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Research Article

EVALUATION OF ANTI-EPILEPTIC ACTIVITY OF ETHANOL EXTRACT OF ROOTS OF *ACALYPHA FRUTICOSA* WITH DIAZEPAM

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ABSTRACT

The Present study is to evaluate the antiepileptic activity of ethanol extract of roots of Acalypha fruticosa . The antiepileptic activity of ethanol extract of Acalypha fruticosa at the doses of 30, 100 and 300 mg/kg, p.o. was evaluated by Maximum electroshock (MES), Pentylenetetrazole (PTZ)-induced convulsions in mice. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's test. In PTZ-induced convulsions the administration of EEAF (30, 100 and 300 mg/kg p.o.) increased the onset time of jerks, tonic and clonic convulsions in mice compared to control group mice EEAF decreased the duration of PTZ-induced seizure reflexes in mice when compared to control group animals and showed increased protection against seizure susceptibility. In MES model the administration of EEAF (30, 100 and 300 mg/kg p.o.) increased the onset time of tonic hind limb extension in mice when compared to control group mice and it decreased the duration of extension of hind limbs when compared to control group animals. The ethanol extract of roots of Acalypha fruticosa exhibited significant and dose-dependant antiepileptic activity in both MES and PTZ-induced convulsions.

KEYWORDS: *Acalypha fruticosa, Diazepam, Epilepsy, Pentylenetetrazole, MES model.*

INTRODUCTION

Epilepsy is a group of chronic neurological disorders characterized by sporadic episodes of convulsive seizures, sensory disturbance, abnormal behavior and loss of consciousness or all of these symptoms resulting from a brain dysfunction or an abnormal discharge of cerebral neurons [1]. Higher prevalence, lack of awareness, cultural and social stigma and non-availability of proper diagnostic and treatment facilities are among the major problems in the developing countries. Drug therapy of epilepsy with currently available AED is associated with side effects, dose-related and chronic toxicity that involves virtually every organ system [2, 7]. There is a pressing need for further research especially in the field of pharmacotherapy of epilepsy to find drugs. Search for anti-epileptic agents has made man turn to alternative sources, indigenous system of medicine.

Acalypha fruticosa (EEAF) is a plant commonly known as "Chinnichedi" and "Birch-leaved acalypha" a shrub belonging to the family of *Euphorbiaceae*. According to literature, the Suiei hunter-gatherers of northern Kenya drunk root decoction to treat convulsions. Root decoction of *Acalypha fruticosa* in traditional and folklore medicine is used to treat convulsions and it is scientifically not yet proved. Hence, the present study is undertaken to explore the possibility of using roots of *Acalypha fruticosa* in folklore medicine with proper scientific evidence.

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A. fruticosa is one such plant whose roots are used in folklore medicine to treat convulsions. The ethanol extract was evaluated for antiepileptic activity by two animal models involving gamma-amino butyric acid (GABA) ergic neurotransmission i.e. MES and PTZ-induced convulsions in mice [9, 10].

MATERIALS AND METHODS

Drugs and chemicals:

Diazepam (Calmpose inj., Ranbaxy), Dimethyl sulfoxide (DMSO), Pentylenetetrazole (PTZ) (Sigma Aldrich Chemical Co.) were used in this study.

Plant collection:

The Roots of *Acalypha fruticosa* were collected from Tirupati, Andhra Pradesh, India. The roots were identified and authenticated by Prof. K. MadhavaChetty, Department of Botany, Sri Venkateswara University. The plant specimen was deposited at Botany Department of Venkateswara University With Voucher number 1252.

Preparation of the extract:

The fresh Roots of *A. fruticosa* were collected and washed under running tap water. They were shade dried at room temperature and 1 Kg of the dried roots was made in to coarse powder. The powder was passed through a No. 60 mesh sieve. Then ethanol extract was prepared by following maceration method [4].

Preliminary phytochemical investigations:

The extract was subjected to qualitative chemical tests for various phytoconstituents like alkaloids, carbohydrates, saponins, tannins, proteins, lipids, flavonoids and steroids [5].

Animals:

- All the study protocol was approved by the institutional animal ethical committee (IAEC).(1047/ac/07/CPCSEA, dated 24-04-2007)
- Animals were procured from Mahaveera Enterprises, Hyderabad. The animals were housed in groups of 6 in cages with paddy husk as bedding, fed with normal commercial pellet diet, given water *adlibitum* and maintained under laboratory conditions (temperature 24 - 28°C, relative humidity 60 - 70%, and 12h light-dark cycle) and were acclimatized for a minimum of 7 days before experiment was performed. Food was withheld for 12h before the start of experiments.

Acute oral toxicity studies:

Male swiss albino mice were used and the procedure was followed by using OECD 423, annexure D (Acute Toxic Class Method) [6].

Evaluation of Antiepileptic Activity:

Pentylenetetrazole (PTZ)-induced convulsions:

Procedure: Male swiss albino mice with a body weight between 18-22g were used. Animals were divided into five groups of six animals each. Mice belonging to Group-I received DMSO, Group-II received diazepam (4mg/kg, i.p.), Group-III, IV, V received ethanol extract of *Acalypha fruticosa* (EEAF) at the doses of 30,100 and 300 mg/kg, p.o. Convulsions were induced by administration of pentylenetetrazole (PTZ) at the dose of 100 mg/kg, s.c. one hour after EEAF and 15 min after diazepam administration. Each animal was placed into individual plastic cage for observation lasting for 30mins. Seizures and onset of tonic-clonic convulsions were recorded [6, 10].

Table No. 1: Treatment schedule for PTZ induced convulsions

GROUPS	TREATMENT
I	PTZ (100mg/kg)
II	DZP (4mg/kg) i.p.
III	EEAF (30 mg/kg) p.o.
IV	EEAF (100 mg/kg) p.o.
V	EEAF (300 mg/kg) p.o.

Maximum electric shock (MES):

Procedure: Male swiss albino mice with a body weight between 18-22g were used. Animals were divided into five groups of six animals each. Mice belonging to Group-I received DMSO, Group-II received diazepam (4mg/kg, i.p.), Group-III, IV, V received ethanol extract of *Acalypha fruticosa* (EEAF) at the doses of 30,100 and 300 mg/kg, p.o. An Electroconvulsiometer with Corneal or Ear electrodes is used to deliver a shock. Current used 50 MA, 0.2 second duration. Animals were observed closely for two minutes after 1 hour of EEAF administration and 15 min after diazepam administration. Disappearance of hind limb extensor tonic convulsions is used as positive criteria. Percent inhibiton of seizures relative to control is calculated [6, 10].

Table No. 2: Treatment schedule for MES -induced convulsions

GROUPS	TREATMENT
I	DMSO
II	DZP (4mg/kg) i.p.
III	EEAF (30 mg/kg) p.o.
IV	EEAF (100 mg/kg) p.o.
V	EEAF (300 mg/kg) p.o.

Statistical Analysis:

The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test. $p< 0.05$ was considered as statistically significant. The data were expressed as mean ± standard deviation (SD).

RESULTS

Preliminary phytochemical investigations:

Preliminary phytochemical investigations of EEAF were presented in Table 3 which showed the presence of Triterpinoids, Phenols, Saponins, alkaloids, steroids, flavonoids and Tannins.

Table No. 3: Preliminary phytochemical analysis of the ethanol extract of *Acalypha fruticosa*

S. No.	Phytochemicals	EEAF
1	Triterpenoids	+
2	Steroids	+
3	Flavonoids	+
4	Phenols	+
5	Saponins	+
6	Anthraquinones	-
7	Alkaloids	+
8	Tannins	+
9	Sugars	-

(+) - Present; (-) - Absent

Pharmacological investigations:

Acute toxicity study:

In the acute toxicity study, EEAF was found to be toxic at a dose of 2000 mg/kg, p.o. body weight in mice and EEAF was found to be safe up to 1000 mg/kg, p.o. So, three doses i.e., 30, 100 and 300 mg/kg body weight were selected for the evaluation of antiepileptic activity.

Evaluation of Antiepileptic activity Pentylenetetrazole (PTZ) induced convulsions in mice:

The onset time of jerks, tonic and clonic convulsions, duration of convulsions and percentage protection were presented in Table 4 and figure 1-5.

Effect on Onset time ofjerks: The onset time of Jerks in control group animals was found to be 1.04 min. EEAF treated mice showed the onset of jerks time as 1.75, 4.41 and 8.13 min (p<0.0001) respectively at the doses of 30, 100 and 300 mg/kg, p.o. Animals which received standard i.e., Diazepam (4mg/kg, i.p.) showed 33.71 min.

Effect on onset time of tonic-convulsions: The onset time of tonic-convulsions in control group animals was found to be 1.70 min, EEAF treated mice showed the onset time of tonic-convulsions as 2.53, 4.84 and 8.54 min (p<0.0001) respectively at the doses of 30, 100 and 300 mg/kg, p.o. Animals which received standard i.e., Diazepam (4mg/kg, i.p.) showed 34.11 min.

Effect on onset time of clonic-convulsions: The onset time of clonic-convulsions in control group animals was found to be 3.32 min, EEAF treated mice showed the onset time of clonic-convulsions as 5.23, 5.91 and 9.84 min (p<0.0001) respectively at the doses of 30, 100 and 300 mg/kg, p.o. Animals which received standard i.e., Diazepam (4mg/kg, i.p.) showed 34.73 min.

Effect on duration of convulsions: The duration of convulsions in control group animals was found to be 6.2 min. EEAF treated mice showed the duration of convulsions as 3.4, 1.7 and 1.24 min (p<0.0001) respectively at the doses of 30, 100 and 300 mg/kg, p.o. Animals which received standard i.e., Diazepam (4mg/kg, i.p.) showed 1.02min.

The time of onset of jerks in control group animals was very less when compared to extract and standard group animals. Duration of convulsions in control group of animals was greater when compared to the extract and standard group animals. Albino mice pretreated with EEAF at doses of 30,100 and 300 mg/kg provided significant protection from convulsions induced by PTZ.

Percentage inhibition of convulsions: The percentage inhibition achieved in EEAF treated animals were 44% (30mg/kg), 72% (100mg/kg) and 80% (300mg/kg) p<0.0001 respectively when compared to control group animals. Animals pretreated with EEAF exhibited significant and dose-dependant antiepileptic activity but less potent when compared to diazepam treated animals (85%).

Table No. 4: The effect of EEAF on PTZ-induced convulsions in mice

Groups (n=6)	Treatment	Onset of Jerks (min)	Onset of Tonic convulsions (min)	Onset of Clonic convulsions (min)	Duration (min)	Percentage inhibition (%)
I	PTZ (100mg/kg)	1.046±0.02***	1.70±0.03***	3.32±0.04***	6.2±0.07***	-
II	Diazepam (4mg/kg)	33.71±0.03***	34.11±0.03***	34.73±0.05***	1.02±0.01***	85***
III	EEAF (30mg/kg)	1.75±0.04***	2.53±0.03***	5.23±0.08***	3.4±0.1***	44***
IV	EEAF (100mg/kg)	4.41±0.02***	4.84±0.03***	5.91±0.03***	1.7±0.03***	72***
V	EEAF (300mg/kg)	8.13±0.02***	8.54±0.03***	9.84±0.02***	1.24±0.07***	80***

*Values were Mean±s.d. (n=6) statistical significance was determined by ANOVA followed by Dunnet's test *** P<0.0001 when compared to control group.*

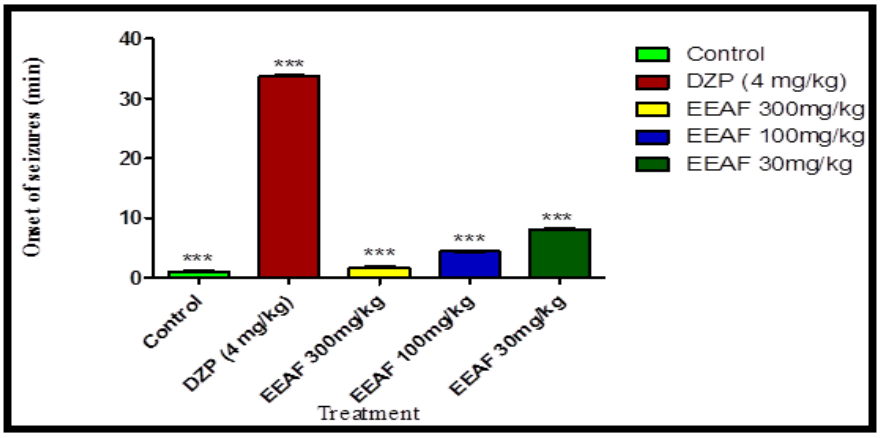

Fig. 1: Effect of EEAF on Onset time ofjerks induced by PTZ

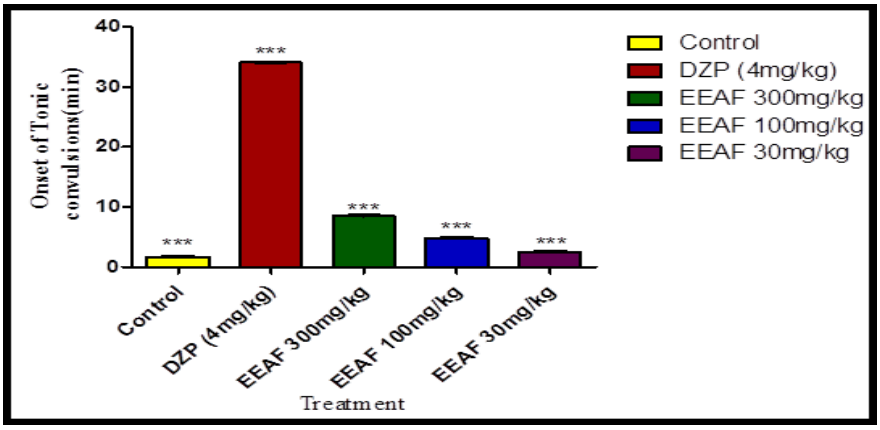

Fig. 2: Effect of EEAF on onset time of tonic-convulsions induced by PTZ

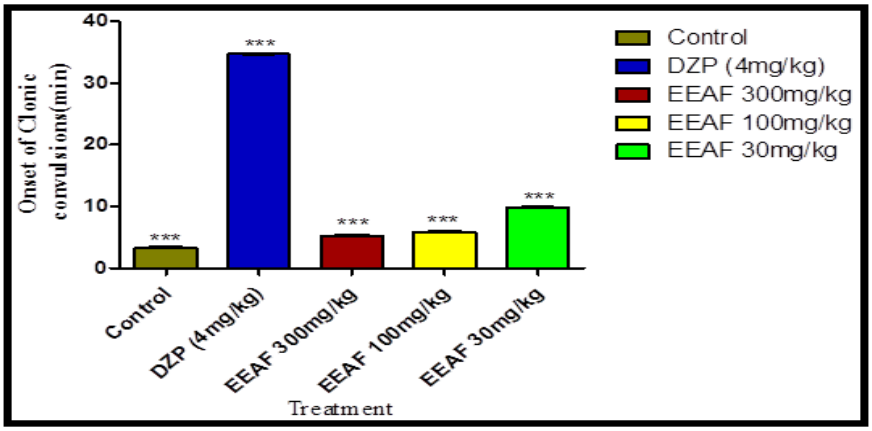

Fig. 3: Effect of EEAF on onset time of clonic-convulsions induced by PTZ

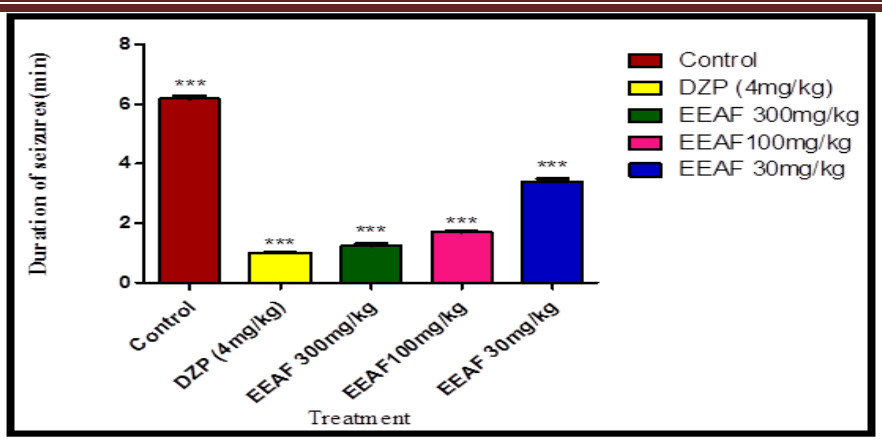

Fig. 4: Effect of EEAF on duration of convulsions induced by PTZ

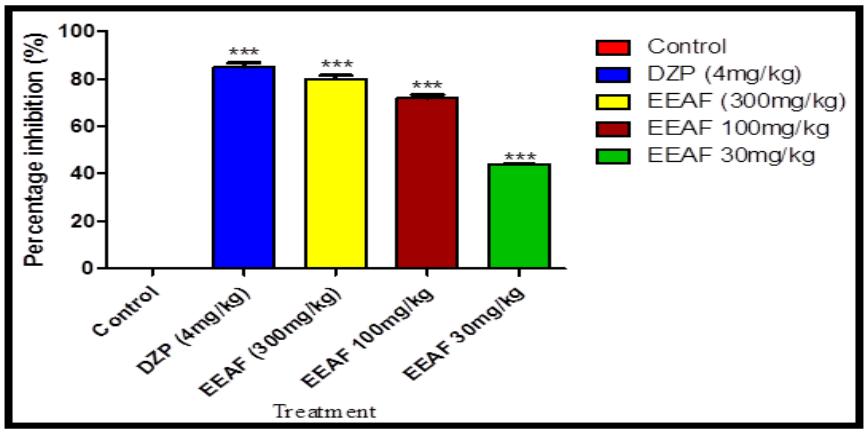

Fig. 5: Effect of EEAF on percentage inhibition (%) of convulsions induced by PTZ

Evaluation of Antiepileptic activity Maximum electric shock (MES)-induced convulsions in mice:

The onset and duration of tonic hind limb extension (THLE) and percentage inhibition of convulsions were presented in table 5 and fig 6-8.

Effect of EEAF on Onset of THLE: The onset of THLE in control group animals were found to be 1.52 sec. EEAF treated mice showed the onset of THLE as 1.82, 2.72 and 3.23 sec (p<0.0001) respectively at the doses of 30, 100 and 300 mg/kg, p.o. Animals which received standard i.e., Diazepam (4mg/kg, i.p.) showed 3.53 sec.

Effect of EEAF on duration of convulsions: The duration of convulsions in control group animals were found to be 120.9 sec, EEAF treated mice showed the duration of convulsions as 56.50, 52.86 and 44.87 sec (p<0.0001) respectively at the doses of 30, 100 and 300 mg/kg, p.o. Animals which received standard i.e., Diazepam (4mg/kg, i.p.) showed 40.86 sec.

The time of onset of THLE in control group animals was very less when compared to extract and standard group animals. Duration of convulsions in control group animals was greater when compared to the extract and standard group animals. Albino mice pretreated with EEAF at the doses of 30,100 and 300 mg/kg provided significant protection from convulsions induced by electric shock.

Effect of EEAF Percentage inhibition of convulsions: The percentage inhibition achieved in EEAF treated animals were 52.9% (30mg/kg), 56.27% (100mg/kg) and 62.8% (300mg/kg) p<0.0001 respectively when compared to control group animals. Animals pretreated with EEAF exhibited significant antiepileptic activity but less potent when compared to diazepam treated animals (66.2%).

Table No. 5: Effect of EEAF on MES-induced convulsions in mice

Treatment Groups	Dose (mg/kg)	Onset of THLE (sec)	Duration of THLE (sec)	Percentage inhibition of convulsions (%)
Control	**DMSO**	1.52±0.054***	120.9±0.02***	-
Standard	**Diazepam (4mg/kg)**	3.53±0.23***	40.86±0.74***	66.20***
EEAF-1	**30mg/kg**	1.82±0.03**	56.50±0.81***	52.9**
EEAF-2	**100mg/kg**	2.72±0.06***	52.86±0.7***	56.27***
EEAF-3	**300mg/kg**	3.23±0.19***	44.87±0.84***	62.8***

*Values were Mean±s.d. (n=6) statistical significance was determined by ANOVA followed by Dunnet's test *** P<0.0001 when compared to control group.*

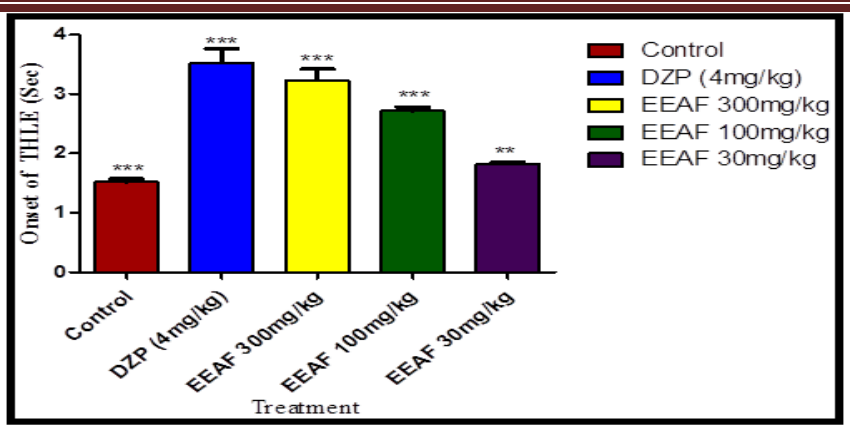

Fig. 6: Effect of EEAF on onset time of convulsions induced by electric shock

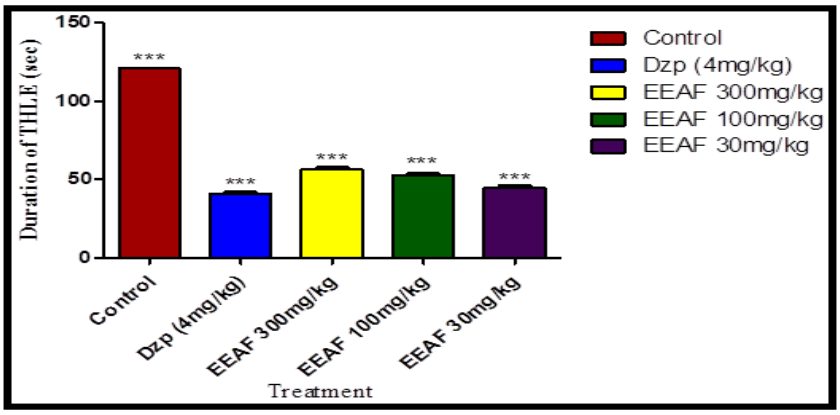

Fig. 7: Effect of EEAF on duration of convulsions induced by electric shock

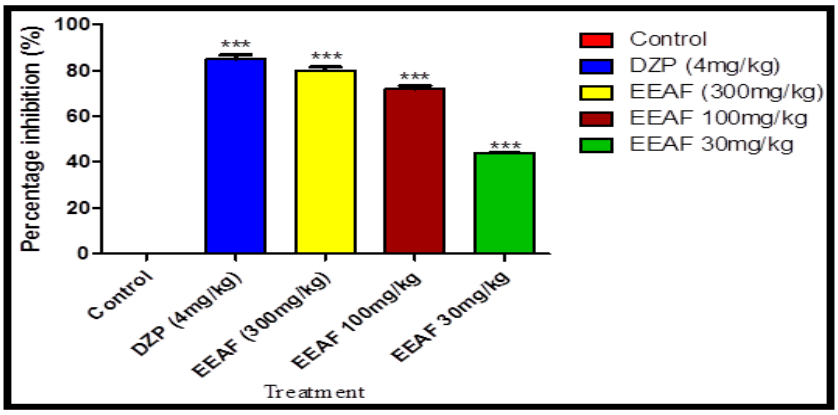

Fig. 8: Effect of EEAF on percentage inhibition of convulsions induce by electric shock

DISCUSSION

Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures that apparently result from complex processes involving several neurotransmitter systems such as glutamainergic, cholinergic, and gabaergic system.

Epilepsy is a group of chronic neurological disorders characterized by sporadic episodes of convulsive seizures, sensory disturbance, abnormal behavior and loss of consciousness or all of these symptoms resulting from a brain dysfunction or an abnormal discharge of cerebral neurons [1, 5].

According to literature survey it is revealed that there are many medicinal plants which are accalaimed the use in traditional and folklore medicine have been proven to possess significant antiepileptic activity. The activity correlates with the scientific investigations. Still there are a number of plants which are used in traditional and folklore medicine whose activity is scientifically not yet proven.

According to literature, the Suiei hunter-gatherers of northern Kenya drunk root decoction to treat convulsion. Root decoction of *Acalypha fruticosa* in traditional and folklore medicine is used to treat convulsions and it is scientifically not yet proved. Hence, the present study is undertaken to explore the possibility of using roots of *Acalypha fruticosa* in folklore medicine with proper scientific evidence.

The ethanol extract was evaluated for antiepileptic activity by two animal models involving gamma-amino butyric acid (GABA) ergic neurotransmission i.e. MES and PTZ-induced convulsions in mice.

GABA is known to be an important inhibitory neurotransmitter in the brain, whereas glutamate is the excitatory neurotransmitter. GABA acts on the GABA receptors and glutamate acts through the N-methyl-D-aspartate (NMDA) and non-NMDA receptors.

Activation of these receptors modifies various voltage-gated Na^+, K^+, Ca^{++} and Cl^- ion channels and excites or inhibits the neuron. Abnormalities in the GABA system have been found in neurological and psychiatric diseases such as Huntingdon's chorea, anxiety, panic attacks, schizophrenia and epilepsy. One major factor in epileptogenesis seems to be a decreased function of $GABA_A$ synapses [6].

The MES test in mice is a suitable model for grand mal epilepsy. MES test in mice is used primarily as an indication for compounds, which are effective in grand mal epilepsy. THLE are evoked by electric stimuli which are suppressed by antiepileptics. PTZ-induced convulsions in mice are a suitable model for petitmal epilepsy. PTZ is GABA antagonist. This assay has been used primarily to evaluate AED. Drugs which antagonize PTZ-induced seizures are generally useful in petitmal epilepsy. It has been indicated that PTZ-induced seizures can be prevented by drugs that reduce T-type Ca^{2+} currents, such as ethosuximide and also by drugs that enhance $GABA_A$ receptor-mediated inhibitory neurotransmission, such as benzodiazepines and Phenobarbital. The results of our study reveal that EEAF significantly inhibited the convulsions induced by MES & PTZ.

Preliminary phytochemical investigations revealed the presence of flavonoids, tannins, terpenoids, steroids, phenols, and saponins.

In PTZ-induced convulsions the administration of EEAF (30, 100 and 300 mg/kg p.o.) increased the onset time of jerks, tonic and clonic convulsions in mice compared to control group mice EEAF decreased the duration of PTZ-induced seizure reflexes in mice when compared to control group animals and showed increased protection against seizure susceptibility.

In MES model the administration of EEAF (30, 100 and 300 mg/kg p.o.) increased the onset time of tonic hind limb extension in mice when compared to control group mice and it decreased the duration of extension of hind limbs when compared to control group animals.

In both MES and PTZ models, all the three doses of EEAF showed significant and dose-dependant antiepileptic activity but less potent than diazepam.

CONCLUSION

Based on the above investigations, it may be concluded that the ethanol extract of roots of *Acalypha fruticosa* exhibited significant and dose-dependant antiepileptic activity in both MES and PTZ-induced convulsions and the antiepileptic activity of EEAF was less potent when compared to diazepam. These findings justify the folklore use of this plant. Flavanoids act as benzodiazepines like molecules in CNS and modulate GABA-generated chloride currents in animal models of anxiety and sedation. Preliminary phytochemical investigations revealed the presence of flavonoids, tannins, terpenoids, steroids, phenols, and saponins. Hence the presence of flavanoids may partially contribute the significant activity of ethanol extract of roots of *Acalypha fruticosa* by enhanced GABAergic neurotransmission. Further detailed phytochemical investigations are required to identify the phytoconstituent/s responsible for the antiepileptic effect.

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