WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 1632-1647. Review Article ISSN 2277–7105

A REVIEW: ANIMAL MODELS FOR SCREENING ANTIEPILEPTIC DRUGS & IMPORTANT UNANI ANTICONVULSANT DRUGS

Zeba Afrin[1]*, Aisha Siddiqui[2], M.A. Jafri[3], Mohd. Asif[2] and Hushan Jahan[1]

[1]PG Scholar, [2]Assistant Professor, [3]Professor, Department of Ilmul Advia, Faculty of Medicine (Unani), Jamia Hamdard, New Delhi. India.

Article Received on
23 April 2017,

Revised on 12 May 2017,
Accepted on 01 June 2017

DOI: 10.20959/wjpr20176-8726

***Corresponding Author**
Dr. Zeba Afrin
PG Scholar, Department of Ilmul Advia, Faculty of Medicine (Unani), Jamia Hamdard, New Delhi. India.

ABSTRACT

Epilepsy is the world's most common serious disorder of the brain according to WHO. It is defined as the occurrence of transient paroxysms of excessive or uncontrolled discharges of neurons due to a number of causes leading to epileptic seizures. The identification of potential therapeutic agents for the treatment of epilepsy requires the use of seizure models. A large number of traditional drugs have been used in epilepsy since centuries. So, screening/investigating these drugs for their anticonvulsant activity to make an effective medicine in the treatment of epilepsy is auspicious. Different types of experimental models have been used to assess the anticonvulsant activity of these drugs such as Maximal electroshock seizure (MES) test, Pentylenetetrazole (PTZ) test, kindling animal model, strychnine model etc. These models can be either in vivo or in vitro, mechanism specific, mechanism independent, or seizure specific. The present article explains the types of experimental models used for screening of anticonvulsant activity of traditional drugs and some important scientifically proved Unani anticonvulsant drugs.

KEYWORDS: Epilepsy, anticonvulsant, experimental models, in vivo, in vitro.

INTRODUCTION

According to WHO, Epilepsy is the world's most common serious disorder of the brain.[1] It is the second most common chronic neurological condition seen by neurologists. In India it is estimated that about 55,00,000 people are affected with epilepsy.[2] It is becoming the most serious brain disorder and affects about 50million people and about 100 million will be

affected at sometime in their life. Overall, it accounts for 1% of the world's burden of diseases, and the prevalence rate is reported at 0.5-1%.[3]

Epilepsy may be defined as an abrupt and transient disturbance of cerebral function with or without loss of consciousness.[4, 5] It is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures which are caused by an abnormal discharge of cerebral neurons. Different type of seizures can be identified on the basis of their clinical phenomena.[6]

Currently many antiepileptic drugs (AEDs) are available. However, almost one-third of the epileptic patients remain uncontrollable and refractory to AEDs.[7] Meanwhile, most of the epileptic patients experience serious central and peripheral adverse effects of AEDs. AEDs have dose related & chronic toxicity involving virtually every major organ system, adverse effects on cognition and behaviour and teratogenic effects.[8] Clinically available anticonvulsant drugs fail to control seizures in around 30% of epileptic patients, which constitute some 15million people in the world. In most of the patients antiepileptic drugs are prescribed for lifelong treatment as patients who become seizure free with the use of antiepileptic drugs have higher chances of relapse following the discontinuation of medication. Despite the introduction of new drugs, the problem of pharmacoresistance has not been solved.[3] Treatment options for epileptic seizure are limited and treatment with modern medicines have profound side effects. Hence, the discovery and development of new drugs with minor side effects is the need of hour. Clinical studies demonstrated safety and efficacy of many herbal products in the treatment of seizures.[9] Traditional medicines provide a treasure of anticonvulsant drugs. A number of single and compound preparations of traditional medicine have been used in the management of seizures since decades. Therefore, many traditional drugs from plant origin are tested for its potential anticonvulsant activity in experimental animal model and validated scientifically.

The use of animal seizure models is imperative in the discovery and development of new drugs for the treatment of epileptic seizures. In recent decades despite the successful development of various new antiepileptic drugs (AEDs), the search for new therapies with better efficacy and tolerability remains an important goal.[10] The discovery and development of a new AED relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans.[11] Animal models deliver different purposes in epilepsy research. Firstly, they are used in development of new antiepileptic drugs. Secondly, they are

used to assess or evaluate specific efficacies of drugs on different type of seizures and also to determine preclinical efficacy of new drugs during chronic administration. Moreover, animal models are also employed to study the pharmacodynamics, mechanism of drug resistance and also to assess whether epileptogenesis alters the adverse effect potential of a given drug.[12]

Historical development of epilepsy models

PHT was the first major advance in epilepsy therapy and it was identified using an electroshock-induced seizure model. In this model, rectangular current was applied to the animal's head via mouth and scalp electrodes attached to a 45-V battery and tonic seizures were induced by it in cats.

Subsequently, in 1945, Toman and Swinyard established that once the thresholds for electrically induced or chemically induced seizures were exceeded by >20%, the pattern and duration of the seizures were relatively constant and free of intensity or duration of the stimulus. This finding was imperative in the development of animal models for seizures as it showed that drug effect on seizure pattern was indistinguishable. Hence, agents that inhibit seizure spread would debilitate the extensor tonic component of the seizure, regardless of how the maximal seizure is produced (i.e., electrically or chemically).

In 1946, Toman and Goodman compiled the then state-of-the-art method for identifying new therapeutic agents and concluded that no single test could satisfactorily determine the anticonvulsant properties of a drug.

In the early 1950s, many articles explained the properties of electrically induced seizures and also the effect of chemical agents on them. The classic article of Swinyard et al. defined the parameters for identifying the anticonvulsant properties of eight AEDs using a battery of four anticonvulsant tests in rats and mice. The tests that composed the battery were maximal electroshock seizure pattern, minimal electroshock seizure threshold, pentylenetetrazol seizure threshold and hyponatremic electroshock seizure threshold.

By 1951, PHT had opened a new generation for the treatment of generalized tonic–clonic seizures. It was soon followed by the discovery of trimethadione and paramethadione for "petit mal triad". Nevertheless, an animal model for assessing therapeutic agents for the treatment of partial onset seizures was lacking.

In 1952, Toman et al. outlined a "psychomotor" test in which low frequency stimulation was subjected to mice and that produced seizures similar to those seen in patients with partial-onset epilepsy. In this model, a rectangular pulse of 6 Hz (1-ms pulse width) and a current of 50 mA were delivered for 3 s through electrodes placed on the cornea. The mice advertised an abnormal behavior for an average duration of 25s that the authors described as "stunned." The mice remained immobile and did not respond to painful stimuli. This method was discontinued by the pharmaceutical industry as a primary screen. The animal seizure models mentioned earlier were used to identify the majority of agents presently used for the treatment of epileptic seizures. These models can be considered nonmechanistic in nature; that is, they are useful for determining whether a compound prevents seizure spread or increases seizure threshold, but they do not provide understanding into the mechanism by which they elicit their pharmacodynamic effect.[13]

Animal models used in anti-epileptic drug research

In order to study the antiepileptic effect of drugs and discovering their mechanism of action, various *in vitro* and *in vivo* models of epilepsy have been devised. Some of them which are used commonly are described below.

Maximal electroshock (MES) induced convulsions

Merritt and Putnam developed the MES test and discovered the anticonvulsive effect of diphenyl hydantoin using this test.[14] This is a model of acute seizure and one of the gold standards in early stage of antiepileptic drug screening and probably the best validated preclinical test to predict effectiveness of drugs against Grand mal seizure. This model identifies those compounds which prevent seizure spread.[15] The MES produce the spread of seizure similar to grandmal epilepsy.[12]

In MES method brief high intensity shock is applied to the head through corneal or ear electrodes with a stimulator that either delivers constant current on constant voltage as a frequency of 50-60 sec^{-1}. The electrodes are moistened with saline solution before application for better conductance. All animals are stimulated with the constant current stimulator typical stimulation parameters include 50mA in mice and 150mA in rats, 50-60 sec^{-1}, current is delivered via corneal/ear electrodes for 0.2sec.[16]

The MES convulsions are divided into five phases such as.

- Phase of tonic limb flexion
- Phase of tonic limb extension
- Phase of clonic convulsions
- Stupor
- Recovery or death

A substance is known to possess anticonvulsant property if it abolishes the extensor phase of MES convulsion.[12]

Suppression of tonic hind limb extension is taken as a measure of efficacy in this test. Drugs effective against generalized tonic clonic seizure such as phenytoin, Carbamazepine, primidone and phenobarbitone are effective while Ethosuximide is ineffective in this test. Disadvantage of the present model is that, they do not give any clue regarding mechanism of action of the compound.

Increasing current electroshock seizure (ICES) test

Increasing current electroshock seizure test, as proposed by Kitano *et al* and modified by Marwah *et al* was used to determine seizure-threshold current (STC) for each animal. It is a widely used test to screen the anti-and pro-convulsant activity of a novel compound. The potential of an agent to increase seizure-threshold current can be assessed in this test. Starting with a current of 2 mA, electroshock was delivered to each mouse via ear electrodes as a single train of pulses (20 Hz for 0.2 s) with linearly increasing intensity of 2 mA/2 s using an electro-convulsometer. The current at which tonic hind limb extension (HLE) occurred was recorded as the seizure threshold current. If no tonic HLE was observed by the current of 30 mA, electroshock was terminated and this cut-off current was used in the analysis.[17]

Pentylenetetrazole (PTZ) test

Pentylenetetrazole test is a tetrazol derivative with consistent convulsive effect in large number of animals like mice, rats, primates etc. It is believed to act by antagonizing the inhibitory GABAergic neurotransmission. In 1944, Everett and Richard's demonstrated that PTZ induced seizures could be blocked by Phenobarbital and Trimethadione but not, by phenytoin.[12]

PTZ test is a model used for screening of drugs effective in petit mal epilepsy or absence seizures. (18) Pharmacologically PTZ is an analeptic agent, possible mechanism of action is potential to alter movement of chloride ions across neuronal membrane. Different routes of administration such as i.p, i.v, s.c have been used by various investigations in this method.[19] In the s.c. PTZ (or metrazol) seizure test, the convulsive dose of PTZ inducing a clonic seizure of at least 5 s duration in 97% of the animals (CD97) is subcutaneously injected and animals are observed for a period of usually 30 min after injection for the occurrence of such a threshold seizure.[13]

Stages of PTZ induced convulsion involve

- one or more isolated jerks
- generalized clonic seizure with loss of righting reflex
- maximum tonic clonic seizures

Efficacy of test drug as an anticonvulsant is measured by determining its ED_{50} for suppression of clonic seizures.

Drugs effective in petitmal epilepsy like valproic acid, ethosuximide are effective in PTZ induced convulsions while phenytoin, carbamazepines are not effective.[16]

Kindling animal model

Kindling is a model of epilepsy produced by repeated administration of an initially subconvulsive electrical or chemical stimulus that produce an increase in seizure activity, culminating in a generalized seizure.[20] The electrographic and behavioral components of kindled seizures are thought to be similar to human partial-onset seizures. In this model, the effect of drugs on both focal and generalized seizure types can be quantitated. Kindling is possible in numerous species. In rats the electrode is implanted in the right amygdale for electrical stimulation. Although kindling can be produced via electrical or chemical stimulation of many, the most common way to produce the kindled state is stimulation of a specific area of the amygdala via implanted electrodes. A fixed current is applied until an after discharge is produced at the site of stimulation.[13]

During the daily electrical stimulation of amygdala, seizures develop into five stages.

- Stage-1: Immobility, eye closure, twitching of vibrissae, stereotypic sniffing
- Stage-2: Facial clonus and head nodding

- Stage-3: Facial clonus, head nodding and forelimb clonus
- Stage-4: Rearing, often accompanied by bilateral forelimb clonus
- Stage-5: Rearing with loss of balance and failing accompanied by generalized clonic seizures.

If the stimulation is given continuously for a few weeks, rats develop spontaneous epileptic seizures that persists for as long as 7 months following termination of the stimulation.[20]
There are advantages and disadvantages to the use of each species. The major advantage of using mice is that a large number of animals can be kindled concurrently at low cost. An additional benefit to the use of mice is that the amount of compound required for testing is smaller than that for rats due to the size difference. In both cases, the efficacy of kindling is dependent on several stimulus parameters, including intensity, duration and interval of the 60-Hz corneal electrode stimulation. In general, mice are stimulated for 2 s at 3 mA once daily, whereas rats are stimulated for 4 s at 8 mA twice daily. Electrical stimulations are continued until a Racine stage 5 generalized seizure is obtained. Typically, 10 consecutive stage 5 seizures (rearing and falling with clonus) are observed before drug testing is done. There is a clear progression of kindling development in rats, which is not seen in mice; lethality in mice also is a problem.[13]

Lothman et al described an alternate method in which fully kindled state in rats is produced, the rapidly recurring hippocampal seizure (RRHS) model. The RRHS model stimulates the hippocampus of rats with 10-strains of suprathreshold tetanic electrical stimuli every few minutes. Seizure intensity was quantified using the duration of after discharge and the accompanying behavioural responses. Severe limbic seizures were evoked on the first day after the initial repeated stimulation of the hippocampus. Seizures intensified on the second day and remained stable thereafter.

Gupta YK et al described an alternative method for Kindling; on every second day, a subconvulsive dose of PTZ (35 mg/ kg body weight) was injected intraperitoneally for (43 days as 22 injections). When the animals showed adequate kindling then PTZ injections were stopped.[20]

Strychnine model

Strychnine-sensitive postsynaptic inhibition in higher centers of the CNS is also mediated by glycine. Strychnine induced seizures are different from those produced by primary GABA

antagonists since they are mainly extensor tonic, with little cortical EEG activity. These seizures are not fully relieved by acceptable doses of any of the classical anticonvulsants including benzodiazepines.

Yamashita *et al* (2004) evaluated the strychnine at a dose 0.8 mg/kg was injected subcutaneous 60 min after the oral administration of test compounds. The animals were observed for 30 min after injection and wild running, clonic seizures, tonic seizures and respiratory arrest were monitored. ED50 values and 95% confidence interval of tonic extension seizures were calculated.[20]

Lithium-pilocarpine model

Pretreatment with lithium provokes limbic seizures following administration of pilocarpine at subconvulsant doses and other cholinergic agoinsts, although lithium does not possess general proconvulsant action in rats. The time of onset of severity of seizures is generalized and reproducible. The convulsions produced always continue unabated for several hours until death ensures.[12] The severity of status epilepticus was observed every 15 min till 90 min and thereafter every 30 min till 180 min using the following scoring system such as

Stage 0 - no response
Stage 1- fictive scratching
Stage 2- tremor
Stage 3-head nodding
Stage 4- Forelimb clonus
Stage 5- Rearing and falling back.[20]

It is reported that activation of muscarinic cholinergic receptors is responsible for initiation of seizure but the mechanism accounting for the sustained maintenance osstatus epilepticus are not known.[12]

Martin ED (2006) described the alternative method on lithium pretreatment, followed by one or several low doses of pilocarpine, produces status epilepticus (SE) and chronic epilepsy with much lower mortality rates than a single dose of pilocarpine. Pretreatment of lithium chloride (3mEq/ kg, i.p) between 2-24 hours before pilocarpine injection potentiates the epileptogenic action of pilocarpine and allows a 10-fold reduction in the drug dose.[20]

Isoniazid (INH) model

At higher dose, Isoniazid acts as convulsant in animal models.[12] The compound is regarded as a GABA-synthesis inhibitor. Clonic tonic seizures are elicited in mice which are antagonized by anxiolytic drugs.[20]

The mechanism of Isoniazid triggered convulsion involves interaction with pyridoxine metabolism. It interferes with the function and supply of pyridoxine which is responsible for epileptogenesis. Isoniazid directly binds with pyridoxine to form Isonicotinyl hydrazine. Deficient GABA and the accumulation of glutamic acid lead to CNS excitation and seizures in animal models. Intraperitoneal administration of 300mg kg^{-1} of Isoniazid induces seizures in mice.[21]

Picrotoxin model

Picrotoxin acts as a GABAA-antagonist modifying the function of the chloride ion channel of the GABAA receptor complex. Picrotoxin induces minimal and maximal seizures in a dose - dependent manner. In rat, doses of 8 mg/kg produce hyperactivity, body tremors and forelimb clonus followed by tonic extension of the hind limbs and generalized tonic-clonic seizures.

Kasture et al (2002) described in mice picrotoxin (3mg/kg, s.c) was administered 30 min prior the test drug. The parameter such as presence or absence of clonic convulsions was studied. The protective effect of classical anticonvulsant against picrotoxin- induced seizures has been studied while diazepam, carbamazepine and phenytoin have a protective efficacy.[20]

Penicillin model

This model is useful for screening of drugs useful in petit mal epilepsy. The convulsive properties of penicillin were first observed by Walker and Johnson. Chen et al established the effective dose of penicillin given i.v or i.p. in rats and cats to induce experimental seizures.[20] The possible mechanism of epileptogenesis of penicillin is via blocking of the GABA's effect when the beta lactam ring binds to GABA receptor. Seizure activity begins after one hour of injection. The seizures are characterized by recurrent episodes of arrested activity, staring myoclonus, facial oral twitching & occasionally progress to generalized tonic clonic seizures. Ethosuximide and valproate are effective in this model.[12]

Kainic acid (KA) model

Systemic administration of the appropriate dose of KA induces generalized tonic-clonic convulsions, teeth chattering and altered motor activity including an initial hypoactivity which at later stage convert to hyperactivity. At this time point, a positive correlation exists between the dose of KA and the extent of the acute neurochemical changes including increases of 3, 4- dihydroxyphenylacetic acid and decrease in noradrenaline levels in all brain regions investigated. By 13 hours to 2 weeks, neuronal somata degenerate and disappear in areas such as the olfactory cortex and parts of the amygdaloid complex, hippocampal formation, thalamus and neocortex.[13]

Common methods used to induce convulsion in animal models[12]

Animal models	Methods to induce convulsion	Types of seizure
Invivo model	**Electrical stimulation:** Maximalelectroshock(MES) Kindling **Chemoconvulsants:** Pentylenetetrazole(PTZ) Strychnine Picrotoxin Isoniazid Lithium pilocarpine Yohimbine Bicuculline 4-aminopyridine n-methyl d- aspartate Penicillin	 Generalised tonic-clonic seizures Chronic partial seizures Myoclonic and absence seizures Acute simple partial seizures Acute simple partial seizures Clonic-tonic seizures Status epilepticus Clonic seizures Acute simple partial seizures Clonic-tonic seizures Status epilepticus Generalised tonic-clonic and absence
Invitro model	Hippocampal slices $GABA_A$ receptor binding Assay	Complex partial seizures
Genetic model	Photosensitive baboons Audiogenic seizures mice Totterer mice and seizures -prone mouse strains Genetically epilepsy- prone rats	Generalised tonic- clonic seizures

Scientific reports on some Unani anticonvulsant herbs

Medicinal plants used in Unani system of medicine for the treatment of epilepsy have been scientifically proved to possess promising anticonvulsant activities in animal model. and can be a source of newer anticonvulsants.

- ***Acorus calamus*:** Methanolic extract of *Acorus calamus* roots significantly increase the latency period in seizure induced by PTZ.[22] Concurrent administration of methanolic

extract of rhizome of Acorus calamus with phenytoin and Phenobarbital showed synergismin anticonvulsant action in mice at their non-effective doses.[23]

- ***Butea monosperma*:** Active constituent triterpene in hexane ethyl acetate fraction significantly inhibited seizure induced by MES, PTZ, electrical kindling and combination of lithium sulphate with pilocarpine nitrate while was not effective against strychnine and picrotoxin induced convulsions.[24]
- ***Delphinum denudatum*:** Ethanolic extract showed dose dependent anticonvulsant action on seizures induced by PTZ and bicuculline while aqueous extract showed activity against PTZ and MES induced convulsions especially inhibition of hind limb extension.[25]
- ***Glycyrrhiza glabra:*** The ethanolic extract of *G. glabra* significantly and dose dependently delayed the onset of clonic convulsion induced by PTZ and also protected the rats against seizure induced by lithium pilocarpine.[26]
- ***Hyoscyamus niger*:** The methanolic extract of *Hyoscyamus niger* produced significant delay in the onset time of seizures, induced by picrotoxin in mice, the most effective dose of extract was 300mg/kg b.w.[27]
- ***Laurus nobilis*:** The essential oil of the leaf of *Laurus noblis* has been used as an antiepileptic remedy in traditional medicine. The scientific evaluation showed protection of mice aginst tonic convulsions induced by MES and PTZ treatment.[28]
- ***Lavandula stoechas*:** Gilani et al validated its anticonvulsant effect. Aqueous methanolic extract of *L. stoechas* flowers significantly reduced the severity and increased the latency of onset of convulsions induced by PTZ.[29]
- ***Myristica fragrans*:** Sonawane et al screened the spectrum of anticonvulsant activity using animal models. In MES model, drug showed significant reduction in duration of HLE. Similar results were obtained in PTZ model by delaying myoclonic spasm. The delayed onset of clonic convulsions were recorded in picrotoxin model while in lithium pilocarpine induced status epilepticus, the gradual decrease in progression as well as severity of status epilepticus was reported with increased dose.[30]
- ***N. Oderum*:** Petroleum ether extract significantly inhibited the PTZ and MES induced convulsions.[31]
- ***Pimpinella anisum*:** The essential oil of fruits of the plant was found to be anticonvulsant as it suppressed the tonic convulsions in mice induced by PTZ and MES treatment.[32]

- ***Rosa damascena*:** Anticonvulsant activity of the essential oil of *Rosa damascena* on the PTZ induced seizure in wistar rats evaluated and found that essential oil delays the start of epileptic seizures.[33]
- ***Solanum nigrum*:** Aqueous extract of leaf of Mako (*Solanum nigrum*) was found to offer protection against electrically, PTZ and picrotoxin induced seizure. 60mg/kg gave 75% protection against picrotoxin induced seizure in rats and also provided complete protection against mortality.[34]
- ***Vitex negundo*:** It possesses anticonvulsant activity particularly against PTZ induced convulsions. Moreover, the potentiation of diphenylhydantoin and valproic acid by *Vitex negundo* indicates that it may be useful as an adjuvant therapy along with standard anticonvulsants.[35]
- ***Calotropis gigantia*:** The methanolic extract inhibited the hind limb extension induced by MES and onset of clonic convulsion or latency of convulsion induced by PTZ.[36]
- ***Cuscuta reflexa:*** It showed significantly reduction in the duration of convulsion in tonic seizure induced by pentylenetetrazole (30mg/kg i.p) in mice. It also reduces the tonic extension convulsion induced by maximum electroshock induced convulsions.[37]

CONCLUSION

The above discussed models can be used to identify new drugs for treatment of epilepsy. Animal models are playing the fundamental role in anticonvulsant screening. Animal models provide only choices for determining which compounds should be developed; they do not predict efficacy in the treatment of human epilepsy. The ultimate test for proof of anticonvulsant activity necessitates the use of patients to validate the conclusions obtained from animal models.

All AEDs available in market have some adverse side effect. AEDs have dose related & chronic toxicity involving virtually every major organ system, adverse effects on cognition and behaviour and teratogenic effects. Herbal anticonvulsant drug like *Cuscuta reflexa, Acorus calamus, Butea monosperma, Lavandula stoechas, Delphinum denudatum* and many more possess a remarkable anticonvulsant effect with lesser or no side effect. Hence present study needs a synthesis and formulation of combined extract of one or more novel herbal drugs to treat the seizure effectively.

REFERENCES

1. Anonymous. *Executive Summary. In Atlas: Epilepsy Care in the world.* Geneva : World Health Organization, 2005; 3.
2. *Epidemiology of Epilepsy.* Sridharan, Ram. 6, Chennai : s.n., 2002, Current Science, 82(82): 664-670.
3. *Difficulties in Treatment and Management of Epilepsy and Challenges in New Drug Development.* Wahab, Abdul. 2 July 2010, Pharmaceuticals, 3: 2090-2110.
4. *A STUDY ON ANTICONVULSANT ACTIVITY OF AQUEOUS EXTRACTS OF FRESH LEAVES OF Ruta chalepensis Linn. IN EXPERIMENTAL ANIMAL MODEL.* Satish. S, Abdul Kareem. M. 1, 2014, Journal of International Pharmaceutical Sciences, 1: 28-32.
5. Warner, E.C. Savill's System of Clinical Medicine, 14th ed. New Delhi : CBS Publishers & Distributors, 2003; 1141.
6. *Plants with anticonvulsant properties - a review.* Lucindo J. Quintans Júnior, Jackson R.G.S. Almeida, Julianeli T. Lima, et al. 2008, Brazilian Journal of Pharmacognosy, 798-819.
7. *In SIlico Screening and Evaluation of the Anticonvulsant Activity of Docosahexaeonic Acid-Like Molecules in Experimental Models of Seizures.* Ali Gharibi Loron, Soroush Sardari, Jamshid Narenjkar, Mohammad Sayyah. 1, January 2017, Iranian Biomedical Journal, 21: 32-39.
8. *Anticonvulsant, Anxiolytic and Neurotoxicity Profile of Aqarqarha (Anacyclus pyrethrum) DC (Compositae) Root Ethanolic Extract.* Syed Mohd Abbas Zaidi, Shadab Ahmad Pathan,Surender Singh,Shakir Jamil,Farhan Jalees Ahmad,Roop Krishen Khar. October 2013, Pharmacology & Pharmacy, 535-541.
9. Bum, E.Ngo. Antiepileptic Medicinal Plants Used in Traditional Medicine To Treat Epilepsy. [book auth.] Dr. Zaid Afawi. *Clinical and Genetic Aspects of Epilepsy.* s.l. : INTECH, 2011; 175-187.
10. *Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs.* Loscher, Wolfgang. 2011, Seizure, European Journal of Epilepsy, 20: 359-368.
11. *Therapeutic Assays for the Identification and Characterization of Antiepileptic and Antiepileptogenic Drugs.* H. Steve White, Misty Smith-Yockman, Ajay Srivastava, Karen S. Wilcox. 2006, Models of Seizures and Epilepsy, 539-549.
12. *Animal Models for Pre-Clinical Antiepileptic Drug Research.* Jagannatha, Lokesh Shetty. 2, 2015, Science, Technology and Development, 34: 82-85.

13. *Animal Models Used in the Screening of Antiepileptic Drugs.* Kupferberg, Harvey. 4, 2001, Epilepsia, 42: 7-12.
14. *Experimental determination of the anticonvulsant properties of some phenyl derivatives.* T.J. Putnam, H.H. Merritt. 1937, Science, 85: 525-526.
15. *MODELS OF EPILEPSY USED IN ANTIEPILEPTIC DRUG DISCOVERY: A REVIEW.* PHULEN SARMA, ANUSUYA BHATTACHARYYA. 11, 2014, International Journal of Pharmacy and Pharmaceutical Sciences, 6: 1-7.
16. [book auth.] S.K.Gupta. *Drug Screening Methods.* New Delhi,India : Jaypee Brothers Medical Publishers Ltd., 2004; 400.
17. *Trimetazidine exerts protection against increasing current electroshock seizure test in mice.* Seema Jain, Nidhi Bharal, Pramod Kumari Mediratta, Krishna Kishore Sharma. 2010, Seizure: Elsevier, 300-302.
18. *Preclinical development of antiepileptic drugs: Past, present and future directions.* White, H.S. 2003, Epilepsia, 44: 2-8.
19. Vogel, G.H. Vogel and W.H. *Drug Discovery and Evaluation of Pharmacological Assay.* Newyork : Springer Varlag Berlin Heidelburg, 2002.
20. *A REVIEW: ANIMAL MODELS USED IN THE SCREENING OF ANTIEPILEPTIC DRUGS* . Kasthuri.S, Karthigadevi.K, Manjulakshmi.P, S. Kavimani. 3, 2013, International Research Journal of Pharmaceutical and Applied Sciences, 3: 18-23.
21. *Evaluation of aantiepileptic activity of aqueous extract of leaves of Gossypium herbaceum in mice.* Sumalatha G, Sreedevi A. 4, 2012, International Journal of Pharmacy and Biological Sciences, 2: 349-353.
22. *ANALGESIC AND ANTICONVULSANT EFFECTS OF ACORUS CALAMUS ROOTS IN MICE.* R. Jayaraman, T. Anitha, Vishal D. Joshi. 1, 2010, International Journal of PharmTech Research, 2: 552-555.
23. *REVERSAL OF NEUROTOXICITY INDUCED COGNITIVE IMPAIRMENT ASSOCIATED WITH PHENYTOIN AND PHENOBARBITAL BY ACORUS CALAMUS IN MICE.* S.R. Yende, U.N.Harle, V.V.Bore, A.O.Bajaj, K.K.Shroff, Y.D.Vetal. 1, 2009, Journal of Herbal Medicine and Toxicology, 3: 111-115.
24. *Anticonvulsive activity of Butea monosperma flowers in laboratory animals.* Veena S. Kasture, S.B. Kasturea, C.T. Chopde. 2002, Pharmacology, Biochemistry and Behavior, 72: 965-972.
25. *Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from Delphinium denudatum.* Mohsin Raza, Farzana Shaheen, M.I Choudhary, Sompong

Sombati, Azhar Rafiq, Amin Suria, Atta-ur- Rahman, R.J DeLorenzo. 1, November 2001, Journal of Ethnopharmacology, 78: 73-78.

26. *Anticonvulsant activity of roots and rhizome of Glycyrrhiza glabra.* SHIRISH D. AMBAWADE, VEENA S. KASTURE, SANJAY B. KASTURE. 2002, Indian Journal of Pharmacology, 34: 251-255.
27. *Effect of methanolic extract of Hyoscymus niger L. on the seizure induced by picritoxin in mice.* Reza HM, Mohammad H, Golnaz E, Gholamreza S. 3, July 2009, Pak J Pharm Sci, 22: 308--12.
28. *Anticonvulsant activity of the leaf essential oil of Laurus nobilis against pentylenetetrazole and maximal electroshock-induced seizures.* M. Sayyah, J. Valizadeh, M. Kamalinejad. 2002, Phytomedicine, 9: 212-216.
29. *Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of Lavandula stoechas L.* A.H Gilani, N Aziz, M.A Khan, F Shaheen, Q Jabeen, B.S Siddiqui, J.W Herzig. 1, July 2000, Journal of Ethnopharmacology, 71: 161-167.
30. *Anxiogenic activity of Myristica fragrans seeds.* G.S. Sonavane, V.P. Sarveiya,V.S. Kasture, S.B. Kasture. 2002, Pharmacology, Biochemistry and Behavior, 71: 239-244.
31. *ANTICONVULSANT ACTIVITY OF FLOWER PART OF N. ODERUM.* POOJA SAINI, N. KANNAPAN,ANUPAMA DIWAN, PARVEEN KUMAR. 2, 2010, International Journal of Pharma and Bio Sciences, 1: 1-4.
32. *The fruit essential oil of Pimpinella anisum exerts anticonvulsant effects in mice.* M.H Pourgholamia, S Majzooba, M Javadia, M Kamalinejadb, G.H.R Fanaeea, M Sayyaha. 2, August 1999, Journal of Ethnopharmacology, 66: 211-215.
33. *Evaluation of the Anticonvulsant Activities of Rosa damascena on the PTZ Induced Seizures in Wistar Rats.* Masoumeh Kheirabadi, Ali Moghimi, Hassan Rakhshande and Morteza Behnam Rassouli. 2, 2008, Journal of Biological Sciences, 8: 426-430.
34. *Anti-seizure activity of the aqueous leaf extract of Solanum nigrum linn (solanaceae) in experimental animals.* Noel N Wannang, Joseph A Anuka,Helen O Kwanashie,Steven S Gyang, Asa Auta. 2, 2008, African Health Sciences, Vol. 8.
35. *AN EXPERIMENTAL EVALUATION OF ANTICONVULSANT ACTIVITY OF VITEX-NEGUNDO.* GUPTA, V. R. TANDON, R. K. GUPTA. 2, 2005, Indian J Physiol Pharmacol, 49: 199-205.
36. *studies on anticonvulsant activity of stem barks of calotropis gigantea in experimental animals.* Subhas S Karki, Suresh Babu A R. 1, 2010, International Journal of Pharmaceutical Sciences Review and Research, 5: 114-116.

37. *Evaluation of anti-epileptic activity of Cuscuta reflexa Roxb.* Sanket P Borole, Rajesh J Oswal, Rishikesh V Antre, Sandip S Kshirsagar, Yogita R Bagul. 1, 2011, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2: 657-663.