Journal of Pharmacy and Practice
www.pavvjopp.com (e-ISSN 2249–5800)
JOPP

Evaluation of Antiepileptic Activity of Ethanolic extract of *Acalypha fruticosa* in mice

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Abstract

The aim of the present study was to investigate antiepileptic activity of ethanolic extract of Acalypha fruticosa (EEAF) in mice.The antiepileptic activity of EEAF at 30, 100 and 300 mg/Kg, p.o. was evaluated by the convulsions induced in mice by maximum electroshock (MES), Pentylenetetrazole (PTZ) and Isoniazid (INH). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. In MES method, EEAF (30, 100 and 300 mg/Kg) inhibited convulsions significantly potent than Diazepam. In PTZ method, EEAF inhibited convulsions potent than Phenobarbitone sodium (PS). In INH method, EEAF delayed the onset of convulsions less potent than Diazepam. In Present investigation, EEAF showed significant dose dependent antiepileptic effect potent than Diazepam and PS.

Keywords: Epilepsy, Pentylenetetrazole, Isoniazid.

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Introduction

The term epilepsy is of Greek origin that originally was known as "falling sickness" and means "Seizure" or "Seized"[1]. Epilepsy is a major neurological disorder and upto 5% of the world population have epilepsy in their lifetime. It affects an estimated 7 million people in India and 50 million worldwide, approximately 40% of them are women. The prevalence of epilepsy is 0.7% in India and higher in tropical/subtropical countries, particularly in South Africa. In developed countries where drugs are easily available, epilepsy responds to treatment in up to 70% of the patients. However, in developing countries 75% of people with epilepsy do not receive effective treatment. It is estimated that up to 5% of people suffer at least one seizure in their lifetime.

Epilepsy is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures, i.e., involuntary contraction of striated muscle repeatedly. Convulsion arises due to sudden excessive and rapid discharge of cerebral neurons in the grey matter of the brain[2]. All the currently available AED have potential for adverse effects on cognition and behaviour[3]. Search for anti-epileptic agents has made man turn to alternative sources, indigenous system of medicine. *Acalypha fruticosa* is a shrub and is a member of the Euphorbiaceae. Traditionally, it is used to treat epilepsy, dyspepsia, stomachache, skin diseases, wounds and poisonous bites[4]. The aim of the present study is to evaluate the potential of ethanolic extract of aerial parts of *Acalypha fruticosa* (EEAF) to protect the mice from convulsions.

Materials and Methods

Drugs and chemicals

Pentylenetetrazole (Sigma Aldrich Chemical Co.), Isoniazid (s.d. Fine-Chem. LTD),

Diazepam (calmpose inj., Ranbaxy) and Phenobarbitone sodium (Luminal, Bayer AG).

Plant Collection

The aerial parts of *A. fruticosa* were collected from Tirupati, Andhra Pradesh, authenticated by Prof. C. Madhava Chetty, department of Botany, Sri Venkateswara University and voucher specimen has been deposited (no. 1024).

Preparation of extract

The fresh aerial parts of *Acalypha fruticosa* were collected and washed under running tap water. They were shade dried at room temperature and the dried parts were made in to coarse powder. The powder was passed through a 60 No mesh sieve. The grounded powder was macerated with ethanol at room temperature. The solvent was then removed by filtration and fresh solvent was added to the plant material. The extraction process was twice repeated. The combined filtrates were then evaporated under reduced pressure [5].

Animals

Swiss Albino mice of either sex weighing 18-22 g were used. They were housed in standard polypropylene cages and kept under controlled room temperature (24±2^{0}C; relative humidity 60-70%) in a 12h light – dark cycle. The mice were given a standard laboratory diet and water *ad libitum.*

Qualitative analysis

The extract was subjected to phytochemical screening for various phytoconstituents like alkaloids, glycosides, steroids, tannins, saponins, flavonoids, lipids and proteins by using different qualitative chemical tests[6].

Acute Toxicity Study

Acute toxicity study will be performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 420 guidelines (OECD)[7].

Antiepileptic activity

Maximum electroshock (MES) in mice

Five groups of six male Swiss albino mice (25 – 30) were used. The test was started one hour after oral treatment with the test compound (EEAF 30, 100, 300 mg/Kg, p.o.) or the vehicle or the standard (Diazepam 3 mg/Kg, p.o.). An apparatus with corneal electrodes was used to deliver the stimuli. The intensity of the stimulus is dependent on the apparatus, eg: 30mA, 50Hz for 0.2 sec has been used. The onset and the duration of tonic himb extension was recorded and percentage of inhibition of seizures relative to controls was calculated[8].

Pentylenetetrazole (PTZ) induced convulsions in mice

Control group received vehicle, test group received EEAF (30, 100 and 300 mg/Kg, p.o.) and standard group received Phenobarbitone sodium, (40 mg/Kg, i.p.). Convulsions were induced by administering PTZ (75 mg/Kg, i.p.), 1hr after EEAF and 15 min after PS administration. The onset and the duration of convulsions were recorded and percentage inhibition was calculated[9].

Isoniazid (INH) induced convulsions in mice

Control group received vehicle, test group received EEAF (30, 100 and 300 mg/Kg, p.o.) and standard group received Diazepam, (4 mg/Kg, i.p.). Convulsions were induced by administering INH (300 mg/Kg, s.c.), 1hr after drug administration. The onset time of convulsions was recorded[10].

Statistical analysis

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

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Treatment	Dose	Onset time (min)	Duration of convulsions (min)	Percentage of Inhibition (%)
Vehicle	1 ml	3.43±0.04	22.54±0.02	-
EEAF	30 mg/Kg	4.38±0.05**	9.55±0.02**	57.62**
EEAF	100 mg/Kg	4.54±0.02**	7.52±0.05**	66.63**
EEAF	300 mg/Kg	5.14±0.03**	6.58±0.01**	70.82**
Phenobarbitone sodium	40 mg/Kg	6.46±0.02**	11.09±0.03**	50.82**

Table 1: Phytochemical constituents of ethanolic extract of *Acalypha fruticosa*

Phytochemical constituents	Ethanolic extract of *Acalypha fruticosa* (EEAF)
Steroids	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Tannins	+
Lipids	+
Saponins	+
Proteins	-

(+): Present (-): Absent

Acute Toxicity Study

Toxicity was found at 2 gm/Kg, p.o. So, 30, 100 and 300 mg/Kg, p.o. were the doses selected for the study.

Antiepileptic activity

Maximum electroshock (MES) in mice

In MES method, EEAF increased the onset time and decreased the duration of tonic hind

Results

Qualitative analysis

In preliminary phytochemical screening, the ethanolic extract shows the presence of steroids, alkaloids, flavonoids, glycosides, tannins, lipids and saponins (Table 1).

Treatment	Dose	Onset time (s)	Duration of tonic hind limb extension (s)	Percentage of Inhibition (%)
Vehicle	1 ml	1.03±0.01	116.53±2.92	-
EEAF	30 mg/Kg	1.47±0.02**	55.33±1.21**	52.52**
EEAF	100 mg/Kg	2.59±0.02**	46.58±0.92**	60.03**
EEAF	300 mg/Kg	3.76±0.04**	36.09±1.84**	69.03**
Diazepam	3 mg/Kg	2.85±0.02**	50.33±1.86**	56.81**

limb extension when compared to control group. EEAF exhibited significant dose dependent antiepileptic activity. EEAF at both 100 and 300 mg/Kg exhibited antiepileptic activity potent than Diazepam (Table 2).

Table 2: Effect of ethanolic extract of *Acalypha fruticosa* on maximal electroshock induced convulsions in mice

Treatment	Dose	Onset of convulsions (min)
Vehicle	1 ml	25.15±0.28
EEAF	30 mg/Kg	30.08±0.05**
EEAF	100 mg/Kg	32.52±0.06**
EEAF	300 mg/Kg	36.06±0.04**
Diazepam	4 mg/Kg	63.27±0.04**

EEAF: Ethanolic extract of *Acalypha fruticosa*; Values are mean ± SD (n = 6). Statistical significance was determined by ANOVA, followed by Dunnett's t test (n=6); $^{**}p < 0.01$.

Pentylenetetrazole (PTZ) induced convulsions in mice

In PTZ method also, EEAF increased the onset time and decreased the duration of convulsions when compared to control group. EEAF exhibited significant dose dependent antiepileptic activity. EEAF at all three doses exhibited antiepileptic activity potent than PS (Table 3).

Table 3: Effect of ethanolic extract of *Acalypha fruticosa* on PTZ-induced convulsions in mice

EEAF: Ethanolic extract of *Acalypha fruticosa*; Values are mean ± SD (n = 6). Statistical significance was determined by ANOVA, followed by Dunnett's t test (n=6); $^{**}p < 0.01$.

Isoniazid (INH) induced convulsions in mice

EEAF at all three doses exhibited significant dose dependent delay in onset of convulsions when compared to control but less potent than Diazepam (Table 4).

Table 4: Effect of ethanolic extract of *Acalypha fruticosa* on INH-induced convulsions in mice

EEAF: Ethanolic extract of *Acalypha fruticosa*; Values are mean ± SD (n = 6). Statistical significance was determined by ANOVA, followed by Dunnett's t test (n=6); $^{**}p < 0.01$.

Discussion

The convulsions induced by MES, PTZ and INH were effectively controlled by EEAF. Normally, antiepileptic drugs may act by modifying excitatory and inhibitory neurotransmission through effects on voltage gated ion channels, GABA(A) receptors and glutamate mediated excitatory neurotransmission. They act by potentiating GABA neurotransmission through inhibiting calcineurin (protein phosphatase 2B) or diminishing activation of NMDA extrasynaptic receptors[11]. Here PTZ act as GABA antagonist and INH act as GABA synthesis inhibitor[8]. So, the antiepileptic effect may be due to enhanced GABA ergic neurotransmission.

Conclusion

In the present investigation, EEAF showed significant dose dependent antiepileptic effect potent than Diazepam and PS. The antiepileptic activity may be due to the phytoconstituents present in the extract which are responsible for the enhanced GABAergic neurotransmission. Further detailed phytochemical investigations are required to

identify the phytoconstituent responsible for antiepileptic effect.

Acknowledgements
The authors are thankful to the Management of Vaagdevi College of Pharmacy, Hanamkonda and Jangaon Institue of Pharmaceutical Sciences, Jangaon, for providing necessary laboratory facilities.

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