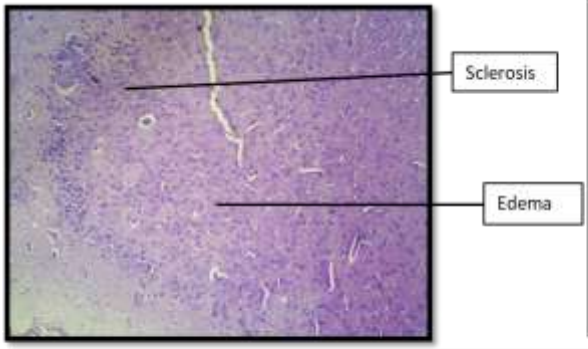
Figure 126: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model**
(10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Nimodipine group [Group X] showing gliosis and edema amounting to the histopathological score 1.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 127: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine model**
(10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Flunarizine group [Group XI] showing sclerosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 128: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model**
(10x; H & E stained)

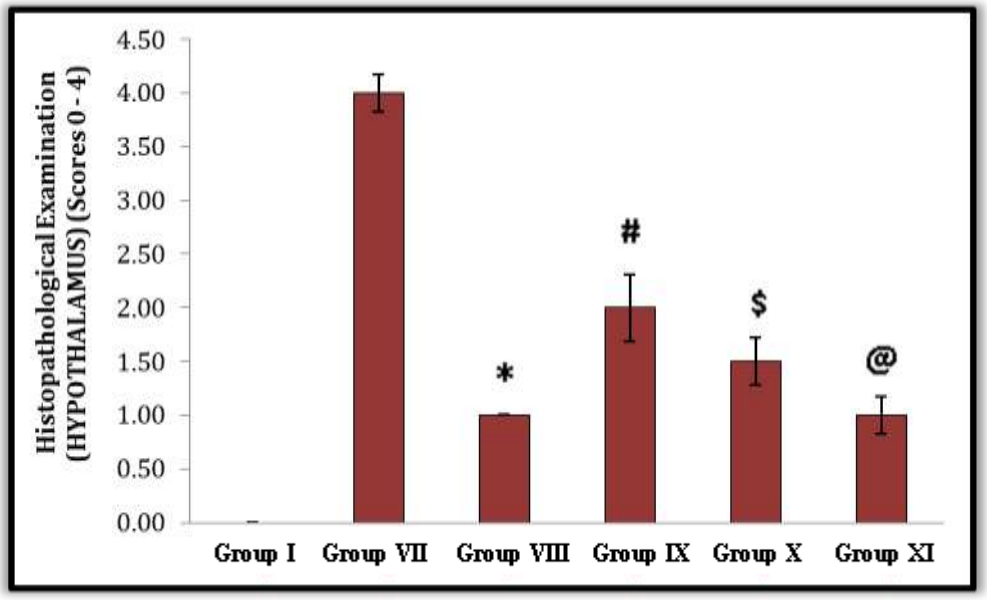


Figure 129: Bar diagram depicting the Histopathological scores of Hypothalamus in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When the negative control group [Group VII] was compared to the vehicle control group [Group I], the histopathological scores in the hypothalamus were substantially (P<0.05) higher in the negative control group [Group VII]. When compared to the negative control group [Group VII], the Standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX – Diltiazem, Groups X – Nimodipine and Groups XI – Flunarizine] showed a decrease in the histopathological scores of the hypothalamus, which was statistically significant (P<0.05).

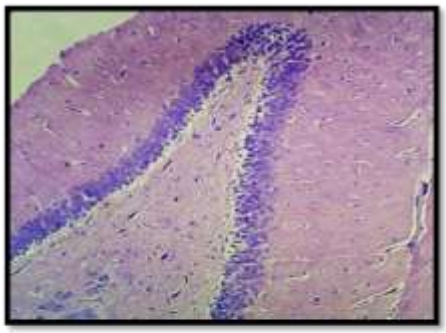
Table 24: Histopathological examination scores for different parts of the brain in Pilocarpine model screening test in Wistar albino rats

Sl. No.	Parameters	Group I	Group VII	Group VIII*	Group IX#	Group X$	Group XI@
1	Hippocampus (Scores 0 - 4)	0.00±0.00	3.50±0.22	1.00±0.17	2.00±0.37	1.50±0.22	1.00±0.17
2	Prefrontal cortex (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.21	2.00±0.21	2.00±0.31	1.00±0.21
3	Corpus striatum (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.17	2.00±0.31	1.50±0.33	1.00±0.17
4	Hypothalamus (Scores 0 - 4)	0.00±0.00	4.00±0.17	1.00±0.00	2.00±0.31	1.50±0.22	1.00±0.17

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus were higher in the negative control group [Group VII], which was statistically significant (P<0.05). When compared to the negative control group [Group VII], there was a significant (P<0.05) decrease in the histopathological scores of the hippocampus, prefrontal cortex, corpus striatum and hypothalamus in the standard drug group [Group VIII] and the experimental groups [Group IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine].

Histopathology of Hippocampus in various groups of MES Model

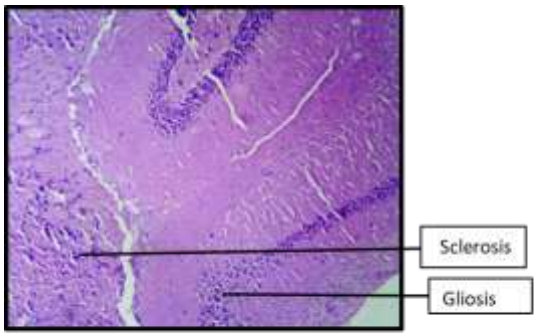


Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 130 : Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **MES model**

(10x; H & E stained)

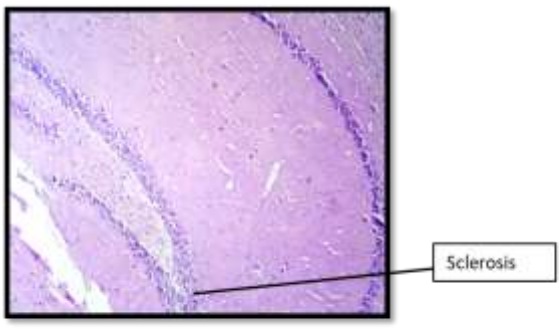


Section studied from the rat brain [Hippocampus] of MES alone group [Group XII] showing severe gliosis, sclerosis and edema amounting to the histopathological score 4*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 131: Section of rat brain [**Hippocampus**] from **MES alone group [Group XII]** in **MES model**

(10x; H & E stained)

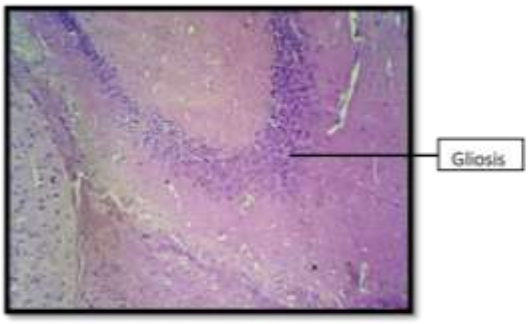


Section studied from the rat brain [Hippocampus] of MES + Positive control group [Group XIII] showing mild sclerosis and edema amounting to the histopathological score 1.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 132: Section of rat brain [**Hippocampus**] from **MES+ Diphenyl hydantoin [Group XIII]** in **MES model**

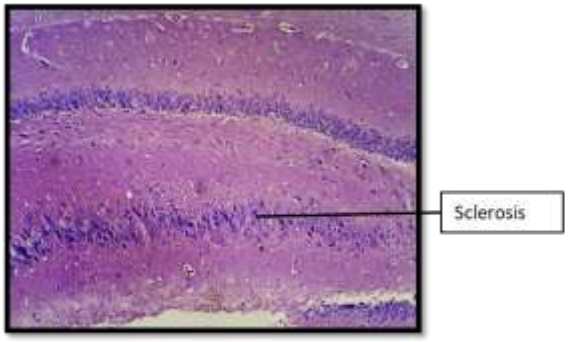
(10x; H & E stained)



Section studied from the rat brain [Hippocampus] of MES + Diltiazem group [Group XIV] showing moderate gliosis and edema amounting to the histopathological score 2*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

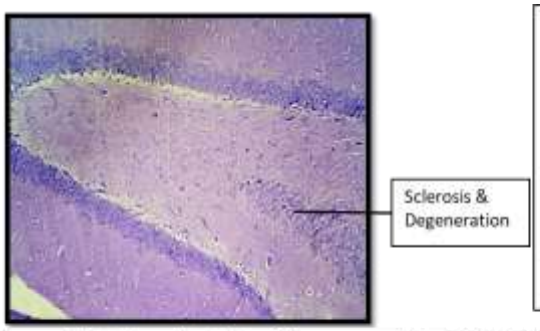
Figure 133: Section of rat brain [**Hippocampus**] from **MES+ Diltiazem [Group XIV]** in **MES model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of MES + Nimodipine group [Group XV] showing mild sclerosis amounting to the histopathological score 1.5*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 134: Section of rat brain [**Hippocampus**] from **MES+ Nimodipine [Group XV]** in **MES model** (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of MES + Flunarizine group [Group XVI] showing mild sclerosis and degeneration amounting to the histopathological score 1*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 135: Section of rat brain [**Hippocampus**] from **MES+ Flunarizine [Group XVI]** in **MES model** (10x; H & E stained)

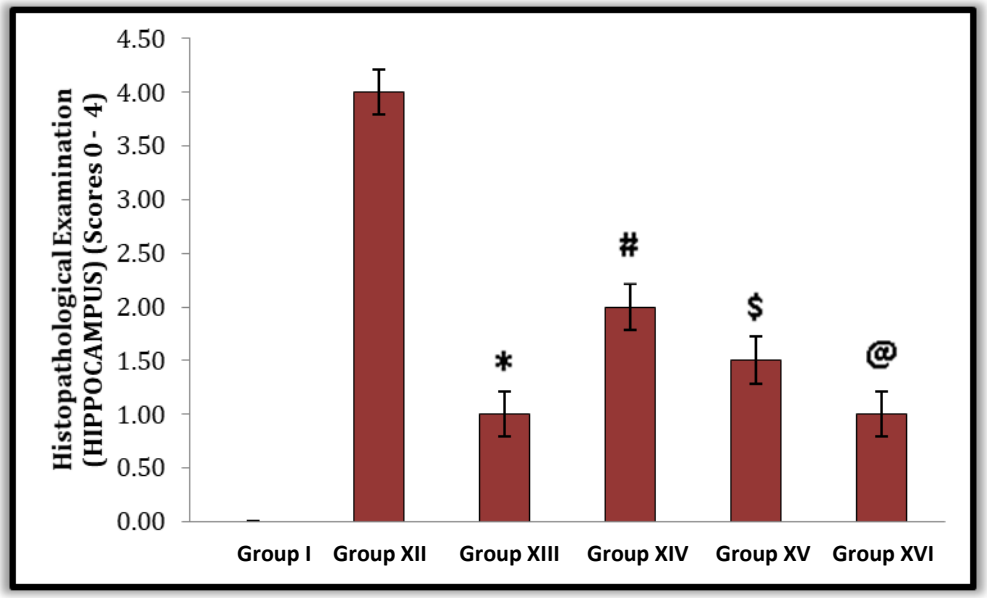
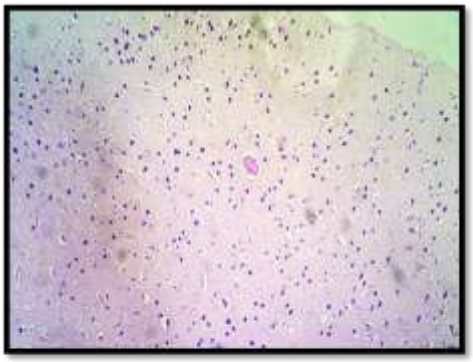


Figure 136: Bar diagram depicting the Histopathological scores in Hippocampus in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When compared to the vehicle control group [Group I], the negative control group [Group XII] had higher histopathological scores in the hippocampus, which was statistically significant (P<0.05). There was also a decrease in the histopathological scores of the hippocampus in the Standard drug group - Diphenyl hydantoin [Group XII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI), which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

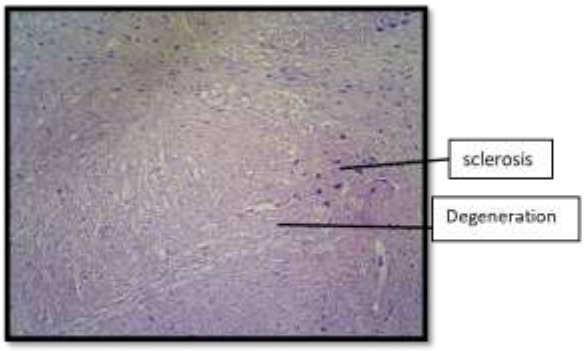
Histopathology of Prefrontal cortex in various groups of MES Model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

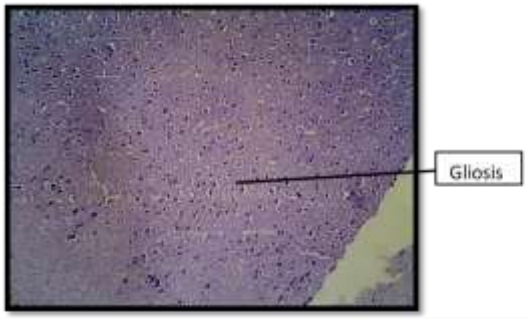
Figure 137: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **MES model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of MES alone group [Group XII] showing sclerosis and degeneration amounting to the histopathological score 3.5*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

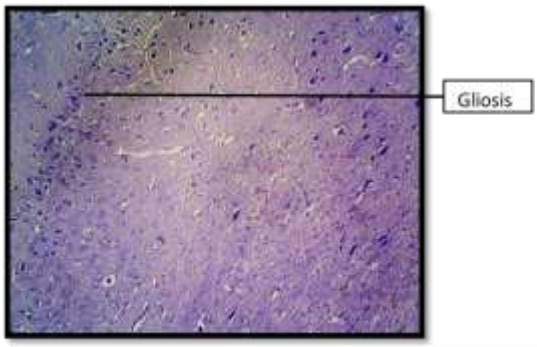
Figure 138: Section of rat brain [**Prefrontal cortex**] from **MES alone group [Group XII]** in **MES model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of MES + Positive control group [Group XIII] showing gliosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 139: Section of rat brain [**Prefrontal cortex**] from **MES+ Diphenyl hydantoin [Group XIII]** in **MES model** (10x; H & E stained)

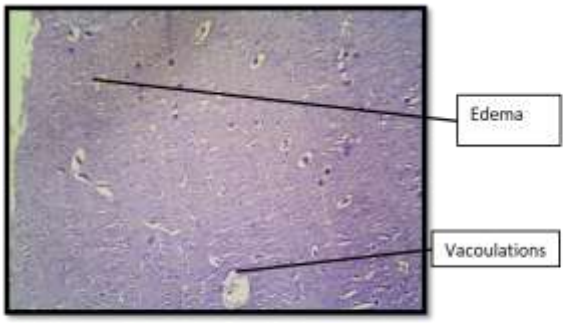


Section studied from the rat brain [Prefrontal cortex] of MES + Diltiazem group [Group XIV] showing gliosis and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 140: Section of rat brain [**Prefrontal cortex**] from **MES+ Diltiazem [Group XIV]** in **MES model**

(10x; H & E stained)

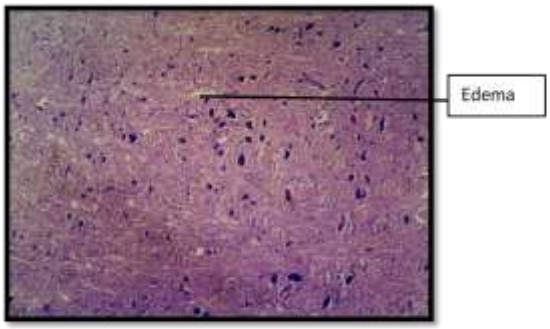


Section studied from the rat brain [Prefrontal cortex] of MES + Nimodipine group [Group XV] showing cytoplasmic vacoulations and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 141: Section of rat brain [**Prefrontal cortex**] from **MES+ Nimodipine [Group XV]** in **MES model**

(10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of MES + Flunarizine group [Group XVI] showing edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 142: Section of rat brain [**Prefrontal cortex**] from **MES+ Flunarizine [Group XVI]** in **MES model**

(10x; H & E stained)

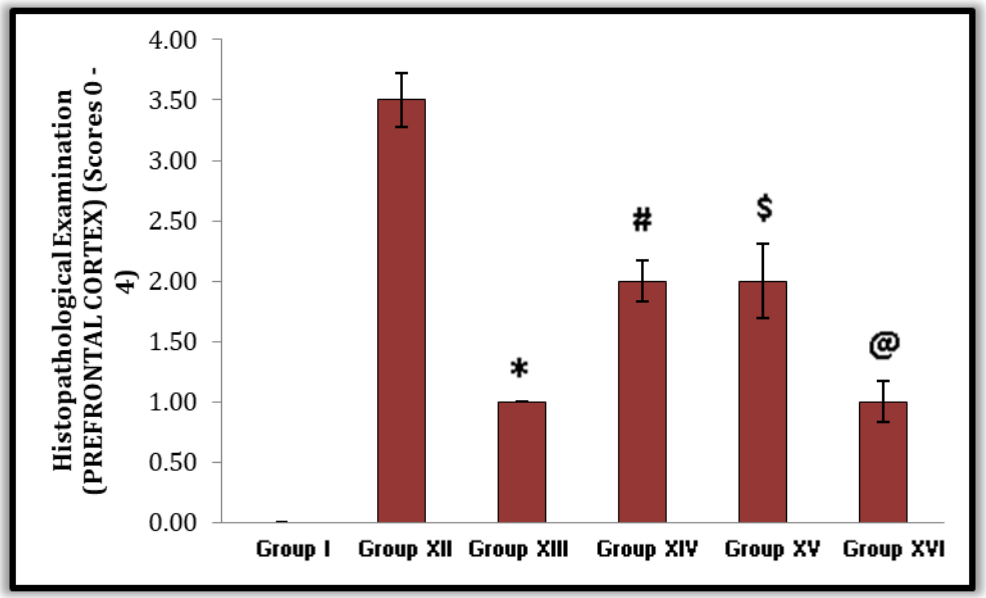
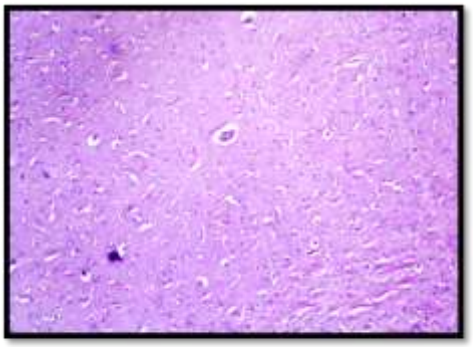


Figure 143: Bar diagram depicting the Histopathological scores in Prefrontal cortex in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When comparing the negative control group [Group XII] to the vehicle control group [Group I], the histopathological scores in the prefrontal cortex [cerebrum] were higher in the negative control group [Group XII], which was statistically significant (P<0.05). The tandard drug group [Diphenyl hydantoin group - Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) both showed a decrease in prefrontal cortex histopathological scores that was statistically significant when compared to the negative control group [Group XII] (P<0.05).

Histopathology of Corpus striatum in MES Model

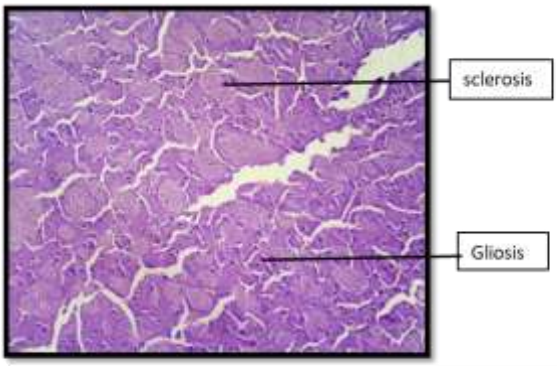


Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 144: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **MES model**

(10x; H & E stained)

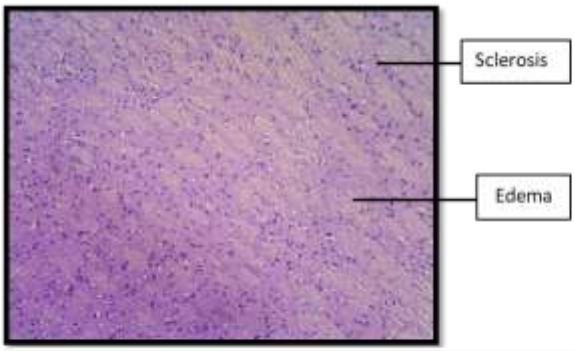


Section studied from the rat brain [Corpus striatum] of MES alone group [Group XII] showing sclerosis and gliosis amounting to the histopathological score 4*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 145: Section of rat brain [**Corpus striatum**] from **MES alone group [Group XII]** in **MES model**

(10x; H & E stained)

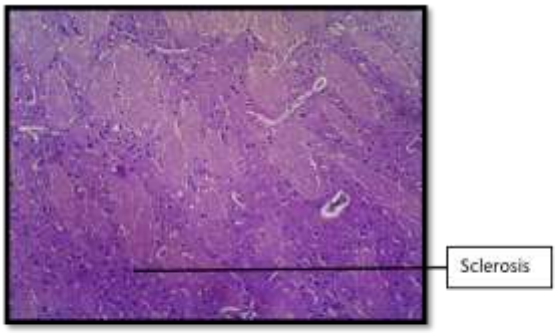


Section studied from the rat brain [Corpus striatum] of MES + Positive control group [Group XIII] showing sclerosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 146: Section of rat brain [**Corpus striatum**] from **MES+ Diphenyl hydantoin [Group XIII]** in **MES model**

(10x; H & E stained)

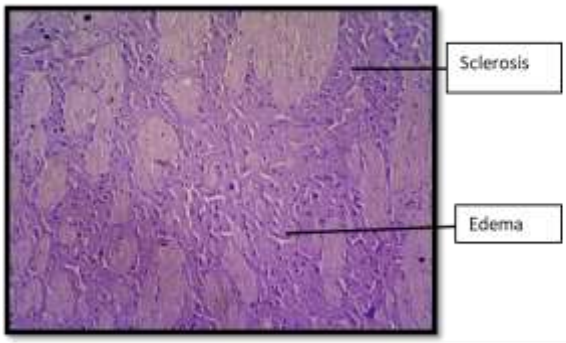


Section studied from the rat brain [Corpus striatum] of MES + Diltiazem group [Group XIV] showing sclerosis amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 147: Section of rat brain [**Corpus striatum**] from **MES+ Diltiazem [Group XIV]** in **MES model**

(10x; H & E stained)

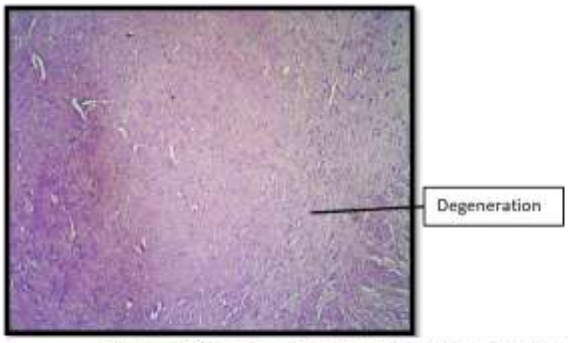


Section studied from the rat brain [Corpus striatum] of MES + Nimodipine group [Group XV] showing sclerosis and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 148: Section of rat brain [**Corpus striatum**] from **MES+ Nimodipine** [**Group XV**] in **MES model**

(10x; H & E stained)



Section studied from the rat brain [Corpus striatum] of MES + Flunarizine group [Group XVI] showing degeneration amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 149: Section of rat brain [**Corpus striatum**] from **MES+ Flunarizine [Group XVI]** in **MES model**

(10x; H & E stained)

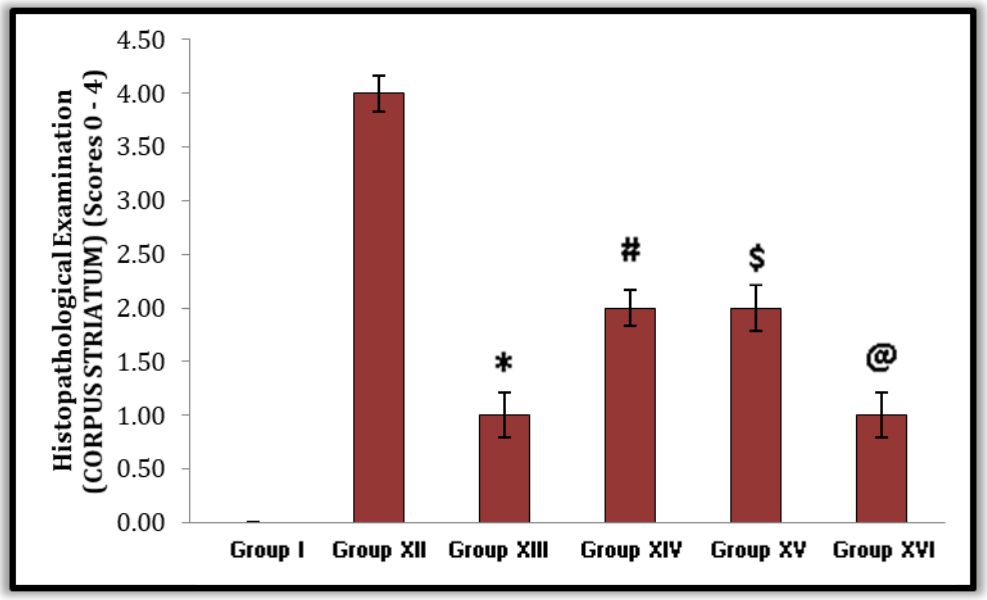
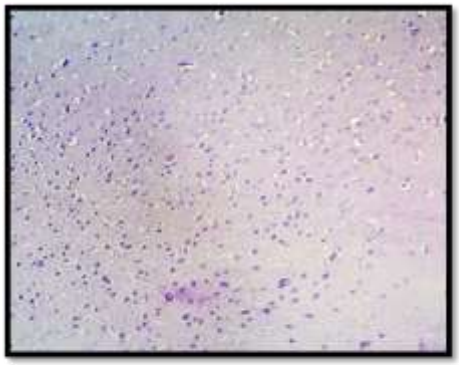


Figure 150: Bar diagram depicting the Histopathological scores in Corpus striatum in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When compared to the vehicle control group [Group I], the negative control group [Group XII] had significantly higher histopathology scores in the corpus striatum) (P<0.05). Furthermore, when the standard drug group (Diphenyl hydantoin - Group XIII) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) were compared to the negative control group [Group I], there was a significant (P<0.05) decrease in the corpus striatum histopathological scores.

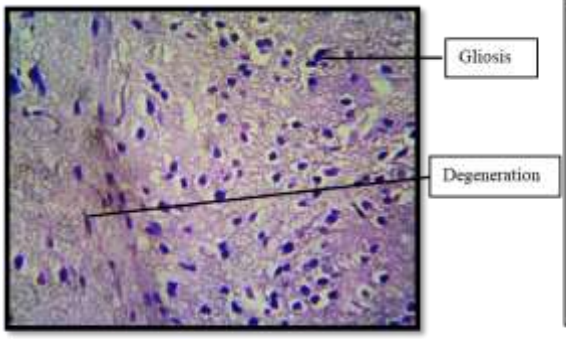
Histopathology of Hypothalamus in various groups of MES Model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

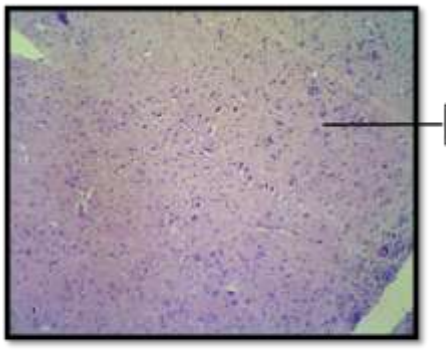
Figure 151: Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **MES model**
(10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of MES alone group [Group XII] showing gliosis and degeneration amounting to the histopathological score 4*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

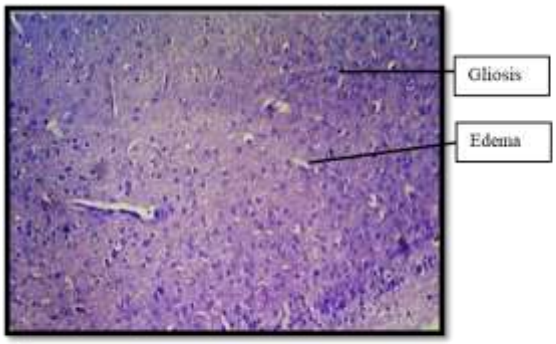
Figure 152: Section of rat brain [**Hypothalamus**] from **MES alone group [Group XII]** in **MES model**
(40x; H & E stained)



Section studied from the rat brain [Hypothalamus] of MES + Positive control group [Group XIII] showing mild gliosis and edema amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 153: Section of rat brain [**Hypothalamus**] from **MES+ Diphenyl hydantoin [Group XIII]** in **MES model**
(10x; H & E stained)

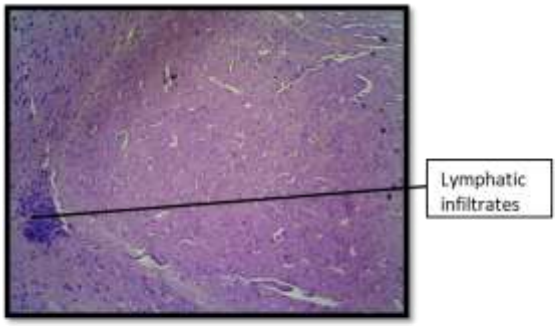


Section studied from the rat brain [Hypothalamus] of MES + Diltiazem group [Group XIV] showing gliosis and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 154: Section of rat brain [**Hypothalamus**] from **MES+ Diltiazem [Group XIV]** in **MES model**

(10x; H & E stained)

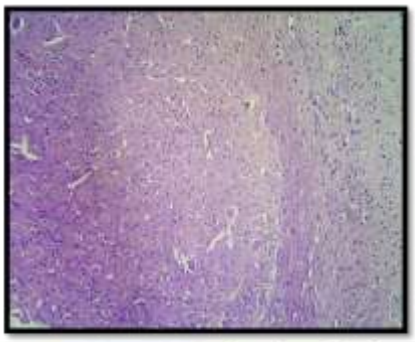


Section studied from the rat brain [Hypothalamus] of MES + Nimodipine group [Group XV] showing lymphatic infiltration and edema amounting to the histopathological score 2*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 155: Section of rat brain [**Hypothalamus**] from **MES+ Nimodipine [Group XV]** in **MES model**

(10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of MES + Flunarizine group [Group XVI] showing degeneration amounting to the histopathological score 1*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 156: Section of rat brain [**Hypothalamus**] from **MES+ Flunarizine [Group XVI]** in **MES model**

(10x; H & E stained)

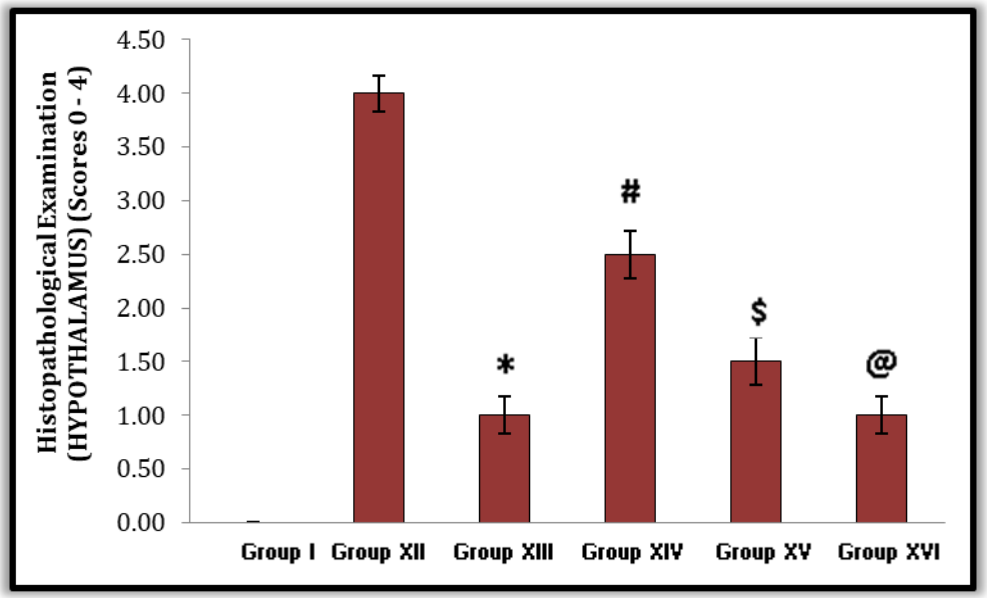


Figure 157: Bar diagram depicting the histopathological scores in hypothalamus in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The negative control group [Group XII] had a statistically significant increase in histopathological scores for the hypothalamus as compared to the vehicle control group [Group I]. Additionally, the results indicated that both the standard drug group (Diphenyl hydantoin [Group XIII]) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) revealed decreased histopathological scores in the hypothalamus, which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

Table 25: Histopathological examination scores in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group XII	Group XIII*	Group XIV#	Group XV$	Group XVI@
1	Hippocampus (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.21	2.00±0.21	1.50±0.22	1.00±0.21
2	Prefrontal cortex (Scores 0 - 4)	0.00±0.00	3.50±0.22	1.00±0.00	2.00±0.17	2.00±0.31	1.00±0.17
3	Corpus striatum (Scores 0 - 4)	0.00±0.00	4.00±0.17	1.00±0.21	2.00±0.17	2.00±0.21	1.00±0.21
4	Hypothalamus (Scores 0 - 4)	0.00±0.00	4.00±0.17	1.00±0.17	2.50±0.22	1.50±0.22	1.00±0.17

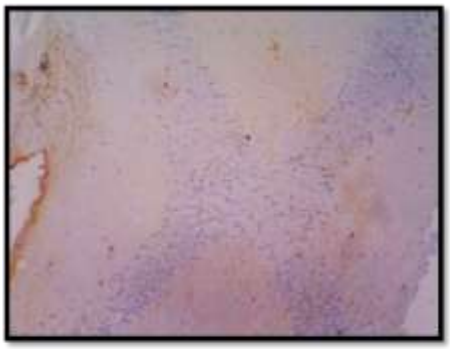
Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The study findings indicated that when compared to the vehicle control group [Group I], the histopathological scores for the hippocampus, prefrontal cortex, corpus striatum, and hypothalamus were significantly (P<0.05) enhanced in the negative control group [Group XII]. Additionally, it was observed that both the standard drug group (Diphenyl hydantoin [Group XIII]) and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) showed a decrease in the histopathological scores of the hippocampus, prefrontal cortex, corpus striatum and hypothalamus, which was statistically significant (P<0.05).

EVALUATION OF IMMUNOHISTOCHEMISTRY IN ALL THE THREE MODELS [PTZ, PILOCARPINE & MES]

IMMUNOHISTOCHEMISTRY IN PTZ MODEL

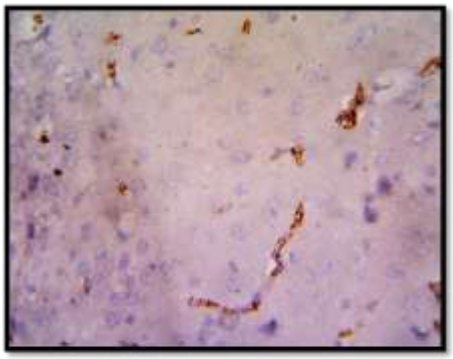
Immunohistochemistry of Hippocampus in various groups of PTZ Model



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

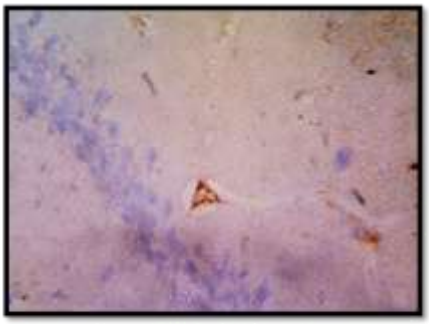
Figure 158: Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **PTZ model** (10x; IHC HSP70)



Section studied from the rat brain [Hippocampus and parahippocampal cortex] of Pilocarpine alone group [Group II] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

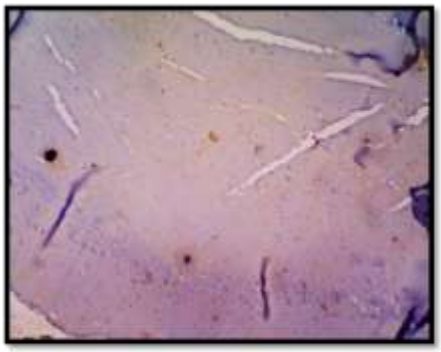
Figure 159: Section of rat brain [**Hippocampus**] from **PTZ alone group [Group II]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of PTZ + Positive control group [Group III] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

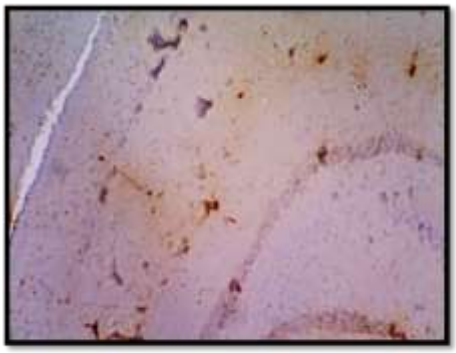
Figure 160: Section of rat brain [**Hippocampus**] from **PTZ + Sodium valproate [Group III]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [hippocampus] of PTZ + Diltiazem group [Group IV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 161: Section of rat brain [**Hippocampus**] from **PTZ + Diltiazem [Group IV]** in **PTZ model** (10x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of PTZ + Nimodipine group [Group V] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 162: Section of rat brain [**Hippocampus**] from **PTZ + Nimodipine [Group V]** in **PTZ model** (10x; IHC HSP70)

Section studied from the rat brain [Hippocampus] of PTZ + Flunarizine group [Group VI] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 163: Section of rat brain [**Hippocampus**] from **PTZ + Flunarizine [Group VI]** in **PTZ model** (10x; IHC HSP70)

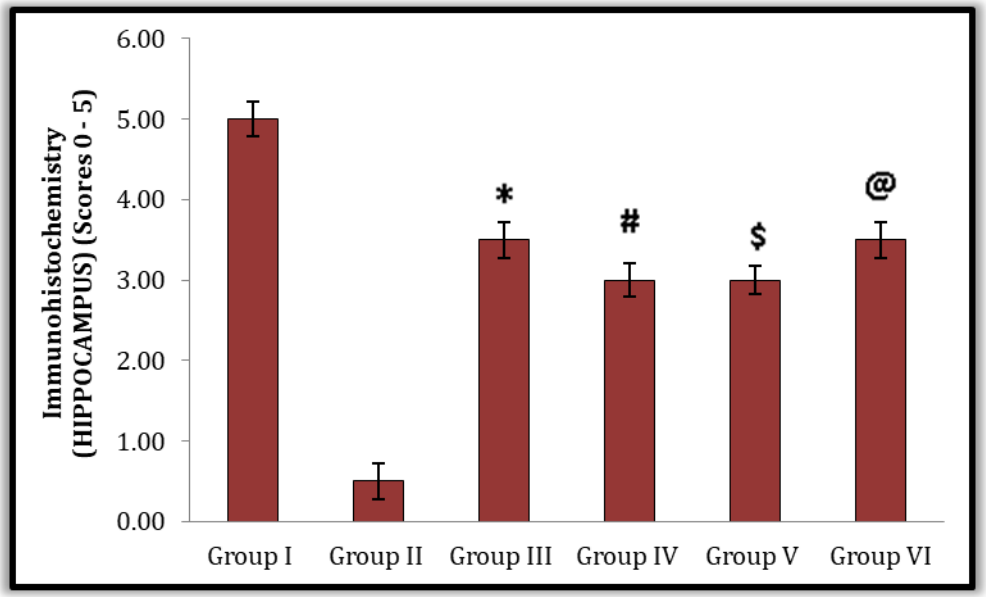
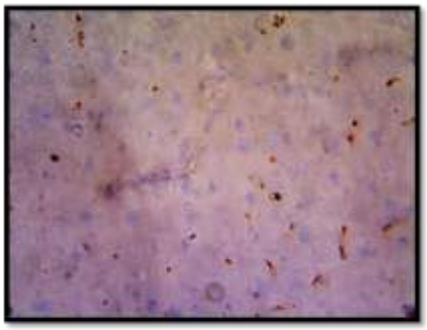


Figure 164: Bar diagram depicting the Immunohistochemistry scores of Hippocampus in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The immunohistochemistry scores for the hippocampus were significantly lower in the negative control group [Group II] than in the vehicle control group [Group I]. Additionally, the standard drug (Sodium valproate) group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] revealed a statistically significant increase in the immunohistochemistry scores for the hippocampus when compared to the negative control group [Group II] (P<0.05).

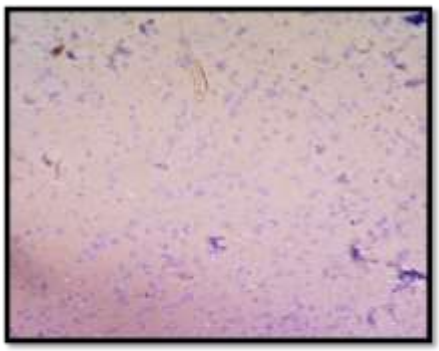
Immunohistochemistry of Prefrontal cortex in various groups of PTZ Model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection)* and **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

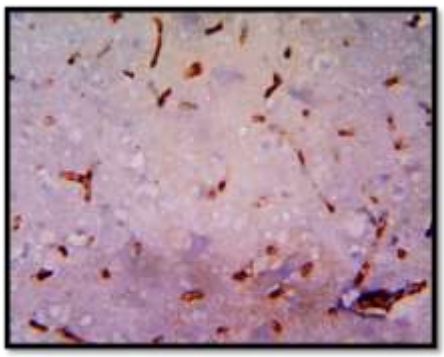
Figure 165: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine alone group [Group II] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection)* and **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

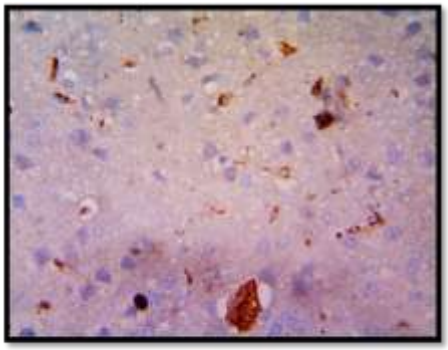
Figure 166: Section of rat brain [**Prefrontal cortex**] from **PTZ alone group [Group II]** in **PTZ model** (10x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Positive control group [Group III] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection)* and **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

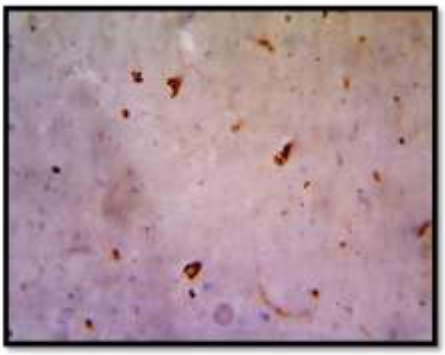
Figure 167: Section of rat brain [**Prefrontal cortex**] from **PTZ + Sodium valproate [Group III]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Diltiazem group [Group IV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

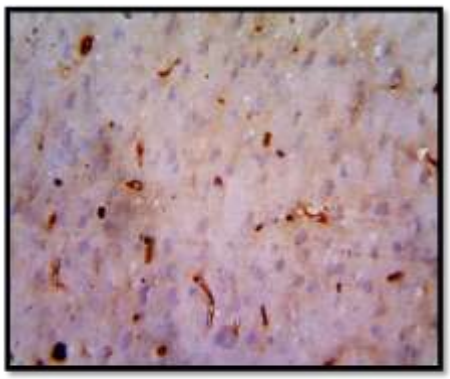
Figure 168: Section of rat brain [**Prefrontal cortex**] from **PTZ + Diltiazem [Group IV]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Nimodipine group [Group V] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 169: Section of rat brain [**Prefrontal cortex**] from **PTZ + Nimodipine [Group V]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of PTZ + Flunarizine group [Group VI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 170: Section of rat brain [**Prefrontal cortex**] from **PTZ + Flunarizine [Group VI]** in **PTZ model** (40x; IHC HSP70)

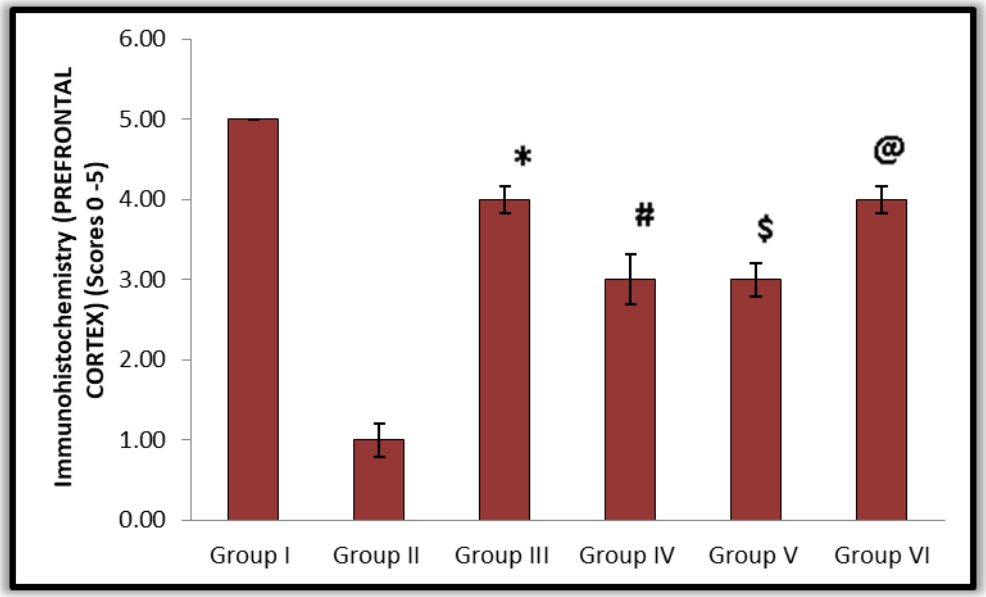
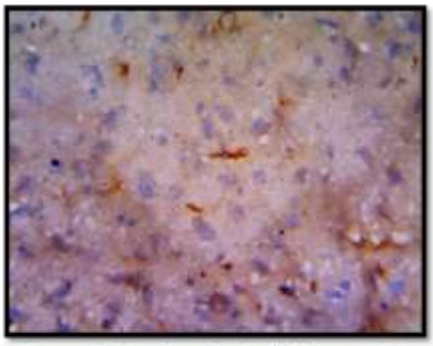


Figure 171: Bar diagram depicting the Immunohistochemistry scores for the Prefrontal cortex in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When compared to the vehicle control group [Group I], the immunohistochemistry scores for the prefrontal cortex (cerebrum) of the negative control group [Group II] were lower, which was statistically significant ($P<0.05$). The prefrontal cortex of the standard drug [Sodium valproate] group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] had higher immunohistochemistry scores, which was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

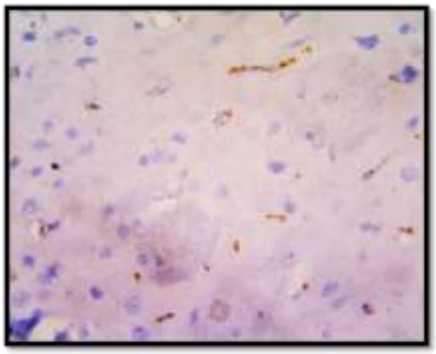
Immunohistochemistry of Corpus striatum in various groups of PTZ Model



Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

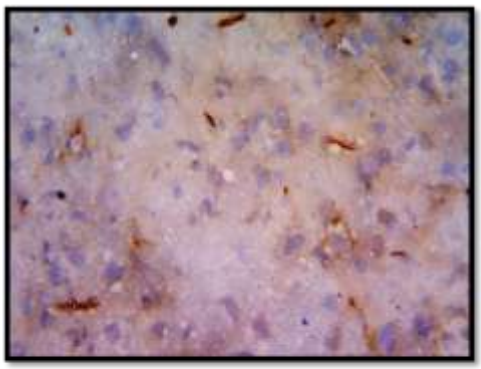
Figure 172: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of Pilocarpine alone group [Group II] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

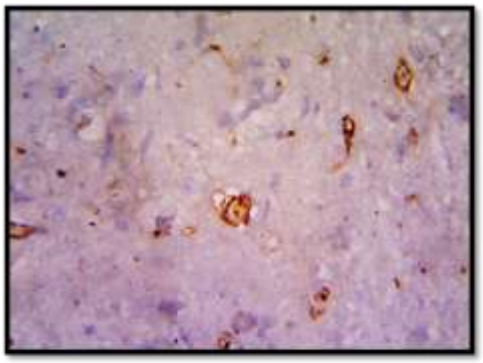
Figure 173: Section of rat brain [**Corpus striatum**] from **PTZ alone group [Group II]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of PTZ + Positive control group [Group III] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

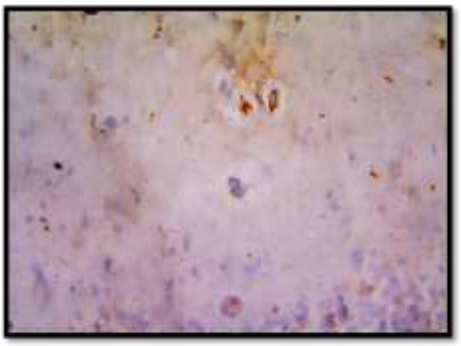
Figure 174: Section of rat brain [**Corpus striatum**] from **PTZ + Sodium valproate [Group III]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of PTZ + Diltiazem group [Group IV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

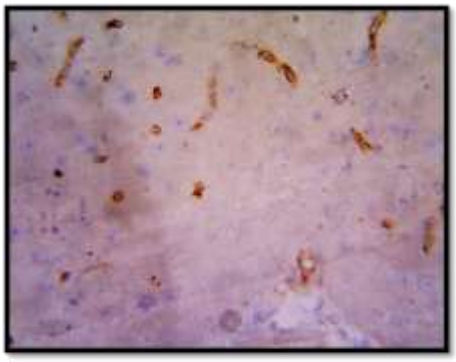
Figure 175: Section of rat brain [**Corpus striatum**] from **PTZ + Diltiazem [Group IV]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of PTZ + Nimodipine group [Group V] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 176: Section of rat brain [Corpus striatum] from **PTZ + Nimodipine [Group V]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of PTZ + Flunarizine group [Group VI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 177: Section of rat brain [**Corpus striatum**] from **PTZ + Flunarizine [Group VI]** in **PTZ model** (40x; IHC HSP70)

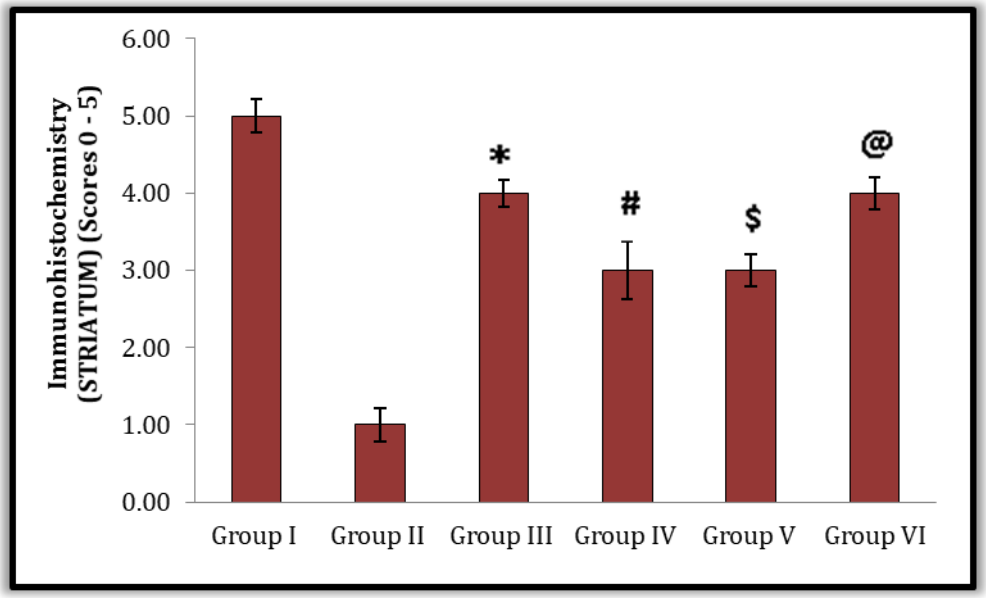
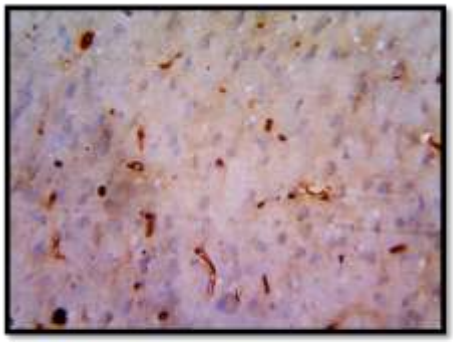


Figure 178: Bar diagram depicting the Immunohistochemistry scores of Corpus striatum in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

The negative control group [Group I] had lower corpus striatum immunohistochemistry scores than the vehicle control group [Group II], which was statistically significant ($P<0.05$). The standard drug [Sodium valproate] group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] both had higher immunohistochemistry scores in the corpus striatum, which was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

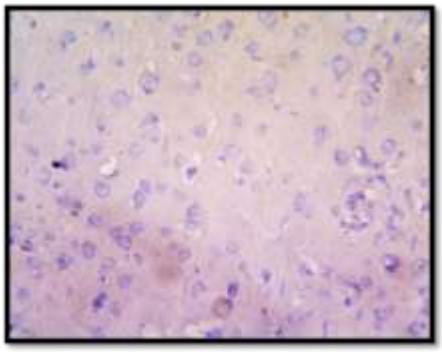
Immunohistochemistry of Hypothalamus in various groups of PTZ Model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

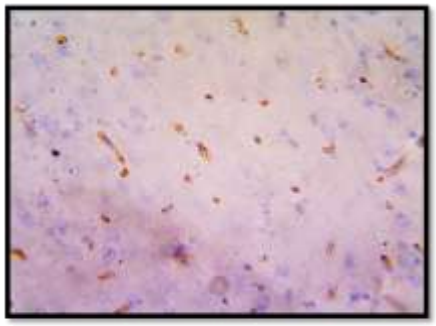
Figure 179: Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Pilocarpine alone group [Group II] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

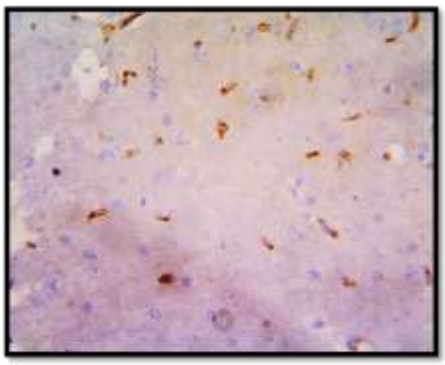
Figure 180: Section of rat brain [**Hypothalamus**] from **PTZ alone group [Group II]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of PTZ + Positive control group [Group III] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

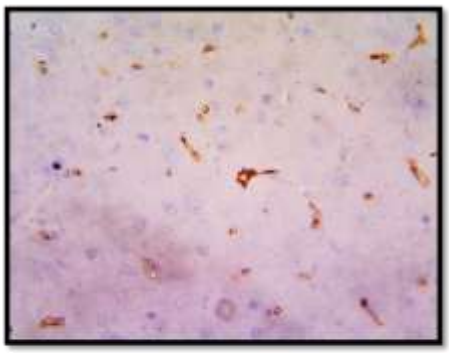
Figure 181: Section of rat brain [**Hypothalamus**] from **PTZ + Sodium valproate [Group III]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of PTZ + Diltiazem group [Group IV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

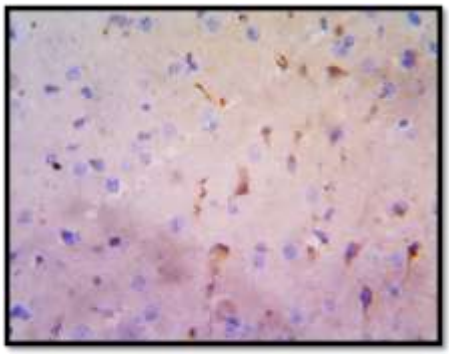
Figure 182: Section of rat brain [**Hypothalamus**] from **PTZ + Diltiazem [Group IV]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of PTZ + Nimodipine group [Group V] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 183: Section of rat brain [**Hypothalamus**] from **PTZ + Nimodipine [Group V]** in **PTZ model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of PTZ + Flunarizine group [Group VI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 184: Section of rat brain [**Hypothalamus**] from **PTZ + Flunarizine [Group VI]** in **PTZ model** (40x; IHC HSP70)

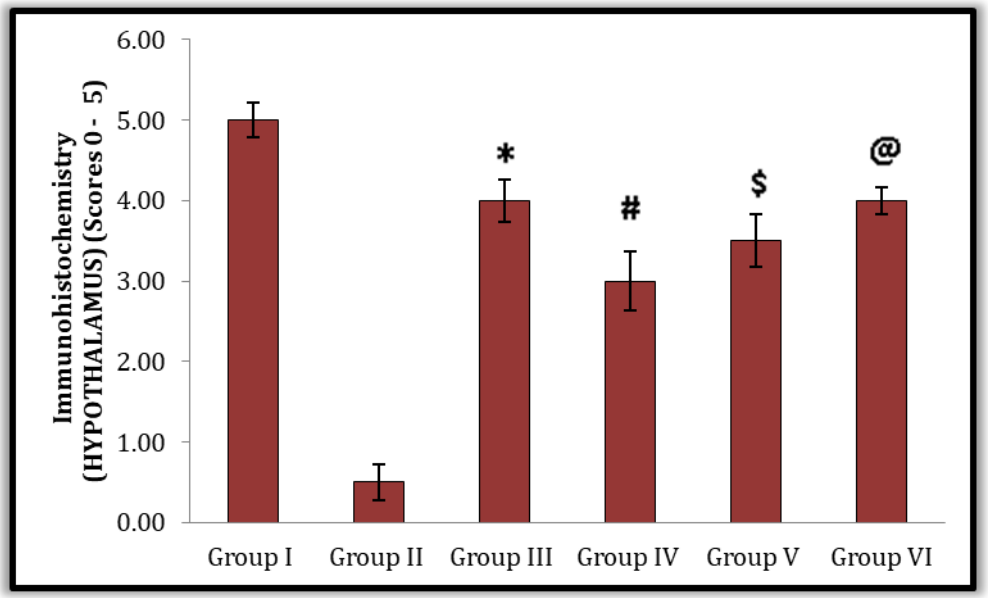


Figure 185: Bar diagram depicting the Immunohistochemistry scores of Hypothalamus in Pentylenetetrazole [PTZ] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hypothalamus were lower in the negative control group [Group I], which was statistically significant (P<0.05). Immunohistochemistry scores in the hypothalamus were shown to be substantially significant in the standard drug group [Group III] and the experimental groups [IV, V and VI - Diltiazem, Nimodipine and Flunarizine] as compared to the negative control group [Group II] (P<0.05).

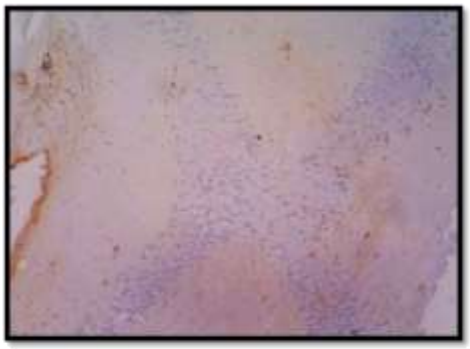
Table 26: Immunohistochemistry scores in PTZ model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
1	Hippocampus (Scores 0 - 5)	5.00±0.21	0.50±0.22	3.50±0.22	3.00±0.21	3.00±0.17	3.50±0.22
2	Prefrontal cortex (Scores 0 - 5)	5.00±0.00	1.00±0.21	4.00±0.17	3.00±0.31	3.00±0.21	4.00±0.17
3	Corpus striatum (Scores 0 - 5)	5.00±0.21	1.00±0.21	4.00±0.17	3.00±0.37	3.00±0.21	4.00±0.21
4	Hypothalamus (Scores 0 - 5)	5.00±0.21	0.50±0.22	4.00±0.26	3.00±0.37	3.50±0.33	4.00±0.17

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The immunohistochemistry scores of the hippocampus, prefrontal cortex, corpus striatum, and hypothalamus were significantly lower in Group II [Negative control group] than in Group I [Vehicle control group] when compared to Group I [Vehicle control group]. Additionally, it was observed that Group I [Standard drug group] and Groups IV, V and VI [experimental drug groups - Diltiazem, Nimodipine and Flunarizine] had considerably higher immunohistochemistry scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus when compared to Group II [Negative control group] (P<0.05).

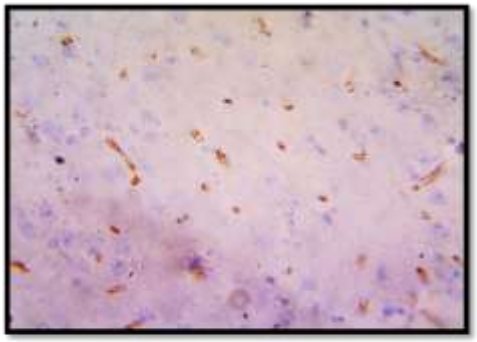
Immunohistochemistry of Hippocampus in various groups of Pilocarpine Model



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

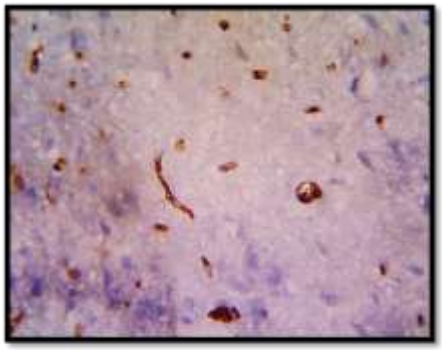
Figure 186: Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **Pilocarpine Model** (10x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of Pilocarpine alone group [Group VII] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.5 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

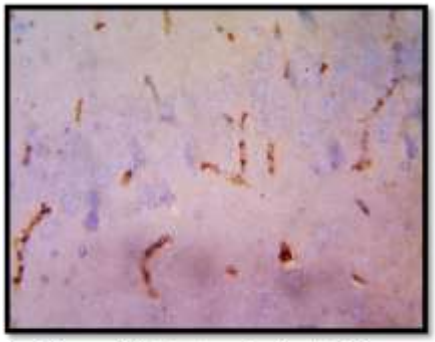
Figure 187: Section of rat brain [**Hippocampus**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine Model** (40x; IHC HSP70)



Section studied from the rat brain [hippocampus] of Pilocarpine + Positive control group [Group VIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.5 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

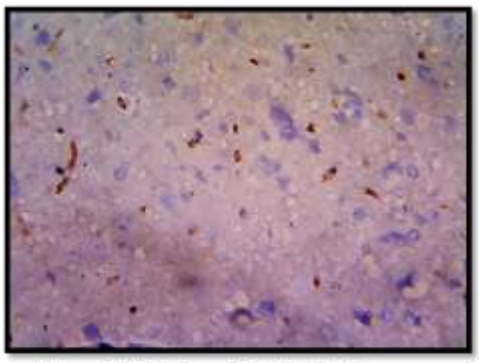
Figure 188: Section of rat brain [**Hippocampus**] from **Pilocarpine + Phenobarbitone [Group VIII]** in **Pilocarpine Model** (40x; IHC HSP70)



Section studied from the rat brain [hippocampus] of Pilocarpine + Diltiazem group [Group IX] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

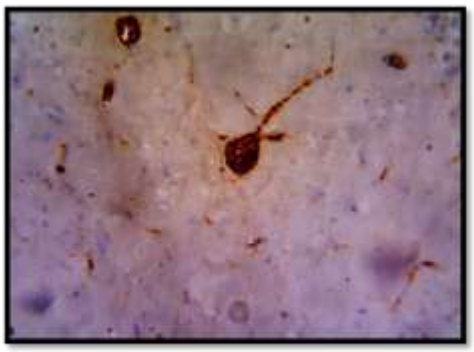
Figure 189: Section of rat brain [**Hippocampus**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine Model** (40x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of Pilocarpine + Nimodipine group [Group X] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.5 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 190: Section of rat brain [**Hippocampus**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine Model** (40x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of Pilocarpine + Flunarizine group [Group XI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 191: Section of rat brain [**Hippocampus**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine Model** (40x; IHC HSP70)

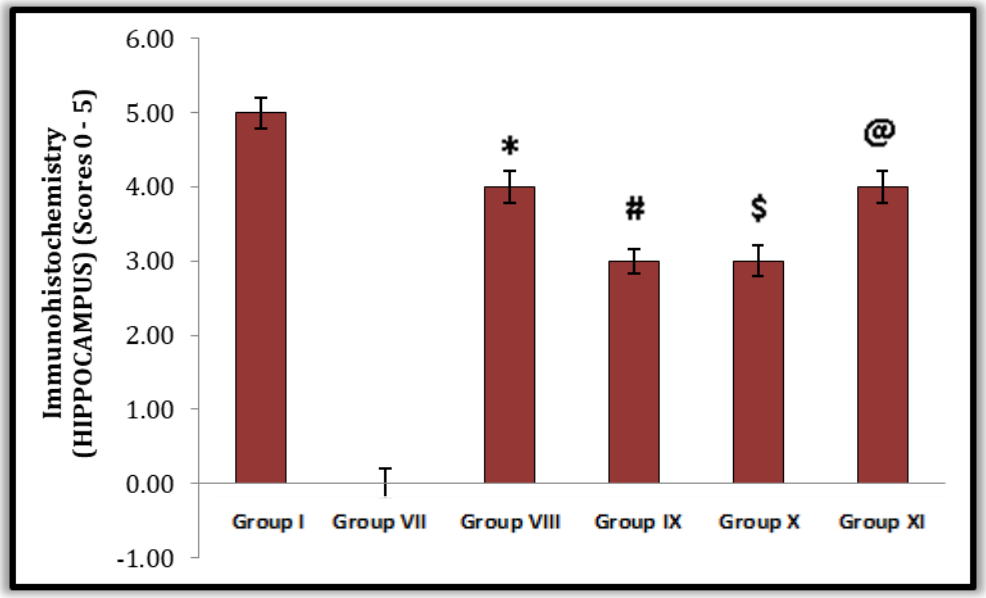
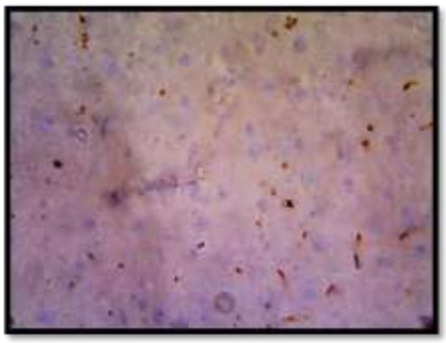


Figure 192: Bar diagram depicting the Immunohistochemistry scores in Hippocampus in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hippocampus were lower in the negative control group [Group VII], which was statistically significant (P<0.05). It was also discovered that when compared to the negative control group [Group VII], the standard drug Phenobarbitone [Group VIII] and the experimental groups [Group IX - Diltiazem, Groups X - Nimodipine and Groups XI – Flunarizine] showed an increase in the immunohistochemistry scores for the hippocampus, which was statistically significant (P<0.05).

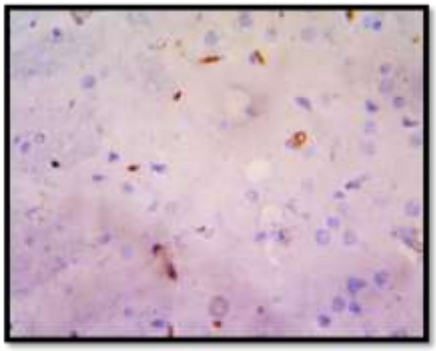
Immunohistochemistry of Prefrontal cortex [Cerebrum] in Pilocarpine Model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

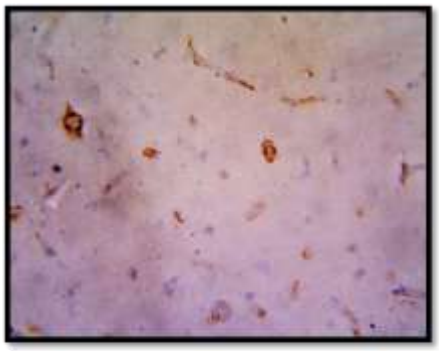
Figure 193: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine alone group [Group VII] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.50 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

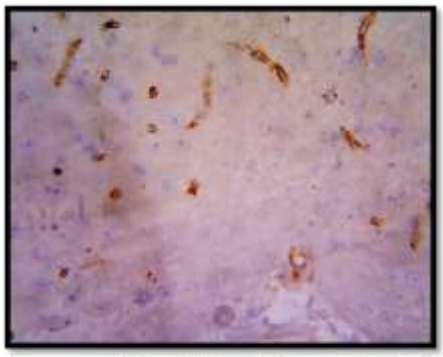
Figure 194: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (10x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Positive control group [Group VIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

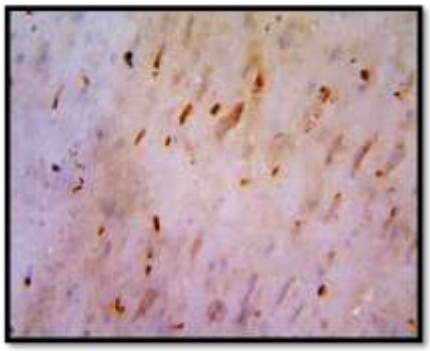
Figure 195: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Phenobarbitone [Group VIII]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Diltiazem group [Group IX] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

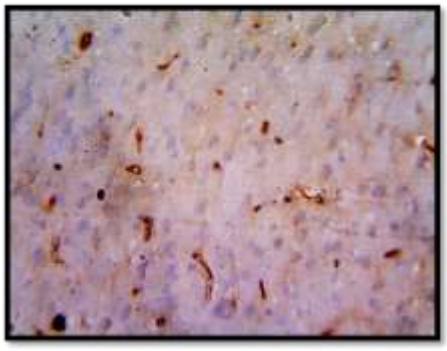
Figure 196: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Nimodipine group [Group X] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 197: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of Pilocarpine + Flunarizine group [Group XI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 198: Section of rat brain [**Prefrontal cortex**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model** (40x; IHC HSP70)

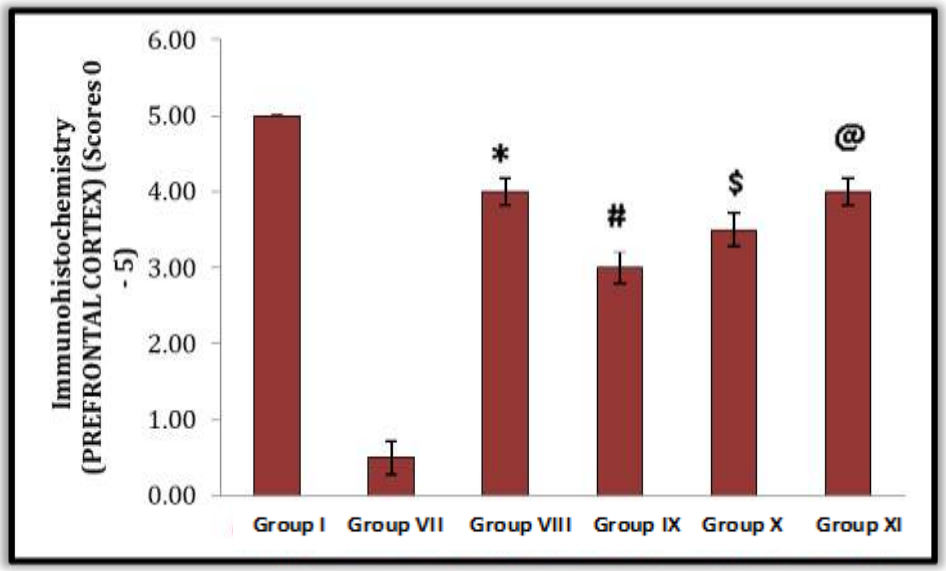
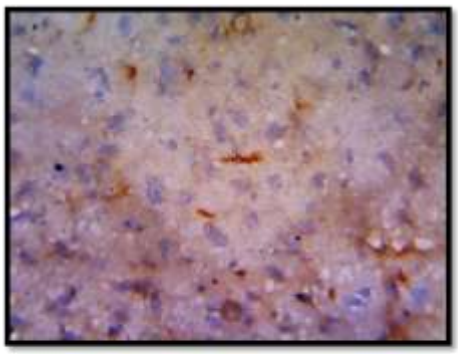


Figure 199: Bar diagram depicting the Immunohistochemistry scores in Prefrontal cortex [Cerebrum] in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], there was a significant (P<0.05) decline in the immunohistochemistry scores for the prefrontal cortex of the negative control group [Group VII]. Similarly, when compared to the negative control group [Group VII], the standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine] showed an increase in the immunohistochemistry scores for the prefrontal cortex that was statistically significant (P<0.05).

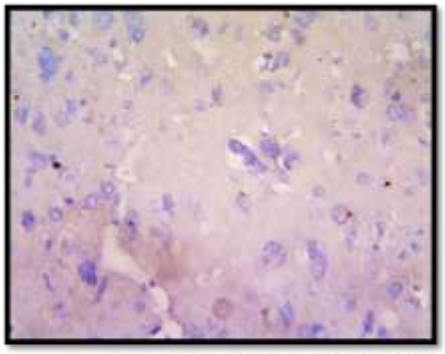
Immunohistochemistry of Corpus striatum in various groups of Pilocarpine Model



Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

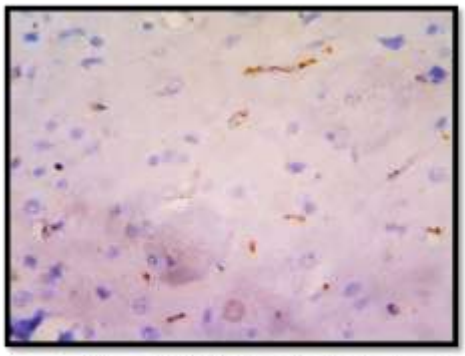
Figure 200: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of Pilocarpine alone group [Group VII] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 201: Section of rat brain [**Corpus striatum**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of Pilocarpine + Positive control group [Group VIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

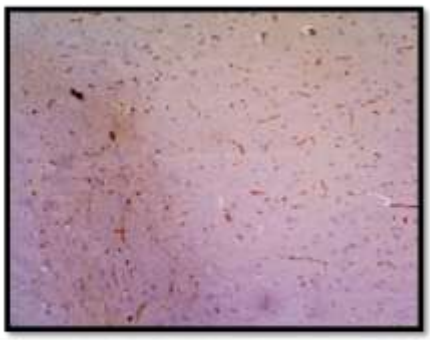
**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 202: Section of rat brain [**Corpus striatum**] from **Pilocarpine + Phenobarbitone [Group VIII]** in **Pilocarpine model** (40x; IHC HSP70)

Section studied from the rat brain [Corpus striatum] of Pilocarpine + Diltiazem group [Group IX] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

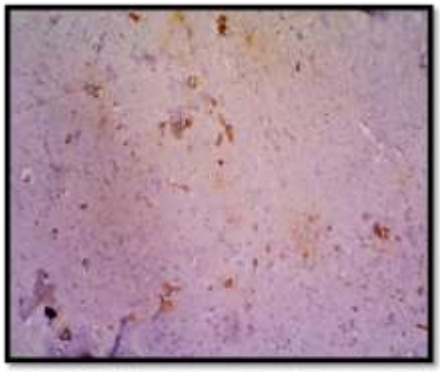
Figure 203: Section of rat brain [**Corpus striatum**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of Pilocarpine + Nimodipine group [Group X] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 204 : Section of rat brain [**Corpus striatum**] from **Pilocarpine + Nimodipine** in **Pilocarpine model [Group X]** (10x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of Pilocarpine + Flunarizine group [Group XI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 205: Section of rat brain [**Corpus striatum**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model** (40x; IHC HSP70)

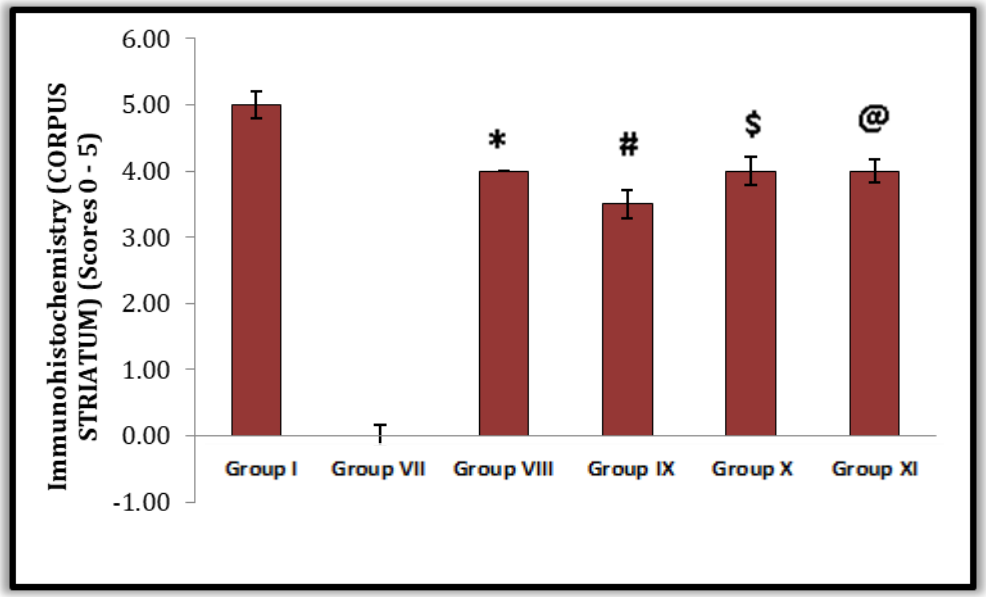
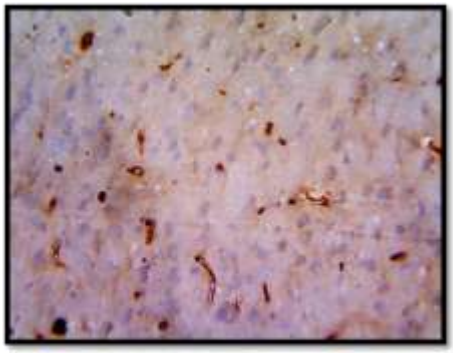


Figure 206: Bar diagram depicting the immunohistochemistry scores of Corpus striatum in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When comparing the negative control group [Group VII] to the vehicle control group [Group I], the immunohistochemistry scores in the Corpus striatum were lower in the negative control group [Group VII], which was statistically significant (P<0.05). When compared to the negative control group [Group VII], there was a significant (P<0.05) increase in the immunohistochemistry scores in the corpus striatum in the standard drug group [Group VIII] and the experimental groups [Group IX - Diltiazem, Group X - Nimodipine and Group XI - Flunarizine].

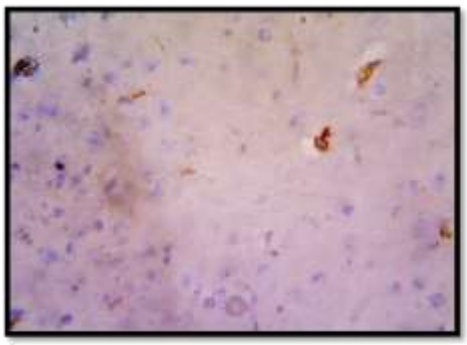
Immunohistochemistry of Hypothalamus in various groups of Pilocarpine Model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

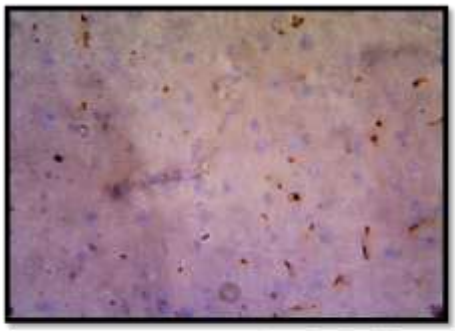
Figure 207: Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Pilocarpine alone group [Group VII] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.50 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

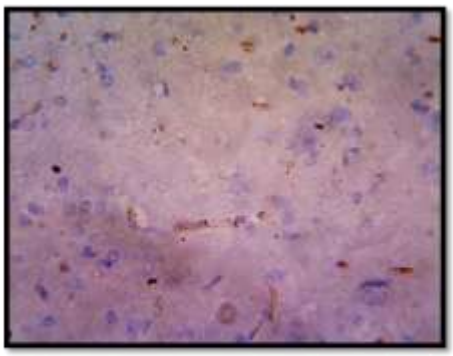
Figure 208: Section of rat brain [**Hypothalamus**] from **Pilocarpine alone group [Group VII]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Positive control group [Group VIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

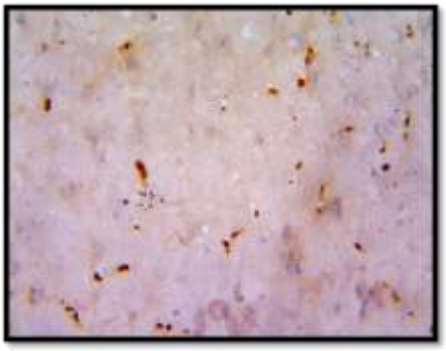
Figure 209: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Phenobarbitone [Group VIII]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Diltiazem group [Group IX] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

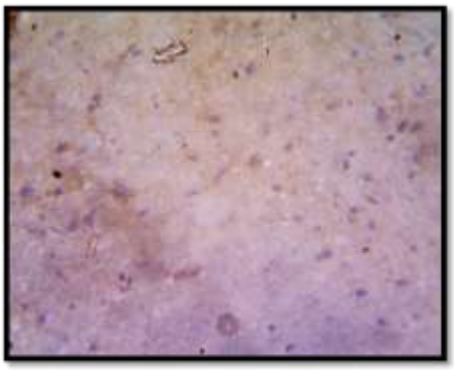
Figure 210: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Diltiazem [Group IX]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Nimodipine group [Group X] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 211: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Nimodipine [Group X]** in **Pilocarpine model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Pilocarpine + Flunarizine group [Group XI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 212: Section of rat brain [**Hypothalamus**] from **Pilocarpine + Flunarizine [Group XI]** in **Pilocarpine model** (40x; IHC HSP70)

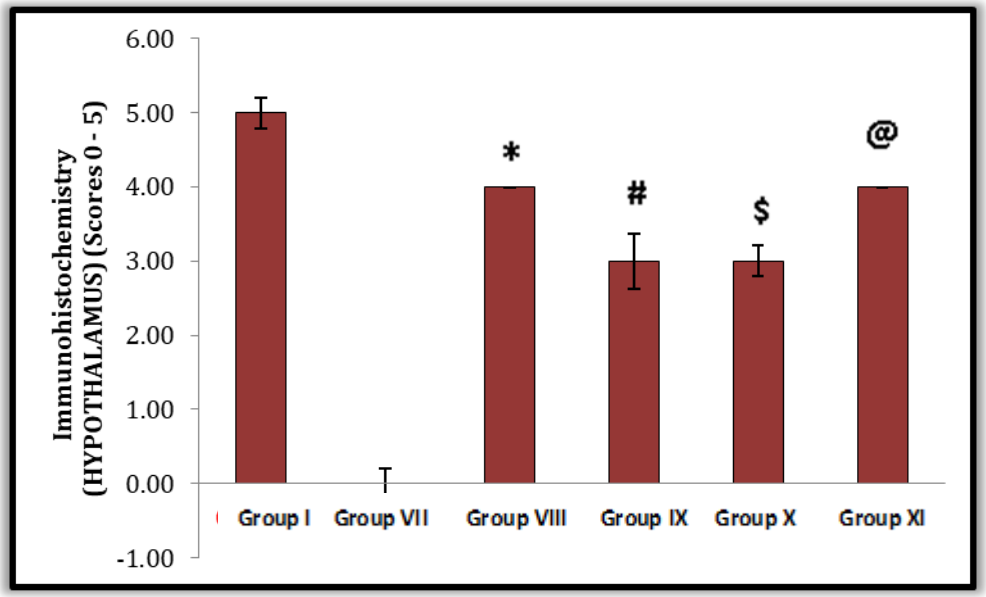


Figure 213: Bar diagram depicting the Immunohistochemistry scores in Hypothalamus in Pilocarpine model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hypothalamus were decreased in the negative control group [Group VII], which was statistically significant (P<0.05). When compared to the negative control group, the standard drug (Phenobarbitone) group [Group VIII] and the experimental groups [Group IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine] revealed an increase in immunohistochemistry scores in the hypothalamus that was statistically significant (P<0.05).

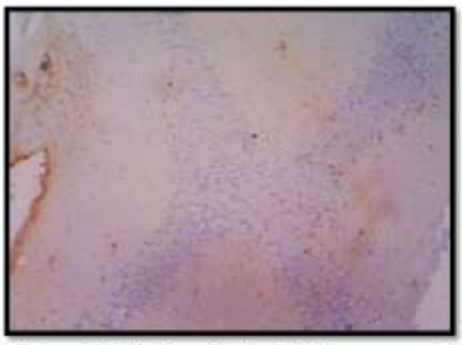
Table 27: Immunohistochemistry scores in Pilocarpine model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group VII	Group VIII*	Group IX#	Group X$	Group XI@
1	Hippocampus (Scores 0 - 5)	5.00±0.21	0.00±0.21	4.00±0.21	3.00±0.17	3.00±0.21	4.00±0.21
2	Prefrontal cortex (Scores 0 - 5)	5.00±0.00	0.50±0.22	4.00±0.17	3.00±0.21	3.50±0.22	4.00±0.17
3	Corpus striatum (Scores 0 -5)	5.00±0.21	0.00±0.17	4.00±0.00	3.50±0.22	4.00±0.21	4.00±0.17
4	Hypothalamus (Scores 0 - 5)	5.00±0.21	0.00±0.21	4.00±0.00	3.00±0.37	3.50±0.21	4.00±0.00

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group VII.

When compared to the vehicle control group [Group VII], immunohistochemistry scores for the hippocampus, prefrontal cortex, corpus striatum, and hypothalamus were significantly (P<0.05) lower in the negative control group [Group VII]. There was also a statistically significant increase in the immunohistochemistry scores of the hippocampus, prefrontal cortex, corpus striatum and hypothalamus in the standard drug group [Group VIII] and in the experimental groups [Groups IX - Diltiazem, Groups X - Nimodipine and Groups XI - Flunarizine] when compared to the negative control group [Group VII].

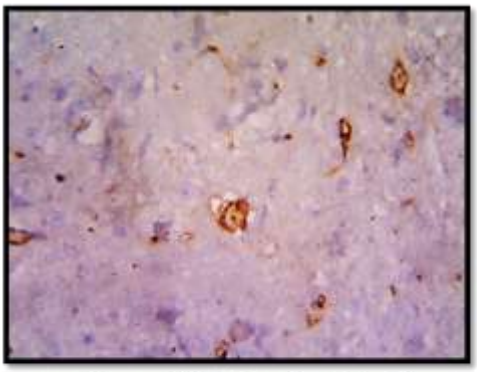
Immunohistochemistry of Hippocampus in various groups of MES Model



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

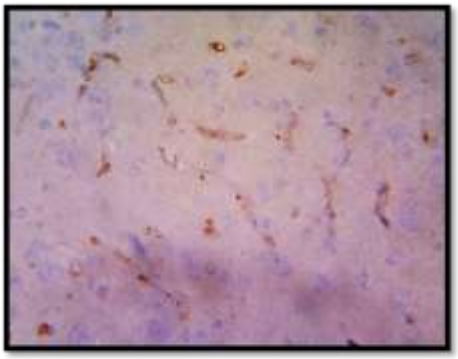
Figure 214: Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **MES model** (10x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of MES alone group [Group XII] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.5 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

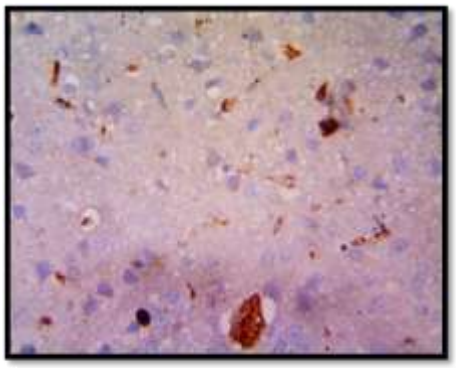
Figure 215: Section of rat brain [**Hippocampus**] from **MES alone group [Group XII]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [hippocampus] of MES + Positive control group [Group XIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.5 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 216: Section of rat brain [**Hippocampus**] from **MES + Diphenyl hydantoin [Group XIII]** in **MES model** (40x; IHC HSP70)

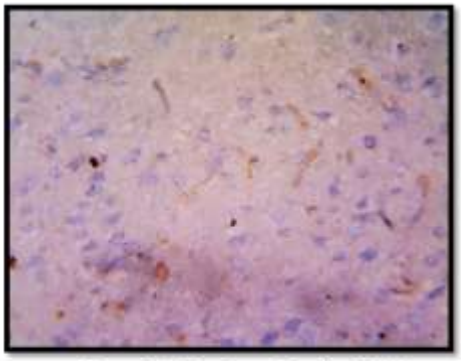


Section studied from the rat brain [hippocampus] of MES + Diltiazem group [Group XIV] showing immunoreactive 1+ with HSP70 amounting to IHC score 1.5 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 217: Section of rat brain [**Hippocampus**] from **MES + Diltiazem [Group XIV]** in **MES model**

(40x; IHC HSP70)

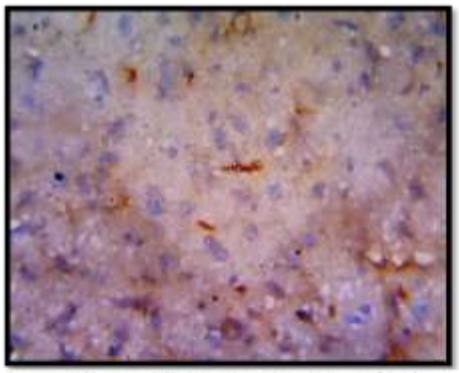


Section studied from the rat brain [Hippocampus] of MES + Nimodipine group [Group XV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 218: Section of rat brain [**Hippocampus**] from **MES + Nimodipine** [Group XV] in **MES model**

(40x; IHC HSP70)



Section studied from the rat brain [Hippocampus] of MES + Flunarizine group [Group XVI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 219: Section of rat brain [**Hippocampus**] from **MES + Flunarizine [Group XVI]** in **MES model**

(40x; IHC HSP70)

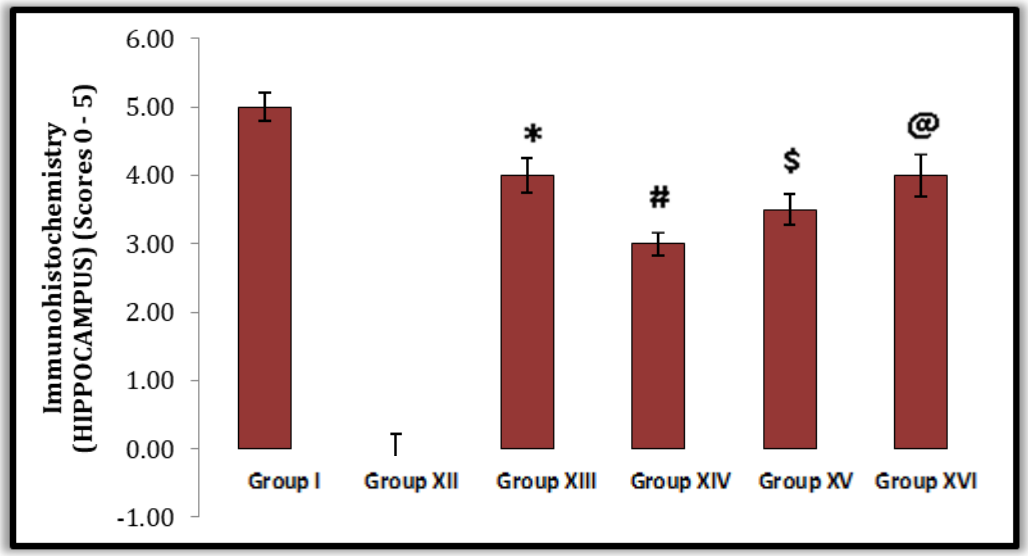
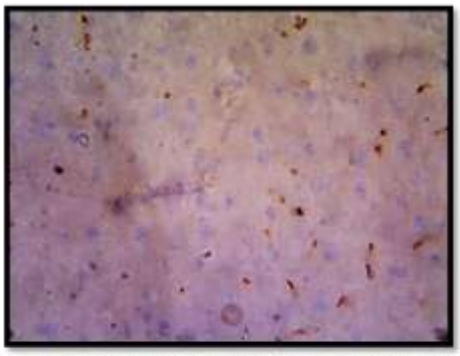


Figure 220: Bar diagram depicting the immunohistochemistry scores in the Hippocampus in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group XII.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hippocampus were considerably ($P<0.05$) lower in the negative control group [Group XII]. The standard drug (Diphenyl hydantoin) group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) both showed an increase in the hippocampus immunohistochemistry scores, which was statistically significant when compared to the negative control group [Group XII] ($P<0.05$).

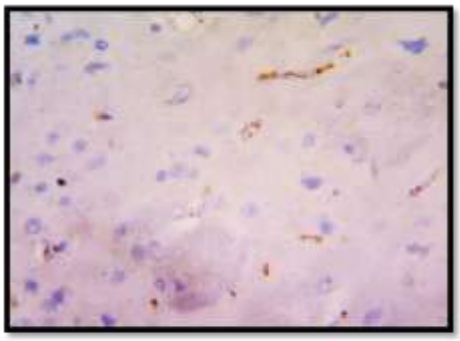
Immunohistochemistry of Prefrontal cortex in various groups of Pilocarpine Model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

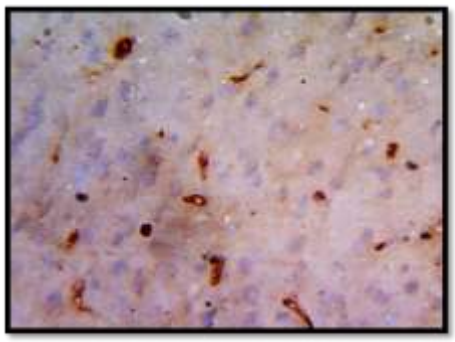
Figure 221: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of MES alone group [Group XII] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

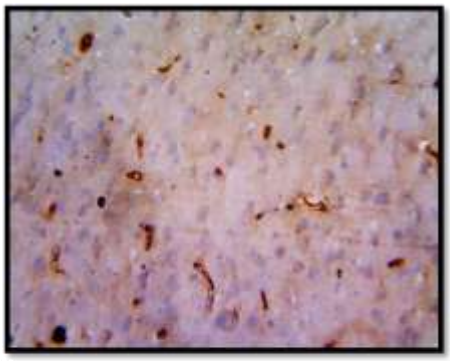
Figure 222: Section of rat brain [**Prefrontal cortex**] from **MES alone group [Group XII]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of MES + Positive control group [Group XIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

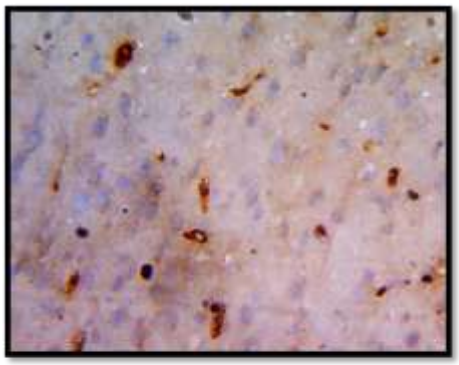
Figure 223: Section of rat brain [**Prefrontal cortex**] from **MES + Diphenyl hydantoin [Group XIII]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of MES + Diltiazem group [Group XIV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

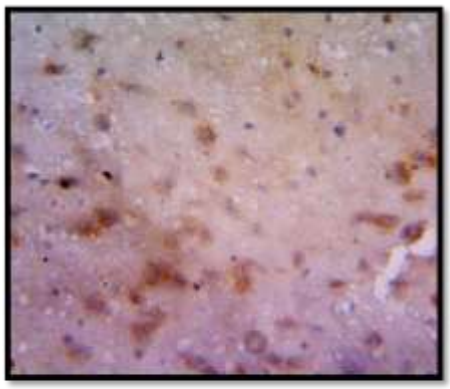
Figure 224: Section of rat brain [**Prefrontal cortex**] from **MES + Diltiazem [Group XIV]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of MES + Nimodipine group [Group XV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 225: Section of rat brain [**Prefrontal cortex**] from **MES + Nimodipine [Group XV]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Prefrontal cortex] of MES + Flunarizine group [Group XVI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 226: Section of rat brain [**Prefrontal cortex**] from **MES + Flunarizine [Group XVI]** in **MES model** (40x; IHC HSP70)

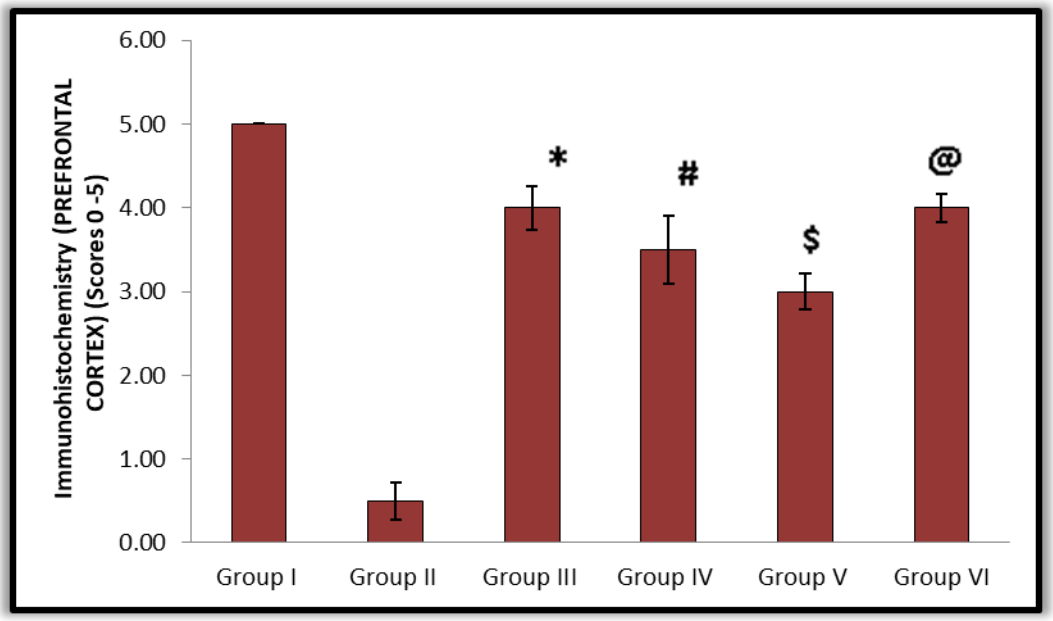
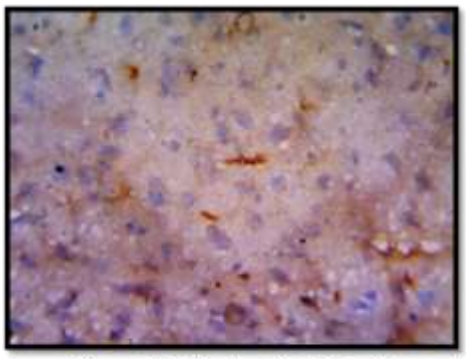


Figure 227: Bar diagram depicting the Immunohistochemistry scores in the Prefrontal cortex in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When comparing the negative control group [Group XII] to the vehicle control group [Group I], the immunohistochemistry scores for the prefrontal cortex [cerebrum] were lower in the negative control group [Group XII], which was statistically significant (P<0.05). It was also found that the standard drug (Diphenyl hydantoin) group [Group XIII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) had higher immunohistochemistry scores in the prefrontal cortex, which were statistically significant when compared to the negative control group [Group XII] (P<0.05).

Immunohistochemistry of Corpus striatum in various groups of MES Model

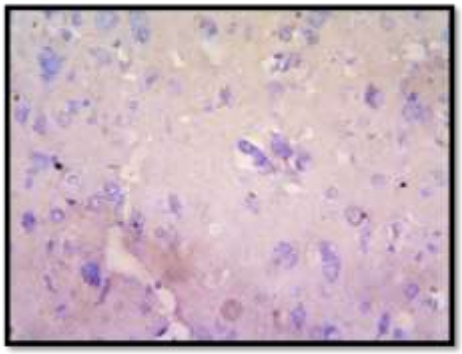


Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 228: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **MES model**

(40x; IHC HSP70)

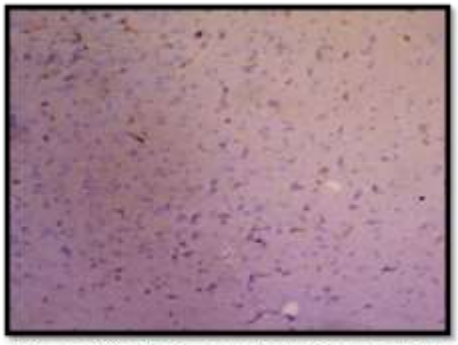


Section studied from the rat brain [Corpus striatum] of MES alone group [Group XII] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 229: Section of rat brain [**Corpus striatum**] from **MES alone group [Group XII]** in **MES model**

(40x; IHC HSP70)

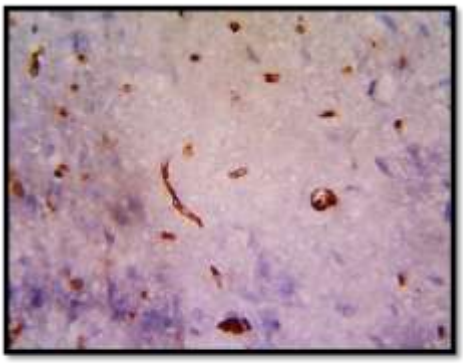


Section studied from the rat brain [Corpus striatum] of MES + Positive control group [Group XIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 230: Section of rat brain [**Corpus striatum**] from **MES + Diphenyl hydantoin [Group XIII]** in **MES model**

(10x; IHC HSP70)

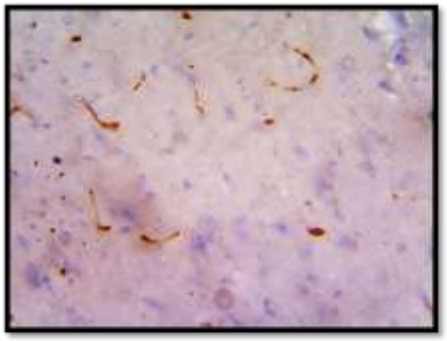


Section studied from the rat brain [Corpus striatum] of MES + Diltiazem group [Group XIV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 231: Section of rat brain [**Corpus striatum**] from **MES + Diltiazem [Group XIV]** in **MES model**

(40x; IHC HSP70)

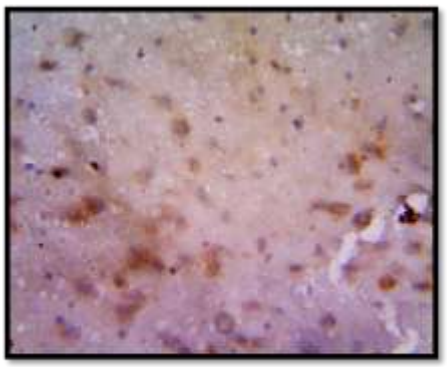


Section studied from the rat brain [Corpus striatum] of MES + Nimodipine group [Group XV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 232: Section of rat brain [**Corpus striatum**] from **MES + Nimodipine [Group XV]** in **MES model**

(40x; IHC HSP70)



Section studied from the rat brain [Corpus striatum] of MES + Flunarizine group [Group XVI] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 233: Section of rat brain [**Corpus striatum**] from **MES + Flunarizine [Group XVI]** in **MES model**

(40x; IHC HSP70)

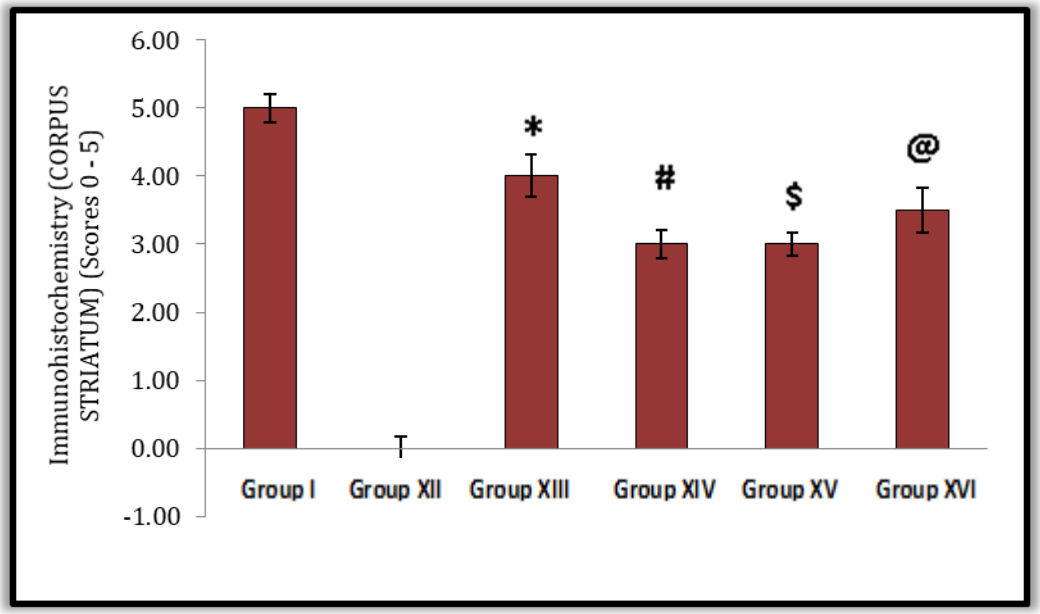
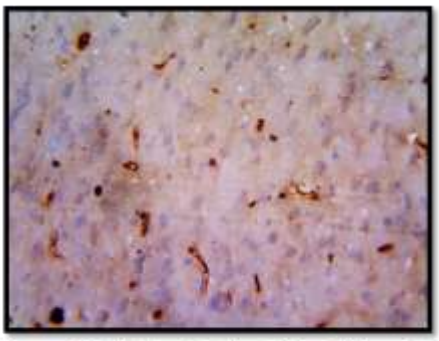


Figure 234: Bar diagram depicting the Immunohistochemistry scores in the Corpus striatum in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the corpus striatum in the negative control group [Group XII] were considerably lower (P<0.05). There was also an increase in immunohistochemistry scores in the corpus striatum in the standard drug group [Diphenyl hydantoin] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI), which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

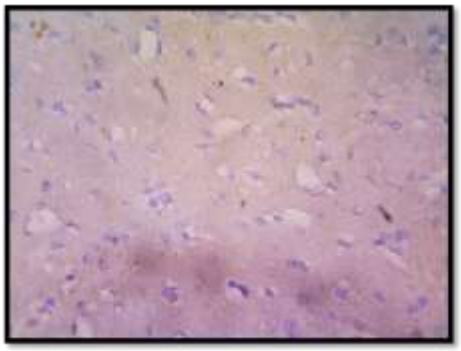
Immunohistochemistry of Hypothalamus in various groups of MES Model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing immunoreactive 1+ with HSP70 amounting to IHC score 5.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

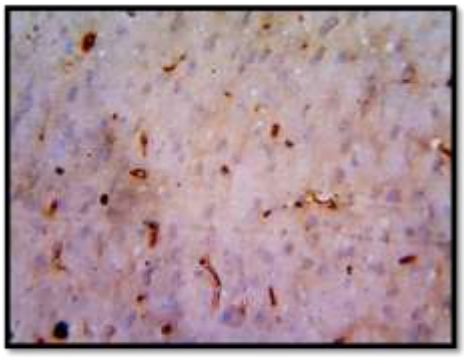
Figure 235: Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of MES alone group [Group XII] showing immunoreactive 1+ with HSP70 amounting to IHC score 0.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

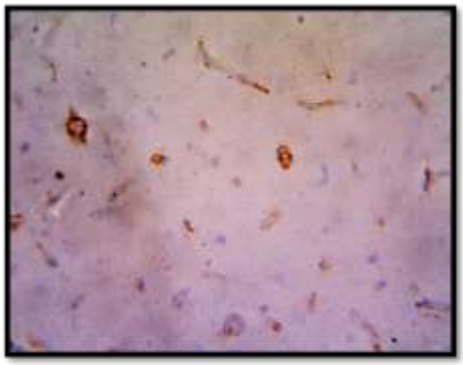
Figure 236: Section of rat brain [**Hypothalamus**] from **MES alone group [Group XII]** in **MES model** (40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of MES + Positive control group [Group XIII] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 237: Section of rat brain [**Hypothalamus**] from **MES + Diphenyl hydantoin [Group XIII]** in **MES model** (40x; IHC HSP70)

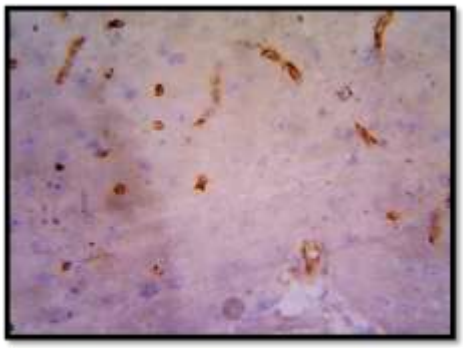


Section studied from the rat brain [Hypothalamus] of MES + Diltiazem group [Group XIV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 238: Section of rat brain [Hypothalamus] from **MES + Diltiazem [Group XIV]** in **MES model**

(40x; IHC HSP70)

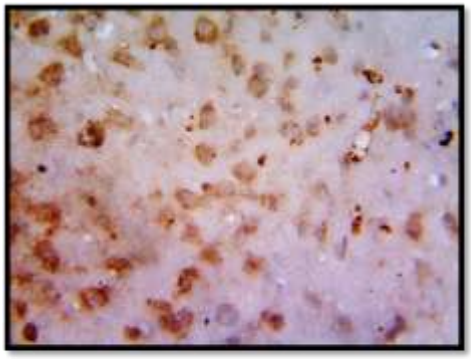


Section studied from the rat brain [Hypothalamus] of MES + Nimodipine group [Group XV] showing immunoreactive 1+ with HSP70 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 239: Section of rat brain [**Hypothalamus**] from **MES + Nimodipine [Group XV]** in **MES model**

(40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of MES + Flunarizine group [Group XVI] showing immunoreactive 1+ with HSP70 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 240: Section of rat brain [**Hypothalamus**] from **MES + Flunarizine [Group XVI]** in **MES model**

(40x; IHC HSP70)

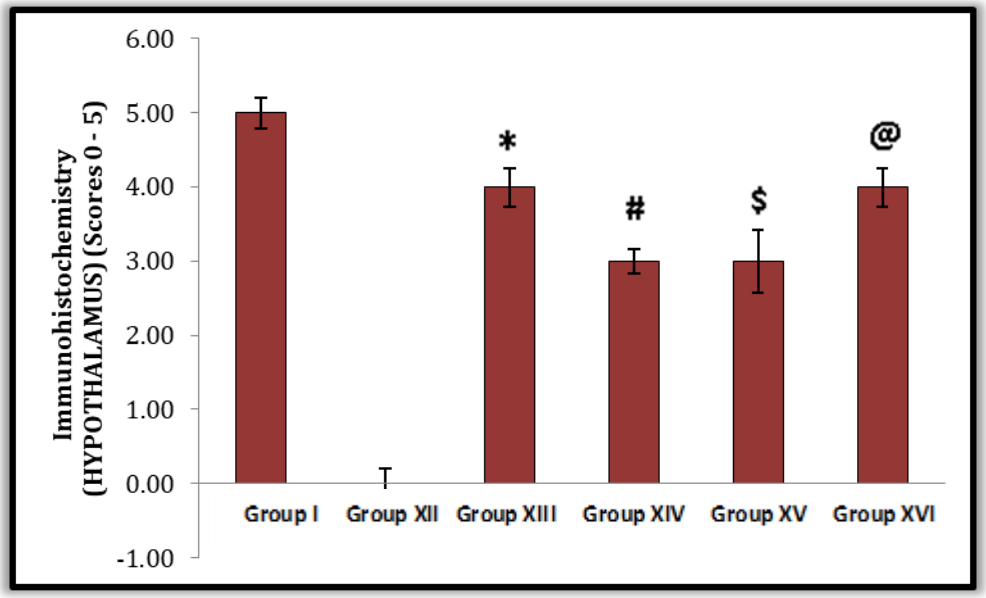


Figure 241: Bar diagram depicting the Immunohistochemistry scores in the Hypothalamus in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hypothalamus in the negative control group [Group XII] were considerably (P<0.05) lower. There was also a statistically significant increase in the immunohistochemistry scores for the hypothalamus in the standard drug group [Diphenyl hydantoin] and the experimental groups [Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI] when compared to the negative control group [Group XII] (P<0.05).

Table 28: Immunohistochemistry scores in Maximal Electroshock Seizure [MES] model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group XII	Group XIII*	Group XIV#	Group XV$	Group XVI@
1	Hippocampus (Scores 0 - 5)	5.00±0.21	0.00±0.21	4.00±0.26	3.00±0.17	3.50±0.22	4.00±0.31
2	Prefrontal cortex (Scores 0 - 5)	5.00±0.00	0.50±0.22	4.00±0.26	3.50±0.40	3.00±0.21	4.00±0.17
3	Corpus striatum (Scores 0 - 5)	5.00±0.21	0.00±0.17	4.00±0.31	3.00±0.21	3.00±0.17	3.50±0.33
4	Hypothalamus (Scores 0 - 5)	5.00±0.21	0.00±0.21	4.00±0.26	3.00±0.17	3.00±0.42	4.00±0.26

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group XII.

The study findings indicated that when the negative control group [Group XII] was compared to the vehicle control group [Group I], the immunohistochemistry scores in the hippocampus, prefrontal cortex, corpus striatum, and hypothalamus fell significantly (P<0.05). Additionally, it was observed that both the standard drug group - Diphenyl hydantoin [Group XII] and the experimental groups (Diltiazem - Group XIV, Nimodipine - Group XV and Flunarizine - Group XVI) enhanced immunohistochemistry scores for the hippocampus, prefrontal cortex, corpus striatum and hypothalamus which was statistically significant when compared to the negative control group [Group XII] (P<0.05).

DISCUSSION

DISCUSSION

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

There are several advantages of calcium channel blockers over the currently available antiepileptic drugs, which include no enzyme induction or inhibition properties, devoid of sedation, safe during pregnancy, minimal effect on quality of life, wide therapeutic range, even best suited for the elderly individuals, stroke preventive role, and a preventive role in hypertensive or diabetic nephropathy.

Among the various calcium channel blockers, diltiazem, nimodipine, and flunarizine had been chosen and their anti-epileptic activity was evaluated using PTZ, Pilocarpine and MES animal models.

In case of PTZ induced convulsion, the result of the present study showed that the flunarizine showed an increase in the recovery time when compared with diltiazem and flunarizine. PTZ may cause convulsions by decreasing GABA receptor activation at GABAa receptors.

The effect of diltiazem, nimodipine, and flunarizine on mean latency period was determined on the pilocarpine induced animal model. Among the three CCBs, the flunarizine gave a 90% protection compared to the mean latency periods of diltiazem and nimodipine. Similarly, when the total protein was estimated, the results showed matching with the control groups when compared with other two drugs.

The decrease in the lipid peroxidation level and increase in the glutathione level in the pilocarpine induced convulsion models indicated that the flunarizine exhibited good antioxidant activity compared with the nimodipine and the flunarizine. The grand mal type of epilepsy was represented by the maximum electroshock generated convulsion in the animals. The drugs that are helpful in generalised tonic-clonic seizures specifically eliminate the tonic extensor phase.[19] The result of the present study showed that the recovery time of diltiazem was increased when compared with other two drug groups [nimodipine and flunarizine].

Oxidative stress was described as an imbalance between generation and elimination of reactive oxygen and reactive nitrogen species. The brain is particularly susceptible to oxidative stress because it utilizes the highest amount of oxygen than other body organs. It has been postulated that the lipid peroxidation may be casually associated with the certain types of epilepsy. A decrease in the free radical scavenging activity may lead to an increased risk of seizure recurrence.[58] The effect of diltiazem, nimodipine and flunarizine on oxidative stress in MES and PTZ induced convulsion was evaluated.

Glutathione reductase is an important free radical scavenging compound that prevents membrane lipid peroxidation. The lower level of reduced glutathione in the control group in this study indicated that there was a higher production of free radicals and that reduced glutathione was depleted during the oxidative stress response.[58] Flunarizine (10 mg/kg dose) showed a significant increase in the GSH levels in the brain tissue. The decrease in the lipid peroxidation level and the increase in the glutathione level in the PTZ and MES induced convulsion models indicated that the flunarizine exhibited a good antioxidant activity compared with diltiazem and nimodipine.

Epilepsy may develop because of an imbalance of nerve signaling chemicals called neurotransmitters. In case of epilepsy, there may be abnormally high level of excitatory neurotransmitter (glutamate) that increases the neuronal activity, while abnormally low level of inhibitory neurotransmitter (GABA) which increases the neuronal activity in the brain. Hence, the GABA hypoactivity and the glutamate hyperactivity can enhance the epileptic seizure. In epileptic foci, the GABA hypoactivity, which reduces the activity of dopaminergic neurons through a presynaptic effect through GABAa receptors. The Glutamate hyperactivity is exerted through presynaptic N- methyl-D-aspartate receptors, which strongly inhibit serotonergic neurons and it induces epileptic seizures through post synaptic ionotropic glutaminergic receptors.[15] The results of the present study showed that the above three calcium channel blockers significantly showed an increase in the level of inhibitory neurotransmitter GABA, and also showed a significant increase in the levels of DA and serotonin when compared to the control group of PTZ, Pilocarpine and MES induced epilepsy animal models.

The microanatomical study showed an alteration in the histopathological picture of neuronal tissue in PTZ, Pilocarpine and MES [negative control groups only] treated groups compared to the standard and three test drugs (diltiazem, nimodipine and flunarizine). Moreever, this histopathological evaluated results indicated that the flunarizine have good anticonvulsant activity compared with diltiazem and nimodipine.

Pentylenetetrazole (PTZ) Model:

In our study, the results showed that, the onset, duration, number and scores of seizures in experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group ($P<0.01$). All these parameters in

the experimental test drug groups were also comparable to the standard drug with P>0.05. The onset of seizures was significantly increased in the experimental test drug groups in comparison with the control group (P<0.05). The duration of the seizures was significantly decreased in the experimental test drug Groups in comparison to the control group (P<0.001). The number of seizures was also decreased in the experimental test drug groups in comparison with the control group which was found to be statistically significant (P<0.01). In this study, it was also seen that, the scores of seizures were significantly reduced in the experimental test drug groups when compared to the control group (P<0.001).

The oxidative stress markers "Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Catalase and Lipid peroxidation (LPO)" in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group (P<0.001). All the above oxidative stress markers in the experimental test drug groups were also comparable with the standard drug and the vehicle control group with P>0.05. Our study showed that, SOD, GPx, GSH and Catalse were significantly increased in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared with the control group (P<0.001). When compared to the control group, LPO levels were lower in the experimental test drug groups (Diltiazem, Nimodipine, and Flunarizine), and this was shown to be statistically significant (P<0.05).

The neurotransmitters Serotonin (5-HT), Dopamine (DA), GABA, Glutamate and Acetylcholine (ACh), in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group (P<0.0001). All the above neurotransmitters in the experimental test drug groups were also comparable to the standard drug and the vehicle control group with P>0.05. Our

study showed that, 5-HT, DA and GABA were significantly increased in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group ($P<0.01$). Glutamate and Ach levels were also shown to be lower in the experimental test drug groups (Diltiazem, Nimodipine, and Flunarizine) when compared to the control group in the study, which was statistically significant ($P<0.001$).

The histopathological [microanatomy] examination of different areas of the brain [Hippocampus, Prefrontal cortex [Cerebrum], Corpus striatum [Basal nuclei] and Hypothalamus], the scoring was in favour of restoring the microanatomical changes of brain in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared with the control group.

In our study, immunohistochemistry (IHC) with HSP-70, the scores were in favour of neuroprotection in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine respectively) when compared to the control group.

Pilocarpine Model:

In this model, our study revealed the same results that of in PTZ model that the onset, duration, number and scores of seizures in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were found to be significantly different from the control group ($P<0.05$). All the above parameters in the experimental test drug groups were comparable to the standard drug group with $P>0.05$. Similarly, in the Pilocarpine model, the onset of seizures was significantly increased in the experimental test drug groups in comparison with the control group ($P<0.05$). When compared to the control group, the test drug groups demonstrated a substantial reduction in the duration of seizures ($P<0.0001$). In addition, the number of seizures was reduced in the experimental test drug groups compared to the control

group, which was statistically significant ($P<0.05$). It was also discovered that the experimental test drug groups had significantly lower seizure scores than the control group ($P<0.01$)

This study results with regard to assays of oxidative stress markers - Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Catalase and Lipid peroxidation (LPO) in Pilocarpine model, the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group ($P<0.01$). All the above oxidative stress markers in the experimental test drug groups were also comparable to the standard drug and the vehicle control group with $P>0.05$. This study revealed that, the SOD, GPx, GSH and Catalase were significantly increased in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group ($P<0.05$). But was observed that, the LPO levels were reduced in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group which was found to be statistically significant ($P<0.01$).

In this study of pilocarpine model, the neurotransmitters Serotonin (5-HT), Dopamine (DA), GABA, Glutamate and Acetylcholine (ACh), the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group ($P<0.05$). All the above neurotransmitters in the experimental test drug groups were comparable to the standard drug and the vehicle control group with $P>0.05$. Our study highlighted that, 5-HT, DA and GABA were significantly increased in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine respectively) when compared to the control group ($P<0.001$). But it was seen that, the Glutamate and Ach level were reduced in the experimental test drug

groups (Diltiazem, Nimodipine and Flunarizine respectively) when compared to the control group and this was found to be statistically significant ($P<0.05$).

The histopathological [microanatomy] examination of different areas of the brain [Hippocampus, Prefrontal cortex [Cerebrum], Corpus striatum[Basal nuclei] and Hypothalamus] with scoring was in favour of restoring the microanatomical changes of brain in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared with the control group.

In the present study of immunohistochemistry (IHC) – Hsp-70, the scores were in favour of neuroprotection in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group.

Maximal Electroshock Seizure (MES) Model:

The results of our study in this model highlighted that, the scores of seizures in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly decreased when compared to the control group ($P<0.001$). The decline in the scores of seizures in the experimental test drug groups were comparable to the standard drug ($P>0.05$). The percentage of protection against Tonic Hind Limb Extension (THLE) in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were comparable to the standard drug group ($P>0.05$). It was seen that none of the animals in the control group were protected against THLE.

In MES model, the similar results of that were observed in other two models [PTZ & Pilocarpine] were observed with respect to the oxidative stress markers namely Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Catalase and Lipid peroxidation (LPO) in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) that were significantly different from the control group ($P<0.05$) and these oxidative stress markers in the

experimental test drug groups were in comparable to the standard drug and the vehicle control group with P>0.05. It was observed that, the SOD, GPx, GSH and Catalase were significantly increased and LPO levels were decreased in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group (P<0.05).

The neurotransmitters results in the MES model, Serotonin (5-HT), Dopamine (DA), GABA, Glutamate and Acetylcholine (ACh), in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group (P<0.0001). All the above neurotransmitters in the experimental test drug groups were also comparable to the standard drug and the vehicle control group with P>0.05. Our study highlighted that, the 5-HT, DA and GABA were significantly increased in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group (P<0.05). But it was also found that, the Glutamate and ACh level were reduced in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared with the control group and this was found to be statistically significant (P<0.01).

The histopathological [microanatomy] examination of different areas of the brain [Hippocampus, Prefrontal cortex (Cerebrum), Corpus striatum (Basal nuclei) and Hypothalamus] with the scoring was in favour of restoring the microanatomical changes of brain in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group.

In the present study the hippocampus, prefrontal cortex of the cerebrum, the corpus striatum and hypothalamus showed edema and gliosis after PTZ alone administration. It had also produced vacuolations, neuronal degeneration, gliosis, sclerosis, and the appearance of pyknotic cells in the prefrontal cortex of the cerebrum

and the hippocampus. Many immature granular cells with pale nuclei, vacuolations around the cells, astrogliosis, and oedema were detected in the cerebrum and hippocampus in the Pilocarpine model without pretreatment with the test drugs.

Histopathological lesions such as mild gliosis, sclerosis, vacuolations, and edoema (in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus), apoptic cells, astrocyte variations, pale granular cells, glial pale bodies (in the cerebrum and corpus striatum), hyperchromatic and pyknotic nuclei (in the cerebrum and corpus striatum). However, when compared to the toxin induced rat brains without pre-treatment, the general neuronal architecture was not significantly altered in the pre-treated groups. The anticonvulsive activity, oxidative stress marker levels, and neurotransmitter levels were all in line with the protecting the neurons against the chemicals used to induce epilepsy.

Eventhough the histopathological evaluation of epilepsy animal models pretreated with diltiazem, nimodipine and flunarizine in the current study showed mild cerebral edema, gliosis and vacuolated cells, it was found to be not significant.

Based on these findings of our histopathological analysis, we concluded that the calcium channel blockers were neuroprotective and useful in the treatment of epilepsy in chemical/electrical induced animal models.

HSP-70 immunohistochemistry is a good indicator of anti-apoptosis. HSP-70 is routinely expressed in the brain tissue. In the current study, flunarizine showed a significant anti-apoptotic property (evidenced by HSP-70 immunohistochemistry) in the PTZ and pilocarpine model. In the PTZ model, nimodipine and diltiazem also had significant anti-apoptotic properties (as shown by HSP-70 immunohistochemistry).

Thus in immunohistochemistry (IHC), with HSP-70 marker, the scores were in favour of neuroprotection in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group.

All the parameters in PTZ, Pilocarpine and MES models, the experimental test drugs (Diltiazem, Nimodipine and Flunarizine) were comparable to the standard drug(s). The experimental test drugs were able to restore the oxidative stress enzymes and the neurotransmitters induced by the PTZ, Pilocarpine and MES. In the literature it was found that, the studies done by Waldbaum et. Al., Aguiar et al., Folbergrova et al. concluded that, pathophysiological basis behind causation of seizures is oxidative stress.[13-16]

Similarly, studies done by Theoharides et. al., Friedman et al., Vezzani et al., Galic et al. shown that, one of the pathophysiological basis behind the causation of seizures is neuroinflammation.[3-12] Epilepsy may develop because of an imbalance in the nerve signaling chemicals called neurotransmitters. In case of epilepsy, there may be abnormally high level of excitatory neurotransmitter (glutamate) that increases the neuronal activity, while abnormally low level of inhibitory neurotransmitter (GABA) that increase the neuronal activity in the brain. Hence, the GABA hypoactivity and the glutamate hyperactivity can enhance the epileptic seizure.[1,2] In epileptic foci, the GABA hypoactivity, which reduces the activity of dopaminergic neurons through a presynaptic effect through $GABA_a$ receptors. The Glutamate hyperactivity is exerted through a presynaptic N–methyl–D-aspartate receptors, which strongly inhibit the serotonergic neurons through the post synaptic ionotropic glutaminergic receptors, which can induce the epileptic seizures.[1,2]

Calcium channel blockers[1,2,17-21,26-33,40,62-66] do have antioxidant, anti-inflammatory and some with promising anticonvulsant activities in few animal

models and have better safety profile when compared to the current available antiepileptic drugs.

There are many studies[3-12] supporting the role of neuroinflammation and immune mediated insults in the seizure initiation and the process of epileptogenesis. The experimental studies in rodents have proved that the neuroinflammation can lead to increase in the permeability of blood brain barrier and excitability of nervous tissue.[7]

Various experimental studies[13-16] have described the role of oxidative stress in the epileptogenesis process. The mitochondrial related oxidative stress plays an important role in the epileptogenesis in the lithium-pilocarpine model of temporal lobe epilepsy in the animals.[13] Antioxidants can reduce the neuronal injury caused due to the oxidative stress and hence, further development of seizures in some animal models.[14]

Calcium channel blockers [CCBs] are often given for the treatment of hypertension, angina, and cardiac arrhythmias by inhibiting L-type and T-type calcium channels found in different tissues, including the brain.[17] There are three important classes of calcium channel blockers which include phenyl alkylamine [verapamil], benzothiazepine [diltiazem] and dihydropyridines [nifedipine, felodipine, amlodipine, nitrendipine, nimodipine, lacidipine, lercanidipine and benidipine].[17] Phenyl alkylamine and benzothiazepine class of CCBs are clinically used in arrhythmias whereas dihydropyridines are preferred in angina and hypertension.[17] The advantages of CCBs include that, they can be safely administered to the patients with chronic obstructive lung diseases and peripheral vascular diseases. Other clinical uses of CCBs include in the treatment of hypertrophic cardiomyopathy, premature labour, migraine and the nocturnal cramps.[17]

Calcium channel blockers have shown to possess the antiinflammatory[18,19] and antioxidant[20,21] properties in various experimental animal models. There are several advantages of calcium channel blockers over the currently available antiepileptic drugs, which include no enzyme induction or inhibition properties, devoid of sedation, safe during pregnancy, minimal effect on quality of life, wide therapeutic range, best suited even for the elderly individuals, stroke preventive role, and the preventive role in the hypertensive or diabetic nephropathy.[17]

A study was done by Kalita et al.[22] in swiss albino mice to evaluate the microanatomical injuries due to administration of pentylenetetrazol. The study showed that the pentylenetetrazol caused the neuronal injuries like meningeal cellular damage, loss of gray matter, presence of pyknotic cells and the presence of huge number of reactive astrocytes on histopathological examination.

Another study carried by Borges et al.[23] in the mouse pilocarpine model exhibited the hippocampal sclerosis, complete loss of hilar neurons, varied death of pyramidal cells, mossy fiber sprouting, astrogliosis and the thalamic axonal death on histoanatomical characterization of mouse brain tissue. Jandova et al.[24] also found the neurological insult specially in the hippocampal and the dentate gyrus regions of the brain on single dose administration of pentylenetetrazol [60 mg/Kg] in immature rats. A review done by Sendrowski et al.[25] on microanatomical changes in the human brain in temporal lobe epilepsy include the hippocampal sclerosis which is characterized mainly by the glial reaction, abnormal proliferation of interneuron networks and the selective loss of pyramidal neurons in CA1 and CA3 regions.

A study conducted by Hadizadeh et al.[26] to evaluate the anticonvulsant activity of two novels 4-[1-(4-fluorobenzyl)-5-imidazolyl] dihydropyridine derivatives in the mice using pentylenetetrazole and electroshock seizure models by using nifedipine,

phenytoin and sodium valproate as positive controls. Their study showed that, the two dihydropyridine derivatives had a significant anticonvulsant activity in both the experimental animal models. Another study by Pattan et al.,[40] to evaluate the anticonvulsant activity of new substituted dihydropyridine derivatives in the rats using pentylenetetrazole, strychnine and electroshock seizure models also confirmed the significant anticonvulsant properties of these drugs.

A study was done at Department of medicinal chemistry and toxicology, Tehran University, Tehran [Iran] by Shafiee et al.,[63] to study the anticonvulsant activities of alkyl, arylalkyl and cycloalkyl ester analogues of nifedipine in pentylenetetrazole induced seizures in the mice. The results of their study concluded that, the alkyl, arylalkyl and cycloalkyl ester analogues of nifedipine had significant effect in reducing the latency and the duration of seizures.

Ullal et al.,[35] at M. S. Ramaiah Medical College [India] performed the experiment to evaluate the anticonvulsant effect of nifedipine on the hyperthermic induced seizures in rats. The author placed the rats in hot water at 55^0C for about 10 minutes for the seizure induction. In this study of them, diphenylhydantoin and phenobarbitone were used. The study inferred that, both the nifedipine and the diphenylhydantoin did not prevent the hyperthermic seizures in the animals. However, phenobarbitone completely suppressed the hyperthermic seizures in the rats.

In Swiss albino mice, a study[64] was conducted to assess the anticonvulsant effect of two calcium channel blockers, verapamil and nifedipine. Convulsions were generated in swiss albino mice by administering strychnine at a dose of 1 mg/kg through the intraperitoneal route in this study. When compared to the control group, both calcium channel blockers extended the onset of seizures in the rats, and this was determined to be statistically significant [$P<0.05$]. Their research also found that both

verapamil and nifedipine- calcium channel blockers, provided 100% protection against strychnine-induced death.

Khobragade et al.[31] did a study on wistar albino rats to evaluate the anticonvulsant effect of flunarizine using maximal electroshock seizure and the pentylenetetrazole models. This study of them found that, the flunarizine had a significant anticonvulsant activities in both the above models when it was used alone as well as used in potentiating the effects of the standard antiepileptic drugs when used in combination.

Some calcium channel blockers, such as flunarizine, nifedipine, nicardipine, nimodipine, nitrendipine, and diltiazem, were found to have considerable anticonvulsant effect in DBA/2 mice in a study.[33] Convulsions were generated in experimental animals by auditory stimulation [109 db] in their study. Verapamil, on the other hand, failed to show substantial anticonvulsant activity in an audiogenic seizure test in DBA/2 mice in this investigation.

Amano et al.[34] evaluated the anticonvulsant activity of a novel L-type calcium channel blocker [methyl-4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)-thieno-[2,3-b]pyridine-5-carboxylate)] in the genetic model of spontaneously epileptic rat [zi/zi, tm/tm]. The above compound showed a significant anticonvulsant activity by reducing the total duration as well as the number of tonic clonic seizures in a spontaneously epileptic rat [zi/zi, tm/tm].

A study[65] was done at Indira Gandhi Medical College, Shimla [India] to reevaluate the anticonvulsant activity of flunarizine in pentyletetrazole induced seizures in Swiss albino mice. This study by them found that, flunarizine at 20 mg/kg showed a significant anticonvulsant activity when compared to the control group. Another study done by Brahmane et al.[30] to evaluate the anticonvulsant activity of

cinnarizine and nifedipine in maximal electroshock seizure and the pentylenetetrazole model in the mice, as per their results, when compared to the controls, mice revealed that both calcium channel blockers had a strong anticonvulsant effect. Both calcium channel blockers considerably potentiated the effect of sodium valproate in both animal models in mice. Khanna et al.[29] carried the experiment on Swiss albino mice and Wistar albino rats to evaluate the anticonvulsant effect of nifedipine and nimodipine in maximal electroshock seizure and the pentylenetetrazole models. In their study, both the calcium channel blockers decreased the total duration of clonic seizures and the tonic hind limb extensor phase in the pentylenetetrazole and the maximal electroshock seizure models respectively.

Sahadevan et al.[28] used maximum electroshock and audiogenic seizure tests in Swiss albino mice to evaluate the anticonvulsant activity of three calcium channel blockers, namely nifedipine, diltiazem, and flunarizine. They found that only nifedipine and flunarizine had significant anticonvulsant effects in both the experimental animal models.

Chattopadhyay et al.[27] had done the experiment in swiss albino mice to evaluate the antiepileptic activity of nifedipine, verapamil and diltiazem. The author studied the effects in the maximal electroshock seizure model. The study results inferred that, none of the calcium channel blockers among these three had significant antiepileptic effect in maximal electroshock seizure model when compared to the vehicle control group. However, their study showed that, both nifedipine and the verapamil potentiated the antiepileptic activity of phenytoin sodium in above said animal model.

The present available antiepileptic drugs[1,2,17] controls the seizure only in 50% of patients but associated with the various serious toxicities and have a narrow

therapeutic index. In epilepsy, the oxidative stress[13-16] and the inflammation[1,3-12] plays an important role in its pathogenesis as evidenced from the experimental animal and the human studies.[13-16] Currently there are no drugs in the market which can prevent the process of epileptogenesis as such.[41]

Calcium channel blockers (Diltiazem, Nimodipine and Flunarizine) possess anti-oxidant, anti-inflammatory properties.[18-21] The anti-seizure properties of experimental test drugs (Diltiazem, Nimodipine and Flunarizine) as resulted from various parameters (seizures, anti-oxidant enzyme levels, neurotransmitter (NTs) levels, histopathological [microanatomy] examination (HPE) and immunohistochemistry (IHC) shown in this study, could be due to their single or combined effects of anti-oxidant/anti-inflammatory/Ca^{2+} channel blocking properties.

SUMMARY & CONCLUSION

GRAPHICAL ABSTRACT OF THE STUDY

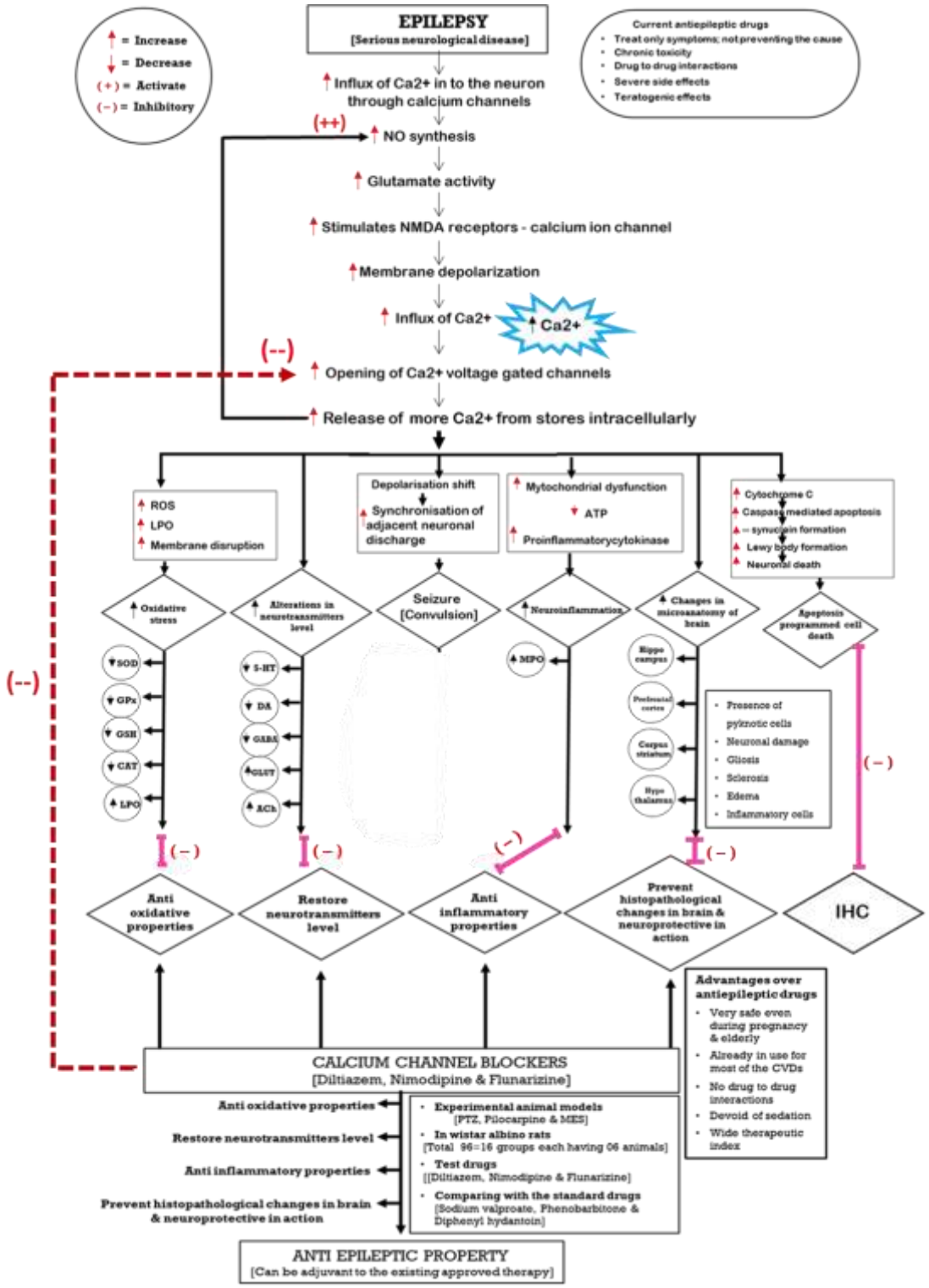


Figure 242: Graphical abstract of the study

SUMMARY

Background and objectives:

The term epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process, and its incidence is around 0.3-0.5% and the prevalence is 5-10 persons per 1000 among world population.

Presently, there are numerous drugs available in the market for treating epilepsy. The antiepileptic drugs act by different mechanisms which include prolongation of Na^+ channel inactivation, inhibition of Ca^{2+} current, facilitation of GABA, mediate Cl^- channel opening, enhancing the opening of K^+ channels, blockade of NMDA receptors, blockade of AMPA receptors and the blockade of kainic acid receptors. The present antiepileptic drugs have certain limitations due to their toxicity profile, drug-drug interactions and resistant in some patients.

There are many studies supporting the role of neuroinflammation and immune mediated insults in the seizure initiation and the process of epileptogenesis. The experimental studies in the rodents have proved that the neuroinflammation can lead to an increase in the permeability of blood brain barrier and the excitability of nervous tissue.

Various experimental studies have described the role of oxidative stress in the epileptogenesis process. The mitochondrial related oxidative stress plays an important role in the epileptogenesis in the lithium-pilocarpine model of temporal lobe epilepsy in the animals. Antioxidants can reduce the neuronal injury caused due to the oxidative stress and hence, the further development of seizures in some animal models.

Thus, further research into the role of calcium channel blockers in preventing microanatomical alterations in the brain and their anticonvulsant characteristics in

different experimental animal models is needed to improve epilepsy pharmacotherapy.

Materials and Methods:

The experimentation was carried on adult healthy wistar albino rats of both sexes (males and females) weighing between 180 - 250 gm. The research protocol received ethical clearance from the Institutional Animal Ethics Committee [IAEC]. CPCSEA guidelines were followed during this study and carried out at Institutional animal laboratory, BLDE, Vijayapura, Karnataka. All the anticonvulsant properties of diltiazem (20 mg/kg), nimodipine (20 mg/kg) and flunarizine (10 mg/kg) were explored using Pentylenetetrazole (PTZ), Pilocarpine and Maximal Electroshock Seizure (MES) animal models in the animal laboratory. The study was done to evaluate onset, duration, number and the scores of seizures, oxidative stress parameters as well as neurotransmitter levels and the results were compared among the groups. Wistar albino rats were sacrificed as per the protocol and CPCSEA guidelines after recording the seizure parameters. The brain tissue was subjected for the biochemical assays (oxidative stress enzymes, neurotransmitters and inflammatory marker, histopathological (microanatomy) and immunohistochemistry study and the scores were given as per the scoring system. The obtained data were tabulated and represented as Mean/Median ±SE and the different statistical tests including one way ANOVA and its non-parametric version were applied wherever required and the ‘p’ value less than 0.05 was considered as statistically significant.

Results:

In this study, in all the three models (PTZ, Pilocarpine and MES) the results showed that, the onset, duration, number and scores of seizures in the test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control

group ($p<0.01$). All these parameters in test drug groups were also comparable to the standard drug group with $p>0.05$.

The oxidative stress markers in all the three models (PTZ, Pilocarpine and MES), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Catalase and Lipid peroxidation (LPO) in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group ($p<0.001$). All the said oxidative stress markers in the experimental test drug groups were also comparable to the standard drug and the vehicle control group with $P>0.05$.

The neurotransmitters - Serotonin (5-HT), Dopamine (DA), GABA, Glutamate and Acetylcholine (ACh) in all the three animal models (PTZ, Pilocarpine and MES), in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) were significantly different from the control group ($p<0.0001$). All the above neurotransmitters in the experimental test drug groups were also comparable to the standard drug and the vehicle control group with $P>0.05$.

The microanatomical/histopathological examination scores of the brain in all the three animal models (PTZ, Pilocarpine and MES) was significantly in favour of restoring the microanatomical changes of brain in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine when compared to the control group.

In immunohistochemistry (IHC) with HSP-70 marker study, the scores were in favour of significant neuroprotection in the experimental test drug groups (Diltiazem, Nimodipine and Flunarizine) when compared to the control group.

CONCLUSION

- Diltiazem, Nimodipine and Flunarizine had significantly improved the seizure parameters in PTZ, Pilocarpine and MES models
    - ☞ Substantially increased the onset of seizures
    - ☛ Significanltly reduced the duration of seizures
    - ☞ Considerable reduction in the frequency of seizures
    - ☛ Remarkable reduction in the seizure scores
- Diltiazem, Nimodipine and Flunarizine had significantly improved/restored the oxidative stress parameters in PTZ, Pilocarpine and MES models
    - ☛ Significant increase in the Superoxide dismutase level
    - ☞ Significant increase in the Glutathione peroxidase level
    - ☞ Increase in the Reduced glutathione level considerably
    - ☞ Catalase level significantly increased
    - ☞ Remarkable decrease in the Lipid peroxidation level
- Diltiazem, Nimodipine and Flunarizine had significantly improved the neurotransmitter levels in PTZ, Pilocarpine and MES models
    - ☞ Significant increase in the Serotonin level
    - ☞ Elevated Dopamine level significantly
    - ☞ Significant increase in the GABA level
    - ☞ Substantial reduction in the glutamate level
    - ☞ Significant decrease in the Acetylcholine level

- Diltiazem, Nimodipine and Flunarizine had significantly improved the antiinflammatory condition levels in PTZ, Pilocarpine and MES models

    ☞ Substantial decrease in the Myeloperoxidase level

- Diltiazem, Nimodipine and Flunarizine showed a significant decrease in the microanatomical /histopathological examination scores in PTZ, Pilocarpine and MES models
- Diltiazem, Nimodipine and Flunarizine had significantly showed an increase in the scores of immunohistochemistry in PTZ, Pilocarpine and MES models

☞ Among the three test drugs, **Flunarizine** had shown a significant anti-epileptic properties in comparison with the standard treatment as evidenced through

- Significant increase in the onset and decrease in the duration, number and scores of seizures
- Significant increase in the Superoxide Dismutase, Glutathione peroxidase, Reduced glutathione and Catalase levels and decrease in the Lipid peroxidation level
- Significant increase in the Serotonin, Dopamine, GABA, and decrease in the Glutamate and Acetylcholine level
- Significant decrease in the inflammatory marker
- No significant alterations in the histopathology of the brain sections leading to decreased histopathological score
- It has shown a significant anti-apoptic property with HSP-70 immunohistochemistry giving high score

Overall conclusion

Calcium channel blockers (Diltiazem, Nimodipine and Flunarizine) possess significant anti-convulsant properties in PTZ, Pilocarpine and MES models in wistar albino rats.

Limitations of the study

- A study design that focused on the specific death of neurons, either through microscopic or molecular analysis of apoptotic markers, would have yielded clear neuroprotection results.
- All neurotransmitters are quantified in homogenised brain hemisections. Estimating the effects of interventional drugs using specific brain areas such as the hippocampus, prefrontal cortex, corpus striatum, and hypothalamus would have resulted in a more precise quantification.
- The use of UV spectrophotometry to estimate neurotransmitter activity is a crude approach of measuring overall effects. These methods may be unable to distinguish between synaptic, presynaptic, and dendritic concentrations, effects, and outcomes in astrocytes. Quantification of receptors (by autoradiography) would have provided a better understanding of the distinct neuroprotective effects of these experimental drugs in epilepsy.

The study's findings and future directions

- The screening of calcium channel blockers (Diltiazem, Nimodipine, and Flunarizine) for anticonvulsant effects in various experimental animal models revealed promising results in this study. The study took into account a variety of variables, including behavioural analysis, oxidative stress marker assays, neurotransmitter assays, inflammatory marker assays, and microanatomical/histopathological/immunochemistry of brain tissue. The experimental drugs showed a considerable favourable effect on all parameters and standard drug employed in this study were comparable to them.
- However, larger research in additional animal models, like the kindling model, and human clinical trials are required to determine the efficacy of experimental drugs as an adjuvant to currently authorised epileptic therapy.

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ANNEXURES

ANNEXURE(s)

MASTER CHART

PTZ Model : Seizure parameters						
Sl. No	**Parameters**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Onset of Seizures (Seconds)	976.33±39.89	2055.67±58.76	1787.17±40.41	1973.5±52.42	1985.83±47.53
2	Duration of Seizures (Seconds)	231.17±16.90	30.67±4.01	69.83±3.86	36.17±5.29	33.5±3.70
3	Number of Seizures (6 h)	10.17±1.42	1.33±0.21	1.67±0.21	1.67±0.21	1.5±0.22
4	Scores of Seizures (0 - 5)	5.00±0.21	0.50±0.22	2.00±0.21	1.00±0.31	1.00±0.31

PTZ Model: Anti-oxidant enzymes							
Sl. No	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Superoxide Dismutase (SOD) (*U/g*)	33.42±1.92	5.90±0.82	31.62±1.42	20.23±1.35	27.64±1.93	29.76±0.50
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	69.07±2.42	9.39±0.87	67.46±0.75	53.55±1.75	64.10±1.50	65.16±1.12
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	518.59±22.33	52.91±4.33	503.52±7.21	466.07±14.61	487.67±11.42	491.09±13.96
4	Catalase (CAT) (*U/g*)	4.68±0.46	0.51±0.14	4.59±0.31	3.42±0.29	3.86±0.27	4.37±0.24
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	38.40±2.65	144.09±6.34	51.95±3.50	58.09±2.62	55.75±1.46	52.09±3.31

PTZ Model: Neurotransmitters							
Sl. No.	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Serotonin (*ng/g of tissue)*	419.78±28.12	91.35±7.02	397.37±27.90	366.09±37.79	373.59±28.10	388.57±20.59
2	Dopamine (*ng/g wet weight*)	55.59±5.41	7.08±0.85	47.80±3.58	42.07±3.47	42.81±2.60	44.05±2.62

3	GABA (*P mol/Sample*)	2.64±0.35	0.12±0.03	2.46±0.35	1.71±0.29	1.89±0.16	2.44±0.22
4	Glutamate (*P mol/Sample*)	3.89±0.42	24.13±3.57	5.18±0.69	3.93±1.04	7.35±0.38	5.57±0.68
5	Acetylcholine (*µg/mg protein*)	27.68±3.02	120.21±7.42	35.34±4.04	46.29±4.20	41.77±3.10	38.09±2.78
PTZ Model: Inflammatory marker							
Sl. No.	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Myeloperoxidase (MPO) (*U/mg protein)*	0.27±0.04	4.93±0.39	0.75±0.11	1.95±0.12	1.76±0.21	0.87±0.12
PTZ Model: Histopathological examination scores							
Sl. No.	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Hippocampus (Scores 0 - 4)	0.00±0.00	3.50±0.22	1.00±0.21	2.00±0.31	2.00±0.21	1.00±0.21
2	Prefrontal Cortex (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.17	2.00±0.31	1.00±0.21	48.70±1.18
3	Corpus striatum (Scores 0 - 4)	0.00±0.00	4.00±0.00	1.00±0.17	2.00±0.21	2.00±0.26	1.00±0.17
4	Hypothalamus (Scores 0 -4)	0.00±0.00	4.00±0.21	1.00±0.17	2.00±0.37	1.50±0.22	1.00±0.17
PTZ Model: Immunohistochemistry examination scores							
Sl. No.	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Hippocampus (Scores 0 - 5)	5.00±0.21	0.50±0.22	3.50±0.22	3.00±0.21	3.00±0.17	3.50±0.22
2	Prefrontal Cortex (Scores 0 - 5)	5.00±0.00	1.00±0.21	4.00±0.17	3.00±0.31	3.00±0.21	4.00±0.17
3	Corpus striatum (Scores 0 - 5)	5.00±0.21	1.00±0.21	4.00±0.17	3.00±0.37	3.00±0.21	4.00±0.21
4	Hypothalamus (Scores 0 -5)	5.00±0.21	0.50±0.22	4.00±0.26	3.00±0.37	3.50±0.33	4.00±0.17

Pilocarpine Model: Seizure parameters						
Sl. No.	**Parameters**	**Group VII**	**Group VIII**	**Group IX**	**Group X**	**Group XI**
1	Onset of Seizures (Seconds)	2771.33±102.61	4341.17±180.49	3436.50±193.42	3843.67±143.55	4069.83±113.68
2	Duration of Seizures (Seconds)	466.83±18.23	56.17±4.97	109.67±9.22	71.67±3.25	59.50±3.63
3	Number of Seizures (6 h)	13.67±1.54	1.83±0.31	8.50±0.43	3.50±0.56	2.33±0.33
4	Scores of Seizures (0 -5)	5.00±0.00	1.50±0.33	2.00±0.31	2.00±0.31	2.00±0.21

Pilocarpine Model: Anti-oxidant enzymes							
Sl. No.	**Parameters**	**Group I**	**Group VII**	**Group VIII**	**Group IX**	**Group X**	**Group XI**
1	Superoxide Dismutase (SOD) (*U/g*)	33.42±1.92	4.41±0.35	32.08±1.57	23.41±1.54	29.95±1.43	30.28±2.11
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	69.07±2.42	7.03±0.94	65.38±2.28	56.91±1.84	61.39±2.02	63.26±2.29
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	518.59±22.33	38.79±3.25	508.99±8.97	485.23±8.51	493.93±9.98	499.31±8.00
4	Catalase (CAT) (*U/g*)	4.68±0.46	0.22±0.03	4.35±0.14	3.83±0.25	4.01±0.32	4.23±0.37
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	38.40±2.65	165.31±5.42	47.35±1.93	55.25±3.38	51.63±2.04	49.79±2.84
Pilocarpine Model: Neurotransmitters							
Sl. No.	**Parameters**	**Group I**	**Group VII**	**Group VIII**	**Group IX**	**Group X**	**Group XI**
1	Serotonin (*ng/g of tissue)*	419.78±28.12	80.08±3.80	407.09±18.60	395.27±14.62	384.46±21.18	396.43±11.95
2	Dopamine (*ng/g wet weight*)	55.59±5.41	5.31±0.52	47.60±3.29	42.62±1.30	43.73±2.04	45.12±3.41
3	GABA (*P mol/Sample*)	2.64±0.35	0.09±0.03	2.20±0.29	1.81±0.23	1.89±0.13	1.97±0.29
4	Glutamate (*P mol/Sample*)	3.89±0.42	31.60±2.00	4.89±0.37	7.71±0.48	7.60±0.60	6.16±1.02
5	Acetylcholine (*µg/mg protein*)	27.68±3.02	132.75±10.46	35.16±3.01	45.62±5.56	43.37±4.33	40.90±3.23
Pilocarpine Model: Inflammatory marker							
Sl. No.	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Myeloperoxidase (MPO) (*U/mg protein)*	0.27±0.04	5.11±0.31	0.61±0.12	1.89±0.18	1.61±0.36	0.79±0.08
Pilocarpine Model: Histopathological examination scores							
Sl. No	**Parameters**	**Group I**	**Group VII**	**Group VIII**	**Group IX**	**Group X**	**Group XI**
1	Hippocampus (Scores 0 - 4)	0.00±0.00	3.50±0.22	1.00±0.17	2.00±0.37	1.50±0.22	1.00±0.17
2	Prefrontal Cortex (Scores	0.00±0.00	4.00±0.21	1.00±0.21	2.00±0.21	2.00±0.31	1.00±0.21

	0 - 4)						
3	Corpus striatum (Scores 0 - 4)	0.00±0.00	4.00±0.21	1.00±0.17	2.00±0.31	1.50±0.33	1.00±0.17
4	Hypothalamus (Scores 0 -4)	0.00±0.00	4.00±0.17	1.00±0.00	2.00±0.31	1.50±0.22	1.00±0.17

Pilocarpine Model: Immunohistochemistry examination scores

Sl. No.	Parameters	Group I	Group VII	Group VIII	Group IX	Group X	Group XI
1	Hippocampus (Scores 0 -5)	5.00±0.21	0.00±0.21	4.00±0.21	3.00±0.17	3.00±0.21	4.00±0.21
2	Prefrontal Cortex (Scores 0 -5)	5.00±0.00	0.50±0.22	4.00±0.17	3.00±0.21	3.50±0.22	4.00±0.17
3	Corpus striatum (Scores 0 -5)	5.00±0.21	0.00±0.17	4.00±0.00	3.50±0.22	4.00±0.21	4.00±0.17
4	Hypothalamus (Scores 0 -5)	5.00±0.21	0.00±0.21	4.00±0.00	3.00±0.37	3.50±0.21	4.00±0.00

MES Model: Seizure parameters

Sl. No.	Parameters	Group XII	Group XIII	Group XIV	Group XV	Group XVI
1	% Protection Against THLE	0	100	50	66.67	83.33
2	Scores of Seizures (0 -4)	4.00±0.00	0.50±0.22	1.00±0.17	1.00±0.17	0.50±0.22

MES Model: Anti-oxidant enzymes

Sl. No.	Parameters	Group I	Group XII	Group XIII	Group XIV	Group XV	Group XVI
1	Superoxide Dismutase (SOD) (*U/g*)	33.42±1.92	6.19±0.38	29.63±1.58	25.69±2.43	28.99±1.95	29.23±2.48
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	69.07±2.42	10.72±1.29	61.33±3.31	54.79±4.09	56.89±3.93	59.38±4.57
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	518.59±22.33	65.05±4.22	493.12±14.95	481.61±8.47	485.73±21.61	491.49±22.03
4	Catalase (CAT) (*U/g*)	4.68±0.46	0.71±0.19	4.55±0.26	3.59±0.34	4.33±0.22	4.47±0.41
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	38.40±2.65	167.13±6.33	44.65±4.66	56.13±6.35	53.81±3.55	46.67±3.11

MES Model: Neurotransmitters

Sl. No.	Parameters	Group I	Group XII	Group XIII	Group XIV	Group XV	Group XVI
1	Serotonin (*ng/g of tissue)*	419.78±28.12	76.63±2.97	411.22±10.39	398.91±16.87	403.91±25.93	408.71±17.06
2	Dopamine (*ng/g wet weight*)	55.59±5.41	3.84±0.60	50.89±4.38	44.60±3.60	48.15±4.18	48.70±1.18

3	GABA (*P mol/Sample*)	2.64±0.35	0.05±0.01	2.22±0.22	1.71±0.21	1.92±0.22	2.05±0.33
4	Glutamate (*P mol/Sample*)	3.89±0.42	45.74±2.65	4.49±0.44	6.70±0.42	6.51±0.70	5.15±0.62
5	Acetylcholine (*µg/mg protein*)	27.68±3.02	148.48±6.42	30.94±4.87	46.66±2.13	39.74±1.57	33.05±2.65
MES Model: Inflammatory marker							
Sl. No.	**Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1	Myeloperoxidase (MPO) (*U/mg protein)*	0.27±0.04	5.39±0.33	0.84±0.14	2.07±0.27	1.83±0.24	1.12±0.24
MES Model: Histopathological examination scores							
Sl. No.	**Parameters**	**Group I**	**Group XII**	**Group XIII**	**Group XIV**	**Group XV**	**Group XVI**
1	Hippocampus (Scores 0 -4)	0.00±0.00	4.00±0.21	1.00±0.21	2.00±0.21	1.50±0.22	1.00±0.21
2	Prefrontal Cortex (Scores 0 -4)	0.00±0.00	3.50±0.22	1.00±0.00	2.00±0.17	2.00±0.31	1.00±0.17
3	Corpus striatum (Scores 0 - 4)	0.00±0.00	4.00±0.17	1.00±0.21	2.00±0.17	2.00±0.21	1.00±0.21
4	Hypothalamus (Scores 0 -4)	0.00±0.00	4.00±0.17	1.00±0.17	2.50±0.22	1.50±0.22	1.00±0.17
MES Model: Immunohistochemistry examination scores							
Sl. No.	**Parameters**	**Group I**	**Group XII**	**Group XIII**	**Group XIV**	**Group XV**	**Group XVI**
1	Hippocampus (Scores 0 - 5)	5.00±0.21	0.00±0.21	4.00±0.26	3.00±0.17	3.50±0.22	4.00±0.31
2	Prefrontal Cortex (Scores 0 - 5)	5.00±0.00	0.50±0.22	4.00±0.26	3.50±0.40	3.00±0.21	4.00±0.17
3	Corpus striatum (Scores 0 - 5)	5.00±0.21	0.00±0.17	4.00±0.31	3.00±0.21	3.00±0.17	3.50±0.33
4	Hypothalamus (Scores 0 -5)	5.00±0.21	0.00±0.21	4.00±0.26	3.00±0.17	3.00±0.42	4.00±0.26

Shri B. M. Patil Medical College, Hospital and Research Centre

BLDE (Deemed to be University), Vijayapura, Karnataka, India

Department of Anatomy

Proforma for Collection of Sample

Title of the Study: Evaluation of Preventive Role on Microanatomical Changes in Brain and Anticonvulsant Properties of Calcium Channel Blockers in Experimental Animal Models

Date: ……. Strain: Wistar albino rats Animal Model: …….

Group No.: ……. Group Description: …….

Sex of Animal: ……. Weight of Animal [Grams]: …….

Test Drug: ……. Experimental Drug: …….

Dose of Test Drug: ……. Dose of Experimental Drug: …….

Humidity: ……. Lab. Temperature: …….

Parameters [Chemical Models: Pentylenetetrazol and Pilocarpine]				
Seizures				
Animal No.	**Onset [in Seconds**	**Duration [in Seconds**	**Number [in 6 Hours]**	**Scores* [Severity]**
1.				
2.				
3.				
4.				
5.				
6.				

***Scores 0**= No behavioral changes, **1**= Isolated myoclonic jerks [Ear and facial twitching], **2**= Atypical minimal seizures [Convulsive wave through the body], **3**= Fully developed minimal seizures, clonus of head muscles and forelimbs, righting reflex present, **4**= Major seizures [Generalized without the tonic phase] and **5**= Generalized tonic-clonic seizures beginning with running.

Animal No.	Parameters [Electrical Model: Maximal Electroshock Seizure]	
	Seizures	
	Tonic Hind Limb Extension [Present/Absent]	Scores[$] [Severity]
1.		
2.		
3.		
4.		
5.		
6.		

[$]**Scores 0**= No seizure, **1**= Forelimb extension without hind limb extension, **2**= Complete forelimb extension and partial hindlimb extension, **3**= Complete tonic hind limb extension [THLE] [Hind limb become parallel to the tail] and **4**= Post ictal depression.

Biochemical assay and Microanatomical changes in different parts of brain of wistar albino rats for all the three models

	Total Protein	Anti-oxidants					Neurotransmitters					Infl.marker	HPE**				IHC***			
		SOD	GPx	GSH	CAT	LPO	5-HT	GABA	GLU	ACh	LPO	MPO	H	PC	CS	Hypo	H	PC	CS	Hypo
1																				
2																				
3																				
4																				
5																				
6																				

Scores 0= Histological section undistinguishable from control group [Number of healthy neurons appeared normal, even if few pyknotic cells found], **1**= More than 75% of healthy pyramidal cells with others 25% with clear evidence of cell death, **2**= 50-74% of healthy pyramidal cells, **3**= 25-49% of healthy pyramidal cells and **4**= Less than 25% of healthy pyramidal cells.

*****Scores:** [**0:** Nil/No neuroprotection, **1:** Plus^{+} (Mild Neuroprotection), **2:** Plus^{++} (Borderline Neuroprotection), **3:** Plus^{+++} (Good Neuroprotection), **4:** Plus^{++++} (Excellent/Normal Neuroprotection) and **5:** >Plus^{++++} (Excellent/Normal Neuroprotection)]

Signature of Guide/Co-

Signature of Principal Investigator

PRESENTATION IN CONFERENCES

Oral Presentation

PHA033

Dihydropyridines ameliorates oxidative stress produced by chemically induced seizures in experimental animal models

Saniya K, Patil BG, Madhavrao C, Prakash KG, Mythili Bai K

Presented at **MED Inspire**, an international multidisciplinary medical summit, organized by **Dr. D Y Patil University,** at **Dr. D Y Patil University Capmus, Navi Mumbai** on 14th to 16th February 2019.

Poster Presentation

PHAP29

T – Type of Calcium Channel Blocker Possess Significant Anticonvulsant Properties in Electrically Induced Seizure Screening Tests

Saniya K, Patil BG, Madhavrao C, Prakash KG, Mythili Bai K

Presented at **MED Inspire**, an international multidisciplinary medical summit, organized by **Dr. D Y Patil University,** at **Dr. D Y Patil University Capmus, Navi Mumbai** on 14th to 16th February 2019.

Poster Presentation

PR/P/08

Dihydropyridines ameliorates oxidative stress produced by electrically induced seizures in experimental animal models

Saniya K, Patil BG, Madhavrao C, Prakash KG, Mythili Bai K

Presented at **12thAnnual International Conference** (SAC-ACCP), Clinical Pharmacology: Preparing for the future organized by South Asian College of Clinical Pharmacology, An Affiliate of American College of Clinical Pharmacology in association with Indian Council of Medical Research (ICMR), Maharashtra University of Health Sciences and Department of Pharmacology, Seth GS Medical College and **KEM Hospital Mumbai** conducted at Mumbai

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Poster Presentation

Effect of T-Type of Calcium Channel Blockers on Behavioral, Biochemical, Immunohistochemical, Oxidative and Histopathological Parameters in Pilocarpine Induced Seizure Tests in Wistar Albino Rats

Saniya K, Patil BG, Madhavrao C, Prakash KG, Mythili Bai K

Presented at **ECON 2019 20th Joint Annual Conference of Indian Epilepsy Society and Indian Epilepsy Association** held at Eros hotel, **New Delhi** from 8th - 10th March 2019.

RESEARCH PUBLICATIONS

Saniya K, BG Patil, Madhavrao, Prakash KG, Acute Anticonvulsant Activity of Diltiazem, Nimodipine and Flunarizine in Wistar Albino Rats by Maximum Electroshock induced Seizure. Indian Journal of Public Health Research & Development, 2019;10 (7) : 372-378

Available from: Website: www.ijphrd.com

K. Saniya, B. G. Patil, C. Madhavrao, K. G. Prakash, Effect of Calcium Channel Blockers on the Seizures, Oxidative Stress, and Histoarchitecture in the Rats. Journal of Pharmacology and Pharmacotherapeutics, 2019;10 (3) : 93-100.

[Download from http://www.jpharmacol.com on Tuesday, November 19, 2019, IP: 137.97.98.2] Saniya

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K. Saniya, B. G. Patil, Madhavrao C. Chavan, K. G. Prakash, Kumar Sai Sailesh, R. Archana, MinuJohny, Review Article: Neuroanatomical Changes in Brain Structures Related to Cognition in Epilepsy: An Update, Journal of Natural Science, Biology and Medicine, 2017; 8 (2) :139-143

[Download from http://www.jnsbm.org on Sunday, January 12, 2020, IP: 137.97.18.9]

Institutional Ethical Clearance Certificate

Chairman,
Institutional Animal Ethics Committee (IAEC),
Prof. & HOD, Dept. of Pharmacology,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

BLDEU'S DEPT. OF PHARMACOLOGY VIJAYAPUR-586103 OUTWARD No. 32/16 Date: 16/01/16 SHRI B.M. PATIL MEDICAL COLLEGE

Mrs. Saniya K
PhD Scholar, Dept. of Anatomy,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 07.12.2015 at 10.30am to scrutinize the Research Project submitted by faculty member of this College.

After scrutiny the following research project has been accorded ethical clearance.
Title: **"Evaluation of Preventive Role on Microanatomical Changes in Brain and Anticonvulsant Properties of Calcium Channel Blockers in Experimental Animal Models"**.

Principal investigator: Mrs. Saniya K, PhD Scholar, Anatomy,

Guide: Dr. B.G Patil.

16.01 .2016

16/1/2016
Dr. R. S. Wali
Chairman, (IAEC).
Professor & HOD
Dept. of Pharmacology
BLDEU's Shri B. M. Patil
Medical College, VIJAYAPUR

BLDE (DEEMED TO BE UNIVERSITY)

PLAGIARISM VERIFICATION CERTIFICATE

1. **Name of the Student:** Mrs Saniya K **Reg No:** 13PHD001

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3. **Department:** Anatomy

4. **Name of the Guide & Designation:** Dr. B G Patil, Professor

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