CHAPTER – 9

ANTI-EPILEPTIC ACTIVITY OF COMBINED EXTRACT OF

*Cissus Quadrangularis & Aegle Marmelos*

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ANTI-EPILEPTIC ACTIVITY OF COMBINED EXTRACT OF 
CISSUS QUADRANGULARIS & AEGLE MARMELOS

9.1. Introduction

Epilepsy is a neurological state that can cause disorders called seizures in the brain’s electrical function. According to the NINDS, group of nerve cells, neurons produce electrochemical impulses that takes action on another neurons, glands and muscles to generate human views, mind-set and actions.

The general kinds of seizure in adults are partial and generalized seizures. Collectively representing about 80% of common cases. In young people there are roughly identical records of partial and generalized seizures\(^1\).

9.1.1. Types of Seizures

Generalized seizures without focal onset, but with bilateral symmetry and loss of consciousness

- Grand mal (Tonic clonic movement)
- Petit mal (Absence seizures)
- Atonic seizures (Akinetic epilepsy)
- Myoclonic seizures
- Infantile spasms (Hypsarrhythmia)

Local motor - Extending into generalized seizures

Partial seizures without impairment of consciousness

- With motor march over one side of the body arising in thumb, index finger, hallux (Jacksonian motor).
• With sensory march over the body from a local region (Jacksonian sensory).

Partial seizures with temporal lobe symptomatology (generally with impairment but without loss of consciousness) characterized by

• Automatism (Psychomotor seizures, temporal lobe epilepsy, complex partial)
• Hallucinations (Psychological seizures)
• Autonomic accompaniments
• Simple partial seizures (cortical focal epilepsy)

**Generalized seizures**

Generalized seizures involve the whole brain including the reticular systems, thus producing abnormal electrical activity throughout both hemispheres. Immediate loss of consciousness is the characteristic feature of generalized seizures².

**Grand mal seizure**

It is typified by a generalized uncontrolled muscular contraction and termination of respiration followed by tonic and clonic spasms of the muscles. The teeth may perhaps be tightened the tongue bitten and bladder control is lost after the completion of this phase, the individual possibly will fall into a deep sleep for 1 hour or more. Typically the individual has no memory about the seizure on awakening. Grand mal seizures are preceded by an aura³.

**Petit mal seizure**

An epileptic seizure which occurs frequently in children and adolescents especially at the time of puberty, these seizure are characterized by sudden momentary loss of consciousness, with myoclonus of the neck and slight twitching of the face, loss of muscle tone, the patient ceases all voluntary motor activity with a rapid return of consciousness, during and between the seizure the EEG shows 3 cycles-per-second spike and wave discharges.

**Partial seizures**
These are seizures in which the discharge begins locally and often remains localized. The symptoms depend on the brain regions involved and include involuntary muscle contractions, abnormal sensory experiences or autonomic discharge or effects on mood and behavior. Partial seizures can often be attributed to local cerebral lesions and their incidences increases with age.

The neurotransmitter intervening the massiveness of synaptic transmission in the mammalian brain is amino acids i.e. GABA and Glutamate - The partial inhibitory and partial excitatory respectively.

**Jacksonian epilepsy**

These are fits beginning unilaterally or asymmetrically and may occur during any prolonged fits or series of fits but may be observed to fluctuate from side to side clonic jerking beginning in one of the three classical sites, the angle of the mouth, the thumb and index or the great toe is immediately recognizable.

**Psychomotor fits**

Psychomotor fits when occurring in isolation, i.e. without grand mal is often not recognized as epileptic or labeled petit mal. Such difficulties are perhaps inevitable as the precise limits of psychomotor epilepsy cannot be defined. The patient becomes unresponsive, flushed in the face, smacks his lips and mutters while rubbing his hands. The patient who experiences sudden causeless fear followed by brief confusion is probably having psychomotor fits. Frequent examples are aimlessly moving objects, muttering unintelligibly, starting to undress or abruptly leaving the room.

**Automatism**

Automatism may follow grand mal and in this state the patient usually arrives in some inappropriate place with no conscious of how he came there. If prolonged this amounts to fugue, but there is seldom much doubt of its epileptic nature as opposed to the much
commoner hysterical fugue with amnesia lasting for many hours or days. The EEG's of the accused of minor sexual crimes were interpreted and found that excluding epilepsy the records are normal, which shows that such crimes are frequently due to automatism.

9.1.2. Modern methods for diagnosing epilepsy

- **A brain scan**: CT scans (computed tomography) or MRI scans be able to illustrate the structure of various parts of the brain. They are used to discover the wounded or damaged brain.

- **EEG**: An EEG is absolutely painless and it helps to diagnosis and identifies the kind of epilepsy. Fix the sensor pads on the head that verify the electrical activity of the brain cells. The epileptic patients are advised to open and close their eyes and to see the blinking lights and take long breaths. Some kind of seizures creates identifiable EEG patterns. Some will not produce the changes in EEG pattern.

9.1.3. Genetic approaches to the epilepsy

Genetic causes contribute to a wide diversity of human epilepsy. Genetic causes are solely responsible for some rare forms inherited in a Mendelian pattern— for example, autosomal dominant or autosomal recessive. Genetic causes also are mainly responsible for some common forms like juvenile myoclonic epilepsy (JME) or childhood absence epilepsy (CAE), disorders like due to inheritance of two or more susceptible genes. Genetic determinants also may contribute some degrees of risk to epilepsies caused by injury of the cerebral cortex.

Whereas prior to 1994, a specific gene defect had been identified in only a single mouse with phenotype of cortical epilepsy more than 33 single gene mutations have been now linked to an epileptic phenotype. This progress has been paralleled by the genetics of human epilepsy mutations of more than a dozen such genes now have been identified.
Most of the human epilepsies for which mutant genes have been identified are symptomatic epilepsies in which the epilepsy seems to be a manifestation of some profound neurodegenerative disease. The mutant genes have been identified in four distinct forms of idiopathic human epilepsy remarkably each of the mutant genes encodes an ion channel gated by voltage or a neurotransmitter.

The four idiopathic human epilepsies for which the mutant genes have been identified are the following,

- Generalized epilepsy with febrile seizures (GEFS+) is caused by point mutation in the \( \beta \) subunit of a voltage-gated \( \text{Na}^+ \) channel. Interestingly, several anti-seizure drugs act on a \( \text{Na}^+ \) channels to promote their inactivation.
- Two forms of benign familial neonatal convulsions have been shown to be caused by mutations of two distinct but related novel \( \text{K}^+ \) channel genes.
- Autosomal dominant, nocturnal, frontal-lobe epilepsy is a fourth form of idiopathic epilepsy for which a mutant gene has been identified, the mutant gene encoding the \( \alpha - 4 \) subunit of the nicotinic cholinergic receptor\(^5\).

9.1.4. Drug induced seizures

- Lignocaine at normal blood levels act as anticonvulsant but at levels more than 5 \( \mu \text{g/ml} \), toxic effects are observed, generalized convulsions\(^6\).
- Metronidazole potentiates leptazole induced seizures and also produces convulsions when used in very high doses\(^7\).
- Penicillin, Benzyl penicillin and 6-amino penicillin have lowered the threshold for electroconvulsions. This is due to alteration in the storage or transport of GABA in CNS. Benzyl penicillin on administration for 8 days has produced seizures in mice\(^8\).

9.1.5. Seizures induced by Antiepileptic drugs (AED’S)
Some of the antiepileptic drugs are reported to induce seizures in patients with other disorders and in some others they are reported to aggravate the incidence and intensity of seizures.

**Phenobarbital**

This drug induces negative myoclonus with unilateral central spike wave in EEG in a child with atypical benign childhood epilepsy with centrotemporal spikes. It also induces tonic seizures in the children with Lennox-Gastaut syndrome and aggravated absence seizures in children.

**Benzodiazepines**

This AED’S are shown to exacerbate tonic seizures in patients with Lennox-Gastaut syndrome. Clonazepam induced tonic like seizures in infants with west syndrome.

**Phenytoin**

Phenytoin enhanced seizures in human and experimental animals. It also enhanced seizures induced by pentylenetetrazole, quisqualate and penicillin. It increased the severity of amygdaloid kindled seizures and produce brain damage secondary to this effect.

**Carbamazepine**

It aggravates generalized seizures, it exacerbate seizure frequency in patients with angel men and Landau-kleffner syndrome. It can aggravate generalized tonic clonic seizures (GTC) and also status epilepticus⁹.

**Lamotrigine**

It aggravates myoclonic seizure when used as an add on treatment.

**Gabapentin**
The drug is reported to induce myoclonus in patients with partial epilepsy.

**Valproic acid**

It aggravates myoclonic and status epilepticus\(^1\).

### 9.1.6. Role of Neurotransmitters

CNS function can be viewed as the transfer of information from one neuron to another. One neuron influence another by stereo typed sequences of electrical and chemical events that involve action potential along an axon release of neurotransmitter binding of the neurotransmitter to a receptor and transduction of the neurotransmitter signal into a change in the state of the post synaptic level.

Alternation at each step in the sequence of information transmission can occur with aging. The major neurotransmitters in the brain are acetylcholine, dopamine, nor epinephrine, Serotonin (5-HT). The amount of each neurotransmitter varies in different regions of the brain and particular subsets of neurons with in those regions. Alteration in the levels of individual neurotransmitter in their synthesis or release can effect synaptic transmission.

Neurobiological basic research as well as clinical studies has revealed that the monoamines have a crucial role in the development of the depression syndrome. The major systems that are involved in the regulation of functions that are mediated by central nervous system are

- Acetylcholine
- Catecholamines
- Indolamines
- GABA

**Acetylcholine (Ach)**
Acetylcholine plays a major role in regulating the Autonomous Nervous System (ANS) apart from which it performs certain functions in the Central Nervous System that are necessary for the performance of everyday tasks of life, mainly the regulation of learning and memory. Acetylcholine pioneers memory that has been acquired by learning and memory retention\textsuperscript{11}.

The various actions of Acetylcholine (Ach) in CNS are

- Increased firing rate in corpus callosum.
- Decline in cholinergic function in dementia is predominantly related to a decrease in cholinergic neurotransmission.
- Anticholinergic drug, Scopolamine is a drug most widely used to induce amnesia in experimental animals.
- Acetylcholinesterase inhibition, which enhances the availability of Ach in the synaptic cleft, is able to reverse the scopolamine induced memory deficit, indicating a neurotransmitter role of Acetylcholine in learning and memory.
- Muscarinic Type I receptor antagonist- pirenzepine and nicotinic antagonist- mecamylamines also have a negative effect on learning and memory\textsuperscript{12}.
- Antidepressants like Amitryptyline and Imipramine have anticholinergic effect and this may attribute to the most adverse effects on memory, whereas fluoxetine, a newer antidepressant that lacks Anticholinergic effect did not exhibit the memory impairing effect\textsuperscript{13}.
- Alzheimer’s disease, a neurodegenerative condition mainly an apparent memory loss, is due to deficiency of cholinergic neurons and Ach. Thus various beneficial approach to enhance cerebral concentration of Ach like cholinergic precursors, cholinergic receptor agonists, cholinesterase inhibitors and Acetylcholine release modulators have been estimated in Alzheimer’s disease\textsuperscript{14}.
- The learning of inactive prevention task is recognized to be develop by cholinergic synapses moderately than active prevention\textsuperscript{15}.
• The cognition facilitating effect of 5-HT<sub>3</sub> receptor antagonist is believed to be secondary to increased release of Acetylcholine<sup>16</sup>.

• Metronidazole on chronic administration lowers the threshold for convulsions in addition to increasing the intensity. This may be due to sensitization of cholinergic system in CNS<sup>17</sup>.

• Cholinergic mechanisms are involved in leptazole-induced convulsions.

**Catecholamines**

Reduction of Dopamine concentration by Prostaglandin D and E has been considered to be a possible mechanism of anticonvulsant effect has been reported To increase the brain levels of Dopamine and Noradrenaline, prostaglandin synthesis is inhibited which produces an inhibition of seizure activity<sup>18</sup>.

Pharmacological experiments in rodent systems have frequently shown that the treatment which depletes the brain monoamines lowers seizure threshold whereas treatments which inhibit MAO, tend to raise seizure threshold in some test systems this changes appear to depend primarily on Catecholamine while in others on Serotonin, Dopamine and Noradrenaline appear to protest against electroshock induced convulsions in rats. Whereas 5-HT protects against PTZ induced convulsions<sup>19</sup>.

Catecholamine and 5-HT in the brain have been one of the etiology of depression, Adrenaline, Noradrenaline directly introduced in the brain causes CNS depression. Decreased Dopamine leads to catalepsy and depression.

Sedative and hypnotic activity may be due to increased concentration of Adrenaline (ADR) and Noradrenaline (NA) and a decreased concentration of Dopamine, drugs that increase 5-HT in brain cause sleepiness, drugs that decrease 5-HT induce permanent awareness<sup>20</sup>.

Noradrenaline is crucial in certain cognitive functions associated with frontal lobe. Prazosin (α<sub>1</sub> adrenergic antagonists) produced highest degree of catalepsy, which may be due
to a decrease in the dopamine activity, further sub cataleptic doses of Haloperidol (0.2 mg/kg) induced highest degree of catalepsy with prazosin, this may be due to simultaneous decrease in the dopaminergic and adrenergic activity. It also suggests possible involvement of central pre and post synaptic Adrenergic and dopaminergic neurons in catalepsy²¹.

Noradrenaline and dopamine show excitatory and inhibitory effects, rate of firing is not altered by catecholamines but altered by acetylcholine. Caudate nucleus has two distinct types of dopamine receptors²².

D₁ sensitive sites are excitatory mediating receptors and are activated by dopamine, levodopa, dexamphetamine and inhibited by haloperidol D₂ inhibition mediation receptors and are activated by dopamine, levodopa and dexamphetamine, inhibited by ergometrine and noradrenaline.

Chemicals which deplete or antagonize catecholamine and 5-HT (Serotonin) disrupt behavior.

5-HT (Serotonin)

Prostaglandins D and E series are reported to have anticonvulsant effect which was found to be 5-HT mediated. Elevation of 5-HT in the brain confers more protection against seizure induction and possibly severity of seizures. 5-HT₃ receptor antagonist exhibited anticonvulsant and antianxiety activity. The anticonvulsant effect of MAO inhibitors may be due to increase in serotonergic activity.

Decrease in central serotonergic functions may form a basic of action of some anxiolytics including Buspirone, Ondansetron and partly Benzodiazepines (BZD’s). 5-HT₃ receptor antagonists attenuate anxiogenic response and an increase in dopaminergic activity⁰²³. 5-HT₃ receptor antagonists like Ondansetron exhibit significant cognition facilitating effect and have a good anxiolytic activity. It is now generally accepted that stimulation of serotonergic neurotransmitter system impairs learning and memory function. It
has been observed that increased serotonin in brain may mediate the decreased release of acetylcholine, via the 5-HT receptor activation, which simultaneously causes dementia associated with aging. The cognition facilitatory effect of 5-HT receptor antagonist is believed to be secondary to increased release of acetylcholine.

Enhancement in serotonergic transmission elevates the significance of PTZ induced seizures in animal test systems thus protecting against PTZ induced convulsions. In neuroleptic drug induced catalepsy, which occurs primarily due to the blockade of dopaminergic neurotransmitter is generally attenuated by noradrenergic neurotransmitter whereas as serotonergic neurotransmitter exacerbates it.

5-HT2 receptor antagonist, 5-HT2c receptor antagonist, 5-HT4 receptor against and 5-HT3 receptor antagonist increased the cataleptic effect. Apart from central dopaminergic and cholinergic mechanisms in neuroleptic induced catalepsy, serotonin has been recently shown to have an important role as well and involvement of serotonin and prostaglandin in interaction of stress and neuroleptic catalepsy.

Ondansetron, a selective 5-HT3 receptor antagonist reduces haloperidol induced catalepsy. The serotonin regulates mood, sleep, memory, learning and sexual behaviors. Serotonin agonists, precursors and neuronal uptake inhibitors improve neuroleptic catalepsy.

**GABA (γ-Amino Butyric Acid)**

Epileptogenic properties of penicillin in mice could be due to alteration in the storage or transport of GABA in CNS. Benzodiazepine receptor antagonists showed an enhancement of learning and memory in mice and in human GABA and Glycine, in mammals have a function of inhibition of some central neuronal processes, while Glutamate has excitatory function.

**PG’S (Prostaglandins)**
Diclofenac, Paracetamol and Hydrocortisone significantly increase the latency of onset of clonic seizures. PGE$_2$ known to induce catalepsy but is prominent only in large doses. Midbrain raphe lesions, which reduce central serotonergic activity, reduce cataleptic effect of neuroleptics as well as restraint stress induced potential of neuroleptic catalepsy.

Prostaglandins synthesis inhibitors Paracetamol and Indomethacin both diminished catalepsy implicating that prostaglandins are having a modulatory role on brain monoamine activities decreasing central sympathetic and increasing central serotonergic neurotransmitter.

9.2. MATERIALS AND METHODS

9.2.1. Materials

Combined ethyl acetate extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-EACA) and combined ethanol extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-ECA), Vehicle -1% tween 80, Oral needle.

9.2.2. Experimental Animals

Wistar albino rats bearing the weight of 180-230 gm were used. They were maintained in Santhiram College of Pharmacy, Nandyal, Andhra Pradesh, India. The animals were kept in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages. Institutional Animal Ethical Committee (IAEC) clearance was done with reference no 1519/PO/a/11/CPCSEA).

9.3. ANTIEPILEPTIC ACTIVITY

9.3.1. Maximal Electro Shock induced Convulsions

Procedure

In the maximal electro shock induced seizure experiment, the MES method$^{208}$ described previously by Swinyard et al., was employed$^{27}$. By means of an electro
convulsiometer, seizures were stimulated to each group. Maximal electroshock seizures were obtained by a 60Hz discontinuous current of 150 mA strength for 0.2 sec. Earlier to apply for rats, a drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes. It helps to enhance the contact and diminish the occurrence of fatalities. The c-EACA & c-ECA were given for 14 days before stimulation of seizures. The time interval of different stages of epilepsy was noted. The safety percentage was evaluated by monitoring the number of animals showing abolition of Hind Limb Tonic Extension (HLTE) or extension not greater than 90°. The time interval of tonic extension of hind limb was indicated as end point, i.e. hindrance or a reduction in the duration of hind limb extension was considered as a defensive action.

9.3.1.1. Experimental Design

Animals were separated into 6 groups and each group is comprised of six rats.

**Group I** – Animals treated with Vehicle 1% Tween 80 (5 ml/kg, p.o).

**Group II** – Animals treated with Phenytoin (25 mg/kg, i.p)

**Group III & IV** - Animals treated with combined ethyl acetate extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-EACA) 250 & 500 mg/kg, p.o

**Group V & VI** - Animals treated with combined ethanol extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-ECA) 250 & 500 mg/kg, p.o once daily for 14 days respectively.

9.3.2. Pentylenetetrazole Induced Convulsions

**Procedure**
Pentylenetetrazole (PTZ - 90 mg/kg, s.c) was to stimulate clonic convulsions animals were evaluated for a period of 30 mins post – PTZ administration. The factors observed were mean onset time of convulsions, period of convulsion and recovery/Death (% recovery or % of survival) caused by PTZ. The c-EACA & c-ECA were given for 14 days prior to stimulation of seizures.\textsuperscript{28}

9.3.2.1. Experimental Design

Animals were separated into 6 groups comprised of six rats in all group.

**Group I** – Animals treated with Vehicle 1% Tween 80 (5 ml/kg, p.o).

**Group II** – Animals treated with Diazepam, (4 mg/kg, i.p)

**Group III & IV** - Animals treated with combined ethyl acetate extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-EACA) 250 & 500 mg/kg, p.o

**Group V & VI** - Animals treated with combined ethanol extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-ECA) 250 & 500 mg/kg, p.o once daily for 14 days respectively.

9.3.3. Estimation of neurotransmitter concentrations in rat brain after induction of epilepsy

A variety of biogenic amines in distinct section of the rat brain were evaluated.

**Preparation of Reagents**

- HCl – Butanol: 0.85 ml 37% HCl was added to one liter of butanol to get HCl – butanol solution.
- Dilute 0.1 M HCl solution: 0.5 ml of concentrated HCl with 100 ml of distilled water to get 0.1 M HCl.
- O-phthaldialdehyde reagent: 20 mg of reagent in 100 ml of concentrated HCl.
• 0.4 M Hydrochloric acid: 3.4 ml of concentrated HCl in 100 ml of distilled water.
• EDTA (pH 6.9): Disodium Ethylene Diamine Tetra Acetate 37.2 g was dissolved in 950 ml of 1 M sodium acetate and the pH was adjusted to 6.9 with 10 M sodium hydroxide.
• 10 M sodium hydroxide: 40 g of NaOH pellets in 100 ml of distilled water.
• 5 M sodium hydroxide: 20 g of NaOH pellets in 100 ml distilled water.
• Iodine in ethanol: 4 g of potassium iodide and 2.6 g of iodine in 100 ml of distilled water.
• Sodium sulphitein 5 M NaOH: 0.5 g Sodium sulphite in 2 ml distilled water added to 18 ml of 5 M sodium hydroxide.
• 10 M Acetic acid: 57 ml of Acetic acid solution in 100 ml distilled water.

9.3.4. Preparation of Tissue Extracts

By means of decapitation method, on the 15th day of (MES and PTZ) evaluated animals were sacrificed. Remove the brains, Dissect the forebrain and Discard the cerebellum. Brains were put on ice and separate and weigh the cortex, striatum and subcortical regions. Rat brains which are dissected and freezed and were cut on a cooled microtome (-20°) into anterior slices (about 1 mm thick) at programmed anteroposterior levels. The anterior slices were consequently positioned on the iced up stage (-20°C) of a punching apparatus where cylindrical tissue samples (generally 1 mm in diameter, same width as the slice) were punched out of chosen brain regions with a glass tube. The tissue pieces were instantly shifted to pre-cooled micro homogenizers stored at -25°C for the determination of weight.

The tissue was homogenized in 0.1 ml HCl-Butanol for 1 minute in a glass homogenizer the total volume was measured to give 0.105 ml, taking account of the tissue volume (1 mg = 0.001 ml) centrifuge the sample for 10 min at 2000 rpm supernatant (0.8 ml) was removed and add to an eppendorf reagent tube which consists of 0.2 ml heptane (for
spectroscopy) and 0.025 ml HCl 0.1 M after 10 min of continuous shaking, the tube was centrifuged to divide the two phases and the upper organic phase was removed, the aqueous phase (0.02 ml) was then taken either for 5-hydroxy tryptamine or noradrenaline and dopamine assay. This test was done at 0°C.

**Serotonin Assay**

Some alteration in reagent concentration became essential together with change in solvent proportion, so as to attain in a fine fluorescence yield with low volume for 5-HT determination; the O-phtaldialdehyde (OPT) process was used. Add 0.025 ml of OPT reagent to 0.02 ml of the HCl extract. On heating at 100°C for 10 min the fluorophore was developed. When the sample attains the equilibrium with the ambient temperature, intensity was readed at 360-470 nm.

**Nor-Adrenaline and Dopamine Assay**

It denotes a efficiency of the trihydroxide method. Add 0.05 ml 0.4 M and 0.01 ml EDTA/Sodium acetate buffer (pH 6.9) to 0.02 ml of HCl phase, along with 0.01 ml iodine solution (0.1 M in ethanol) for oxidation. It was stored after two minutes by adding 0.01 ml Na$_2$SO$_3$ in 5 m NaOH. Add acetic acid after 1.5 minutes. Heat the solution to 100°C for 6 min. When the sample attains the room temperature, intensity were read in the microcuvette as with 5-HT: the evaluation were limited to the excitation maxima at 395-485 nm for NA and 330-375 nm for DA.

**Estimation of brain GABA content**

The brain amino butyric acid (GABA content was estimated according to the method of Lowe et al., (1958). Animals were sacrificed by decapitation and brains were rapidly removed, and separated forebrain region. It was blotted, weighed and placed in 5ml of ice-cold trichloroaceticacid (10% w/v), then homogenized and centrifuged at 10,000rpm for 10min at 0°C. A sample (0.1ml) of tissue extract was placed in
0.2ml of 0.14 M ninhydrin solution in 0.5M carbonate-bicarbonate 1 buffer (pH9.95), kept in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10min fluorescence at 377/455nm in a spectofluorimeter was recorded.

9.3.5. Statistical Analysis

The data were represented as mean ± standard error mean (S.E.M). The importance of variations between the groups was evaluated by means of one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett’s test p values less than 0.05 were measured as significance.

9.4. RESULTS AND DISCUSSION

9.4.1. Effects of c-EACA and c-ECA on MES Induced Epilepsy

In MES induced epilepsy, negative control group (vehicle treated) showed the duration of tonic hind leg extension was 15.17 ± 0.4773 seconds. The c-EACA at doses of 250 mg/kg and 500 mg/kg protected the animals from seizures and significantly (p<0.01) reduced the duration of tonic hind leg extension to 8 ± 0.3651 and 3.33 ± 0.2108. While the c-ECA at doses of 250 mg/kg and 500 mg/kg protected the animals from seizures and significantly (p<0.01) decreases the duration of tonic hind leg extension to 6.83 ± 0.3073 and 2.33 ± 0.2108 seconds, correspondingly.

The standard drug phenytoin abolished tonic hind leg extension in the animals. Phenytoin treated animals had 100% protection against MES stimulated seizures while c-EACA 250 & 500 mg/kg and c-EACA 250 & 500 mg/kg had 47.26%, 78.05% and 54.98%, 84.64 protection, correspondingly (Table 9.1 and Figure 9.2).
9.4.2. Effect of c-EACA and c-ECA on PTZ stimulated epilepsy

In PTZ stimulated epilepsy, negative control rats treated with vehicle, duration of clonic convulsion appear for $77.83 \pm 1.833$ seconds after PTZ and all rats died after seizures. The c-EACA at doses of 250 and 500 mg/kg considerably postponed the onset of clonic convulsions for $337.67 \pm 4.153$ ($p<0.01$) and $428.5 \pm 2.446$ ($p<0.01$) seconds, correspondingly in a dose dependent manner. The c-ECA at doses of 250 and 500 mg/kg considerably postponed the onset of clonic convulsions for $368.5 \pm 3.106$ ($p<0.01$) and $527.83 \pm 4.045$ ($p<0.01$) seconds, correspondingly.

While, the standard drug diazepam (4 mg/kg, i.p) postponed the onset of clonic convulsions for $707.67 \pm 4.264$ seconds. Diazepam treated animals produced 100% protection against PTZ stimulated seizures. Diazepam treated animals have exposed 100% protection against PTZ stimulated seizures while c-EACA 250 mg/kg and 500 mg/kg have produced 46.89% and 60.81% protection, c-ECA 250 mg/kg and 500 mg/kg have produced 57.18% and 66.67% protection correspondingly against PTZ stimulated seizures.

The c-EACA & c-ECM 250 mg/kg and 500 mg/kg had produced a considerable enhance in onset of clonic convulsion and similar ($p<0.01$ and $p<0.01$) with the standard (Table 9.5 and Fig.9.6). Mortality of c-EACA & c-ECA 250 mg/kg and 500 mg/kg treated rats is decreased as compared to negative control.

9.4.3. Effect of c-EACA & c-ECA on neurotransmitter levels in MES & PTZ stimulated seizure rats

Noradrenaline

In MES and PTZ models, Noradrenaline levels considerably ($p<0.01$) reduced in brain of epileptic control animals. The c-EACA & c-ECA at the doses of 250 & 500 mg/kg,
standard drugs phenytoin and diazepam treated animals produced a considerably (p<0.05 & p<0.01) better Noradrenaline levels in brain (Table 9.2, 9.6 and Figure 9.3, 9.7).

**Dopamine**

In MES and PTZ models, Dopamine levels considerably (p<0.01) reduced in brain of epileptic control animals. The c-EACA & c-ECA at the doses of 250 & 500mg/kg, standard drugs phenytoin and diazepam treated animals produced a considerably (p<0.05 & p<0.01) better Dopamine levels in brain (Table 9.3, 9.7 and Figure 9.4, 9.8).

**Serotonin**

In MES and PTZ models, Serotonin levels considerably (p<0.01) reduced in forebrain of epileptic control animals were noted. The c-EACA & c-ECA at the doses of 250 & 500 mg/kg, standard drugs phenytoin and diazepam treated animals produced a considerably (p<0.05 & p<0.01) better Serotonin levels in brain (Table 9.4, 9.8 and Figure 9.5, 9.9).

**Gamma amino butyric acid**

In MES and PTZ models, GABA levels significantly (p<0.01) decreased in brain of epileptic control animals were observed. The c-EACA & c-ECA at the doses of 250 & 500 mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly (p<0.01) increased in GABA levels in brain of rats (Table 9.5, 9.10 and Figure 9.6, 9.11).
Fig. 9.1: STAGES OF CONVULSION

MYOCLONIC JERKING

RECOVERY

Fig. 9.1: STAGES OF CONVULSION
Table 9.1: Effect of c-EACA and c-ECA on MES stimulated epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of treatment</th>
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<th>Recovery</th>
<th>% protection</th>
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<td>7.67±0.3333</td>
<td>15.17±0.4773</td>
<td>19±0.3651</td>
<td>37.33±0.7601</td>
<td>177.5±4.233</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Phenytoin 25 mg/kg,i.p.</td>
<td>2.33±0.2108**</td>
<td>0.00±0.0000**</td>
<td>9.67±0.3333**</td>
<td>17.5±0.4282**</td>
<td>89.33±1.626**</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>c-EACA 250 mg/kg,p.o</td>
<td>6±0.2582**</td>
<td>8±0.3651**</td>
<td>16.5±0.3416**</td>
<td>35.17±0.9098</td>
<td>155.5±0.7638**</td>
<td>47.26</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 500 mg/kg,p.o</td>
<td>4.83±0.1667**</td>
<td>3.33±0.2108**</td>
<td>13.33±0.4944**</td>
<td>26.17±0.8724**</td>
<td>134.33±1.476**</td>
<td>78.05</td>
</tr>
<tr>
<td>V</td>
<td>c-ECA 250 mg/kg,p.o</td>
<td>5.33±0.3333**</td>
<td>6.83±0.3073**</td>
<td>15.83±0.3073**</td>
<td>32.33±0.6667*</td>
<td>150.67±2.040**</td>
<td>54.98</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 500 mg/kg,p.o</td>
<td>4.17±0.3073**</td>
<td>2.33±0.2108**</td>
<td>12.5±0.500**</td>
<td>24±0.5774**</td>
<td>128.5±1.727**</td>
<td>84.64</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between Group I Vs Group II, Group II Vs Group III, Group IV, Group V & Group VI
A statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test.*p<0.05; **p<0.01.

Fig.9.2: Effect of c-EACA and c-ECA on MES stimulated epilepsy
Table 9.2: Effect of c-EACA and c-ECA on Noradrenaline levels in MES to stimulate epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>Noradrenaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>771.17±3.311**</td>
</tr>
<tr>
<td>II</td>
<td>MES</td>
<td>421.67±3.018</td>
</tr>
<tr>
<td>III</td>
<td>Phenytoin 25 mg/kg.i.p.</td>
<td>719.83±2.072**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg.p.o</td>
<td>543.33±2.836**</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg.p.o</td>
<td>613.17±2.372**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg.p.o</td>
<td>577.83±2.104**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg.p.o</td>
<td>634.17±2.301**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test.

*p<0.05; **p<0.01. Units = ng/mg of wet tissue.
Fig.9.3: Effect of c-EACA and c-ECA on Noradrenaline levels in MES stimulated epilepsy

Table 9.3: Effect of c-EACA and c-ECA on Dopamine levels in MES stimulated epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>646.50±2.405**</td>
</tr>
<tr>
<td>II</td>
<td>MES</td>
<td>464.83±2.994</td>
</tr>
<tr>
<td>III</td>
<td>Phenytoin 25 mg/kg, i.p.</td>
<td>610.67±1.542**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg, p.o</td>
<td>525.33±1.944**</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg, p.o</td>
<td>554.17±2.982**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg, p.o</td>
<td>543.83±2.227**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg, p.o</td>
<td>579±2.989**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII
Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test. *p<0.05; **p<0.01. Units = ng/mg of wet tissue.

![Graph showing the effect of c-EACA and c-ECA on Dopamine levels in MES stimulated epilepsy](image)

**Table 9.4: Effect of c-EACA and c-ECA on Serotonin levels in MES stimulated epilepsy**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>185.17±1.759**</td>
</tr>
<tr>
<td>II</td>
<td>MES</td>
<td>73.33±1.453</td>
</tr>
<tr>
<td>III</td>
<td>Phenytoin 25 mg/kg, i.p.</td>
<td>133.17±2.574**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg, p.o</td>
<td>94.67±2.404**</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg, p.o</td>
<td>112.17±2.072**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg, p.o</td>
<td>101.83±2.120**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg, p.o</td>
<td>120.33±2.011**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII
Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test.

*p<0.05; **p<0.01. Units = ng/mg of wet tissue.

Fig 9.5: Effect of c-EACA and c-ECA on Serotonin levels in MES stimulated epilepsy

Table 9.5: Effect of c-EACA and c-ECA on GABA levels in MES stimulated epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>281±2.477**</td>
</tr>
<tr>
<td>II</td>
<td>MES</td>
<td>219.17±1.35</td>
</tr>
<tr>
<td>III</td>
<td>Phenytoin 25 mg/kg,i.p.</td>
<td>285.83±1.72**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg, p.o</td>
<td>245.00±1.79**</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg, p.o</td>
<td>267.00±1.07**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg, p.o</td>
<td>263.33±1.05**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg, p.o</td>
<td>275.00±1.59**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM
Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test.
*p<0.05; **p<0.01. Units = ng/mg of wet tissue.

Fig 9.6: Effect of c-EACA and c-ECA on GABA levels in MES stimulated epilepsy
Table 9.6: Effect of c-EACA and c-ECA on PTZ stimulated convulsion

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of Treatment</th>
<th>Onset of convulsion (sec.)</th>
<th>Duration of convulsion (sec.)</th>
<th>Protection convulsion %</th>
<th>Protection mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle control</td>
<td>180.33±2.603</td>
<td>77.83±1.833</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (4 mg/kg, i.p)</td>
<td>707.67±4.264**</td>
<td>12.33±0.7601**</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>c-EACA 250 mg/kg, p.o</td>
<td>337.67±4.153**</td>
<td>41.33±1.308**</td>
<td>46.89</td>
<td>66.67</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 500 mg/kg, p.o</td>
<td>428.5±2.446**</td>
<td>30.50±1.057**</td>
<td>60.81</td>
<td>100</td>
</tr>
<tr>
<td>V</td>
<td>c-ECA 250 mg/kg, p.o</td>
<td>368.5±3.106**</td>
<td>33.33±1.801**</td>
<td>57.18</td>
<td>83.33</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 500 mg/kg, p.o</td>
<td>527.83±4.045**</td>
<td>25.17±0.8724**</td>
<td>67.66</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between Group I Vs Group II, Group II Vs Group III, Group IV, Group V & Group VI

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test

*p<0.05; **p<0.01;
Fig. 9.7: Effect of c-EACA and c-ECA on PTZ stimulated convulsion
Table 9.7: Effect of c-EACA and c-ECA on Noradrenaline levels in PTZ stimulated epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>Noradrenaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>771.17±3.311**</td>
</tr>
<tr>
<td>II</td>
<td>PTZ</td>
<td>521±1.592</td>
</tr>
<tr>
<td>III</td>
<td>Diazepam(4 mg/kg, i.p)</td>
<td>738±2.295**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg, p.o</td>
<td>589±2.490**</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg, p.o</td>
<td>673.5±1.784**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg, p.o</td>
<td>634.67±2.836**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg, p.o</td>
<td>710.83±4.214**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test. *p<0.05; **p<0.01. Units = ng/mg of
and c-ECA on Noradrenaline levels in PTZ stimulated epilepsy

Table 9.8: Effect of c-EACA and c-ECA on Dopamine levels in PTZ stimulated epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>646.5±2.405**</td>
</tr>
<tr>
<td>II</td>
<td>PTZ</td>
<td>546.67±2.565</td>
</tr>
<tr>
<td>III</td>
<td>Diazepam(4 mg/kg, i.p)</td>
<td>637.17±2.774**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg, p.o</td>
<td>576±2.017**</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg, p.o</td>
<td>605.33±2.362**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg, p.o</td>
<td>587.5±2.291**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg, p.o</td>
<td>614.83±2.372**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM

Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII
Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test. *p<0.05; **p<0.01. Units = ng/mg of wet tissue.

Fig.9.9: Effect of c-EACA and c-ECA on Dopamine levels in PTZ stimulated epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>185.17±1.759**</td>
</tr>
<tr>
<td>II</td>
<td>PTZ</td>
<td>87.67±1.229</td>
</tr>
<tr>
<td>III</td>
<td>Diazepam(4 mg/kg,i.p)</td>
<td>126.17±2.442**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg,p.o</td>
<td>92.17±1.222*</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg,p.o</td>
<td>99.83±1.400**</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg,p.o</td>
<td>101.67±2.076**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg,p.o</td>
<td>110.83±1.905**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM
Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test. *p<0.05; **p<0.01. Units = ng/mg of wet tissue.

Fig.9.10: Effect of c-EACA and c-ECA on Serotonin levels in PTZ stimulated epilepsy

Table 9.10: Effect of c-EACA and c-ECA on GABA levels in PTZ induced epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Used</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>281±2.477**</td>
</tr>
<tr>
<td>II</td>
<td>PTZ</td>
<td>211.00±1.461</td>
</tr>
<tr>
<td>III</td>
<td>Diazepam(4 mg/kg,i.p)</td>
<td>290.17±0.833**</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 250 mg/kg,p.o</td>
<td>238.00±1.653*</td>
</tr>
<tr>
<td>V</td>
<td>c-EACA 500 mg/kg,p.o</td>
<td>272.00±1.238**</td>
</tr>
<tr>
<td>Group</td>
<td>Treatment</td>
<td>GABA levels (ng/mg of wet tissue)</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 250 mg/kg.p.o</td>
<td>268.50±0.428**</td>
</tr>
<tr>
<td>VII</td>
<td>c-ECA 500 mg/kg.p.o</td>
<td>280.50±0.885**</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SEM.

Comparison between: Group I Vs Group II; Group II Vs III, IV, V, VI and Group VII

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test. *p<0.05; **p<0.01. Units = ng/mg of wet tissue.

Fig.9.11: Effect of c-EACA and c-ECA on GABA levels in PTZ stimulated epilepsy

9.5. CONCLUSIONS

The recent man-made antiepileptic Agents are efficient in about 50% of patients; several cases remain challenging to antiepileptic drug management. These agents are related to side effects with chronic toxicity, teratogenicity, adverse effects on cognition and behavior.

The MES test is the commonly used as an animal model for detection of anticonvulsant activity of agents for the generalized tonic-clonic seizures "grand mal". These models based on examination...
of the stimulus by frequent electrical pulses stimulated in various neuronal structures one typical standard of epileptic action 36.

In our recent study, it is found that treatment with c-EACA & c-ECA on rats considerably reduces in tonic hind leg extensor stage in MES stimulated epilepsy. The MES model to recognize compounds which stop seizure spread, subsequent to generalized tonic-clonic seizures in humans37, 38. At present, used anticonvulsant agents (e.g. phenytoin, carbamazepines) efficient in therapy of generalized tonic-clonic and partial seizures in MES test 39, 40. Since, c-EACA & c-ECA considerably inhibited generalized tonic-clonic seizures in MES test; it proposes the presence of anticonvulsant substances.

Treatment with c-EACA & c-ECA on PTZ stimulated rats considerably lessen the period of convulsion and postponed the onset of clonic convulsion. Though animal models based on pentylentetrazole have still been extensively used to test the drug, the method by which pentylentetrazole obtain its action has not been understood. Pentylentetrazole produce its action is by acting as an antagonist at the picrotoxin sensitive site of the GABA receptor complex 41.

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect42. In addition to the GABA binding site, the GABA\textsubscript{A} receptor complex appears to have distinct allosteric binding sites for benzodiazepines, barbiturates, ethanol etc.43. We therefore studied the effect of c-EACA & c-ECA on brain GABA content. c-EACA & c-ECA showed significant (p<0.01) increased GABA content in brain dose dependently. This suggests that the anticonvulsant activity of c-EACA & c-ECA is probably through elevation of brain GABA content.

As PTZ has been exposed to cooperate with the GABA neurotransmission44 and PTZ stimulated seizures can be avoided by c-EACA & c-ECA that improves GABA receptor-mediated inhibitory neurotransmission like benzodiazepines and phenobarbital45, the antagonism of PTZ- stimulated seizures proposes the interaction of the c-EACA & c-ECA with the GABAergic neurotransmission.
The role of noradrenaline, dopamine and serotonin in seizure remains controversial, but these neurotransmitters convincingly been implicated in the pathophysiology of seizures\textsuperscript{46, 47}. Medicinal plants used for the therapy of epilepsy have been revealed to have promising anti-epileptic activities.

It has been specified that many experimental procedures planned to enhance biogenic amines activity have established anticonvulsant properties\textsuperscript{48-52}.

The recognized antiepileptic drugs like phenytoin and diazepam renovate the biogenic amines level on brain. Likewise c-EACA & c-ECA considerably (p<0.05 & p<0.01) has better monoamines levels in forebrain of rats. The antiepileptic role of endogenous nor epinephrine was secondary from studies that reported injurious effects of nor epinephrine system on seizures stimulated by electrical stimulation of chemo convulsants (PTZ). The c-EACA & c-ECA considerably (p<0.05 & p<0.01) enhanced the noradrenaline in forebrain of rats and confirm the anticonvulsant activity.

Reserpine lacks specificity, since this drug also depletes gamma amino butyric acid (GABA), serotonin (5-HT) and dopamine, in addition to norepinephrine. Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines\textsuperscript{53}. Subsequent the present studies confirmed and c-EACA & c-ECA considerably enhanced the gamma amino butyric acid (GABA), serotonin (5-HT), dopamine and nor epinephrine.

Biogenic amines contribute in enhancing the threshold of Maximal electroshock and pentylenetetrazole stimulated seizure in rat models. Thus it reveals that c-EACA & c-ECA have antiepileptic potential. These consequences sustain the ethno medicinal uses of these plants in the treatment of epilepsy.