



## ANTI-CONVULSANT ACTIVITY OF METHANOLIC EXTRACTS OF *AGERATUM CONYZOIDES L.*

R. Varadharajan and D. Rajalingam

\*Kamalakshi Pandurangan College of Pharmacy, Ayyampalayam, Tiruvannamalai, Tamil Nadu - 606 603.

### ABSTRACT

The present report is an investigation of anti seizure activity of *Ageratum conyzoides L.* (Family - Asteraceae) which is common in disturbed habitats along roadsides and trails, forest margins and openings, clearings, grasslands, and cultivated areas from sea-level to montane. Introduced as an ornamental plant from the Americas, it is now widely cultivated and is present throughout the South Pacific and other warm countries. Essential oils extracted have antibiotic properties. Antinematocidal, anti-inflammatory, anticoagulant, smooth muscle relaxant, haemostatic, analgesic, antifungal, antibacterial and hypothermic activities have been recorded. The methanolic (90%) extract of *Ageratum conyzoides L.* (MEAC) was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylene tetrazole (PTZ) induced seizures models in albino wistar rats. Acute toxicity of extract was non toxic up to the recommended dose 2000mg/kg body weight orally as per OECD guidelines No.423. Animals were pretreated with MEAC at the doses of 200 and 400mg/kg body weight. The study reported the significant delay in clonic seizure induced by PTZ and dose dependent decrease in duration of hindleg extensor phase in MES model. In MES model, MEAC showed significant reduction in duration of hindleg extension with 200 mg/kg dose and effect was dramatically reduced with 400mg/kg. Similar dose dependent results were obtained in PTZ model by delayed the onset of clonic convulsions. The complete protective effect against mortality was reported in both the tests. This study predicted possible mechanism of the formulation mediated through chloride channel of the GABA or benzodiazepine receptor complex. However, the exact mechanism of action is not clear, but attributed to its antiepileptic effect. The methanolic extract of *Ageratum conyzoides L.* deserves further investigation for detailed elucidation of active constituents and the mechanisms of action.

**KEYWORDS:** Antiseizure activity, Traditional Medicine, *Ageratum conyzoides L.* Maximal Electroshock, Pentylene tetrazole

### INTRODUCTION

Epilepsy is among the most prevalent of the serious neurological disorders, affecting from 0.5 to 1.0% of the world's population [1]. In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases per million populations [2]. Despite the optimal use of available antiepileptic drugs (AEDs), many patients with epilepsy fail to experience seizure control. Moreover, many patients suffer with the strong side effects of chronic treatment, which may include chronic toxicity, cognitive impairment, sedation and teratogenesis [3]. The increasing

knowledge on the basic mechanisms of epilepsy, the most important convulsive disorder, has led to the rational development of compounds that block seizure onset or spread targeting specific neuronal substrates [4, 5]. There is still a great demand for new anticonvulsant drugs, as the existing drugs fail to treat all types of convulsive disorders [6]. Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models and can be invaluable sources of new antiepileptic compounds.

*Ageratum conyzoides* L. (Family: Asteraceae) is a Coarse herb up to 1 m tall with opposite, simple hairy leaves. Flowers minute, whitish to pale blue, borne in small sunflowerlike heads 5-8 mm broad. Kaempferol, its glucoside and rhamnoside, quercitrin, scutellarein, eupalestin, chromenes, stigmast-7-en-3-ol, betasitosterol, stigmasterol, fumaric acid, caffeic acid, saponins, pyrrolizidine alkaloids, essential oils, oxygen heterocycles, ageratochromene derivatives, coumarin, alkanes. The plant has been extensively used in various folklore and traditional medicine systems for treatment of several disorders. This plant traditionally used for treat constipation, infective hepatitis, eczema, epilepsy, fresh wounds, dizziness, diarrhoea, dysentery, sore eyes, fever, headaches, intestinal worms, filariasis, vomiting and nausea, wounds and cuts. In Tonga the juice from leaves is applied to infected wounds. Juice from moist leaves is squeezed into sore eyes. Sometimes leaves are directly applied to aid healing of wounds. Carminative agent. Used to treat painful menstruation, cancer of the cervix and itchiness of the eye and to kill head lice [7-13]. Hence, the present study was designed to verify the claims of the native practitioners.

## MATERIALS AND METHODS

### Plant collection

The whole plant of *Ageratum conyzoides* L. was collected from abirami botanicals of Tuticorin, Tamilnadu, India. It was identified and authenticated by Prof. Jayaraman, Taxonomist, Tambaram, Chennai, Tamilnadu, India. The voucher specimen of the plant was deposited at the college for further reference.

### Preparation of extracts

The whole plant was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (220gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The methanolic extract of *Ageratum conyzoides* L. (MEAC) yielded thick violet semi-solid residues. Percentage yield of MEAC was found to be 18.9% w/w.

### Preliminary Phytochemical Screening

The phytochemical examination of the methanolic extract of *Ageratum conyzoides* L. was performed by the standard methods [14]. Further investigation was carried out using the ethanol extract suspended in 1% w/v Sodium carboxy methylcellulose (SCMC).

### Animals used

Albino wistar rats (150-200g) of either sex were

obtained from the animal house in Kamalakshi Pandurangan College of Pharmacy, Tiruvannamalai, Tamil Nadu. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

### Acute Toxicity Study

The acute toxicity of 90% methanolic extract of *Ageratum conyzoides* L. was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10<sup>th</sup> (200mg/kg) and 1/5<sup>th</sup> (400mg/kg) of this dose were selected for further study [15].

### Antiepileptic Activity

#### Effect on Maximal electroshock (MES) induced seizures

Albino wistar rats of either sex weighing 150 to 230 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) intraperitoneally, Group-III and IV, received methanolic extract of the *Ageratum conyzoides* L. (MEAC) (200 and 400 mg/kg body weight) *p.o* respectively for 14 days. On the 14<sup>th</sup> day, Seizures are induced to all the groups by using an Electro convulsimeter. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90° [16].

#### Effect on Pentylentetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 150 to 230 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally, Group-III and IV, received methanolic extract of *Ageratum conyzoides* L. (MEAC) (400 and 200 mg/kg/body weight) *p.o* respectively for 14 days. On the 14<sup>th</sup> day, Pentylentetrazole (PTZ) (90mg/kg body weight, *s.c*) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ [17].

### Statistical analysis

The data were expressed as mean  $\pm$  standard error mean (S.E.M). The Significance of differences among the groups was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

## RESULTS

### Phytochemical screening

The results of preliminary phytochemical screening of the methanolic extract of *Ageratum conyzoides L.* revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenols and absence of saponins and steroids.

### Effects of MEAC on MES Induced Epilepsy

The duration of tonic hindleg extension in rats treated with vehicle was  $15 \pm 0.14$  seconds. The MEAC at doses of 200 mg/kg and 400 mg/kg were protect animals from seizures and significantly ( $p < 0.001$ ) reduced the duration of tonic hindleg extension for  $5.04 \pm 0.42$  and

$3.67 \pm 0.56$  seconds respectively. Whereas, the standard drug Phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures where as MEAC 200 mg/kg and 400 mg/kg have shown 66.40 % and 75.53 % protection respectively (Table-1).

### Effect of MEAC on PTZ Induced epilepsy

In rats treated with vehicle, clonic convulsion appeared for  $182.22 \pm 1.27$  seconds after PTZ and all rats died after seizures. The MEAC at doses of 200 mg/kg and 400 mg/kg significantly delayed the onset of clonic convulsions for  $481.28 \pm 3.64$  ( $p < 0.001$ ) and  $570.57 \pm 3.51$  ( $p < 0.001$ ) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for  $692.54 \pm 2.35$  ( $p < 0.001$ ) seconds. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as MEAC 200 mg/kg and 400 mg/kg have shown 62.37% and 73.82% protection of convulsion and 83.33% and 100% protection of mortality respectively (Table-2).

**Table: 1. Effect of methanolic extract of *Ageratum conyzoides L.* (MEAC) on Maximum electroconvulsive shock (MES)-induced seizures in rats.**

Group	Design of Treatment	Flexion (seconds)	Extensor (seconds)	Clonus (seconds)	Stupor (seconds)	Recovery (seconds)	% Protection
I	Vehicle control (SCMC, 1ml/100g)	$7.42 \pm 0.44$	$15 \pm 0.14$	$20.84 \pm 0.21$	$42 \pm 0.84$	180.24	0
II	Phenytoin 25mg/kg, <i>i.p</i>	$3.89 \pm 0.12^{**}$	0	$9.57 \pm 0.54^{**}$	$17.41 \pm 0.45^{**}$	93.64	100
III	MEAC 200mg/kg, <i>p.o</i>	$5.94 \pm 0.52^{ns}$	$5.04 \pm 0.42^{***}$	$16 \pm 0.72^*$	$32.58 \pm 1.64^*$	138.92	66.40
IV	MEAC 400 mg/kg, <i>p.o</i>	$4.87 \pm 0.15^{**}$	$3.67 \pm 0.56^{***}$	$14.21 \pm 1.12^{**}$	$18.02 \pm 0.52^{***}$	114.57	75.53

Values are expressed as mean  $\pm$  SEM of six observations

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

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

\* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns-non significant.

**Table: 2. Effect of methanolic extract of *Ageratum conyzoides L.* (MEAC) on Pentylentetrazole (PTZ) induced seizures in rats.**

Group	Design of Treatment	Onset of clonic convulsions (seconds)	Duration of convulsion (Seconds)	Protection convulsion %	% Protection mortality
I	Vehicle control (SCMC, 1ml/100g)	$182.22 \pm 1.27$	$75.18 \pm 1.29$	0	50
II	Diazepam (4mg/kg, <i>i.p</i> )	$692.54 \pm 2.35^{***}$	$12.64 \pm 0.12^{***}$	83.18	100
III	MEAC (200 mg/kg, <i>p.o</i> )	$481.28 \pm 3.64^{***}$	$28.29 \pm 1.42^{***}$	62.37	83.33
IV	MEAC (400mg/kg, <i>p.o</i> )	$570.57 \pm 3.51^{***}$	$19.68 \pm 1.45^{***}$	73.82	100

Values are expressed as mean  $\pm$  SEM of six observations

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

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

\* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns-non significant.

## DISCUSSIONS AND CONCLUSIONS

The most popular and widely used animal seizure models are the traditional MES and PTZ tests. The MES test is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal" [18, 19]. This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity [20]. PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" [19] and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type [18].

In our present study, it is found that treatment with MEAC on rats significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. The MES test – to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans [21, 22]. Currently used anticonvulsant drugs (e.g. Phenytoin, carbamazepines) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test [23, 24]. Since, MEAC significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

Similarly, we found that treatment with MEAC on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. PTZ may cause seizures by inhibiting chloride ion channel associated with GABA<sub>A</sub> receptors [18, 25, 26]. Since PTZ has been shown to interact with the GABA neurotransmission [18, 27] and PTZ induced seizures can be prevented by drugs that enhance gamma amino butyric acid type A (GABA<sub>A</sub>) receptor-mediated inhibitory neurotransmission such as benzodiazepines and Phenobarbital [28, 29, 30], the antagonism of PTZ- induced seizures suggests the interaction of the methanolic extract of *Ageratum conyzoides* L. with the GABA-ergic neurotransmission. The effect of the MEAC in the PTZ test could therefore suggest antiepileptic efficacy against the above mentioned seizures type in man.

Preliminary phytochemical analysis performed in this study shows that alkaloids and flavonoids are the major components of the MEAC. Hence, these properties could be mediated by several compounds present in the extract and could explain the use of this plant in traditional medicine in the treatment of epilepsy. The study concluded with significant antiepileptic activity of methanolic extract of *Ageratum conyzoides* L. against various models of epilepsy but was unable to reveal the exact mechanism of its action.

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