Research Article

Anti-Convulsant Activity of Hydroalcoholic Extract of *Anacycclus pyrethrum* Root

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ABSTRACT

Anacyclus pyrethrum is claimed in traditional medical practice in the treatment of epilepsy in Kollimalai hills, Tamilnadu. The present research work was aimed at evaluating the protective effect of ethanolic extract of roots of *Anacyclus pyrethrum* against electrically and chemically induced seizure in experimental animals. Seizure was induced by Maximal Electro Shock and Pentylenetetrazole (80mg/kg I.P.) in mice. A daily dose of 100, 200mg/kg of *EEAP* was administered to the animals for 15 days, after which seizures were induced by MES and PTZ method (at a pretreatment time 30 minutes before seizure induction). The duration of various phases of epilepsy were recorded and compared with the solvent control animals. Administration of EEAP significantly ($p<0.05$, $p<0.01$) delayed the onset of convulsions and reduced the time taken for recovery.(48± 2.07, 96±2.1 sec), significantly ($p<0.05$, $p<0.01$) reduced the time taken for recovery (140±0.01, 122±0.12 sec) in PTZ method when compared to control (176±0.24 sec) respectively. In MES induced seizure, non significant decrease in Extensor phase from 11.5±1.37 sec (control) to 9.66± 1.63, 6.46±1.87 sec was observed. The results suggest that the EEAP root possess significant anti-convulsant activity against PTZ induced seizure.

Keywords: Ethanolic extract of Anacyclus *pyrethrum (EEAP),* Pentylenetetrazole (PTZ), Maximal Electro shock (MES).

INTRODUCTION

A mental or neurological disorder encompasses broad range of conditions that result in dysfunction of brain, spinal cord and nerves[1]. Approximately 1% of the world's population has epilepsy, the second most neurologic disorder after stroke[2] ·Epilepsy is among the disorders that are strongly associated with significant psychological and social consequences for everyday living[3]. Besides a number of allopathic medications available, there is considerable evidence of an increase in demand for medicinal plants, as these plants have no side effects rather it is beneficial to provide sustainability to the body. Hence, the present research work has been undertaken for screening the effect of hydroalcoholic extract of *Anacyclus pyrethrum root for* anti-epileptic activity in experimental animals[3,4]. It was thought worth to explore the antiepileptic activity of *Anacyclus pyrethrum* there by increasing the chances of discovering a lead molecule for the treatment of epilepsy.

MATERIAL AND METHODS

Drugs

Phenytoin, Diazepam (calmpose inj. Ranbaxy, India) were used in this study.

Plant material

The roots of *Anacyclus pyrethrum* were collected from local market of Trishur and authenticated by a Botanist National institute of Siddha.

Extraction of plant material

The roots of *Anacyclus pyrethrum* were shade dried. The dried roots were crushed to a coarse powder and extracted with 70% ethanol by cold maceration. The extract was concentrated by evaporation and stored in desiccators until use. The extract was reconstituted in 2% aqueous tragacanth just before use.

Animals

Albino rats of either sex, weighing between 180-220 gm were used in this study. They received standard diet and water *ad libitum*. Safety evaluation testing: The safety of the hydroalcoholic extract of roots of *Anacyclus pyrethrum* was done according to OECD guidelines 423[5]. The animals were observed for gross behavioral changes for 24hours and up to 14 days.

Acute toxicity study

The extract used in this study was evaluated for its acute toxic symptoms. The rats were subjected overnight with free access to drinking water and divided into six groups each containing six animals. Group I animals served as control and received distilled water orally (2 ml/kg). Group II-VI received (5, 50,300 and 2000mg/kg, p.o) respectively of the extract orally. The animals were observed continuously for 2h and then at one-hourly interval until 24th hour. The animals were then observed for mortality up to the 48th hour.

Phytochemical screening[6]

Phytochemical screening of Anacyclus pyrethrum revealed the presence of saponins, tannins, ß-sitosterol, stigmasterol.

ANTI-CONVULSANT ACTIVITY

Maximal electroshock induced seizure

Rats were divided into four groups consisting of 6 per group. Group 1: Vehicle - 2% CMC(1ml/100g) Group 2: EEAP(200 mg/kg, p.o). Group 3: EEAP(100 mg/kg, p.o). Group 4: Standard (Phenytoin 25 mg/kg, i.p). One hour after the administration of last dose of hydroalcoholic extract of *Anacyclus pyrethrum*, MES seizures were induced by electroconvulsiometer. A current of 150mA was delivered transauricularly for 0.2 sec in rats. This current intensity elicited complete tonic extension of the hind limbs in control. Various phases of convulsions, viz. tonic flexion, extension, clonus and mortality due to convulsions, were timed[7].

Pentylenetetrazol-induced seizures

The EEAP was administered *i.p.* in varying doses (100-200 mg/kg) 30 min before the subcutaneous injection of PTZ (80mg/kg) and mice were observed for onset of myoclonic spasm and clonic convulsions[8]. Group 1: Vehicle - 10% aqueous Tween 80 (10 ml/kg, p.o). Group 2: EEAP (200 mg/kg, p.o). Group 3: EEAP(100 mg/kg, p.o). Group 4: Standard (Diazepam 4 mg/kg, i.p). The animals were observed for onset of convulsion up to 30 min after PTZ administration. Each group contained six animals. After administration of these doses, the following seizure stages were observed in untreated mice (described in the order of appearance): 1: One ore more generalized myoclonic twitches of the whole body; 2: Repeated clonic seizures of fore- and/or hindlimbs for more than 3 s without loss of righting reflexes (corresponding to the threshold seizure proposed by Swinyard for anticonvulsant drug evaluation in the s.c. PTZ test); 3: A generalized clonic seizure of fore- and hindlimbs, during which animals exhibited loss of righting reflexes; 4: Loss of righting followed by tonic forelimb seizure; 5: Loss of righting with tonic fore- and hindlimb seizure.[7,8]

STATISTICAL ANALYSIS

The data are presented as mean±SEM. The significance of differences in PTZ dose between control and treated mice was calculated using Dunnet's 't' test.

RESULTS

Acute oral toxicity

All the doses (5, 50,300 and 2000mg/kg, p.o) of *Ap* employed for acute toxicity studies were found to be non toxic. EEAP did not produce any mortality even at higher dose (2000mg/kg, p.o) employed.

Anticonvulsant activity (MES Model)

In this test EEAP (100mg/kg, 200mg/kg) did not show a significant reduction of time spent in extensor phase. In MES induced seizure, non significant decrease in Extensor phase from 11.5±1.37 sec (control) to 9.66± 1.63, 6.46±1.87 sec was

observed. Results were shown in TABLE.N0.1.

Anticonvulsant activity (PTZ Model)

In this test EEAP (100mg/kg, 200mg/kg) shows a significant reduction in onset of tonic convulsions and Diazepam profoundly antagonized the tonic seizures elicited by PTZ. Table-2 shows data obtained from experiment conducted with PTZ induced convulsions. In animals treated with 2% CMC, the onset of convulsion appeared at 14.33 ± 0.61 min. EEAP in doses of 100, 200 mg/kg non-significantly increased the latency of onset of convulsions. Administration of EEAP significantly ($p<0.05$, $p<0.01$) delayed the onset of convulsions, 96±2.1, 48± 2.07 sec and significantly ($p<0.05$, $p<0.01$) reduced the time taken for recovery 140±0.01, 122±0.12 sec in extract treated when compared to control (176±0.24 sec) respectively in dose dependant manner.

DISCUSSION

In the present study we have evaluated the anticonvulsant activity of *Anacyclucs pyrethrum* using the MES and PTZ models. PTZ seizure threshold is well acknowledged animal model used for screening anticonvulsant effects of various chemical entities. PTZ –induced convulsions represent the petitmal type of seizures and this has been primarily utilized as animal model to evaluate the anti epileptic drugs.PTZ is known to block the post synaptic $GABA_A$receptor mediated cl^- conductance and thus produce seizures[9,10]. GABA is an important endogenous inhibitory neuro-transmitter widely distributed throughout the CNS. As far as the GABA is concerned, the following facts support its involvement. The lowering levels of GABA in the brain results in the appearance of convulsion. Some convulsive drugs found to be GABA antagonists. On the basis of experimental evidences, it may be concluded that the GABA system have a significant role with respect to CNS depressant and anticonvulsive properties of the processed extracts. In the present study, administration of the EEAP showed the anticonvulsant action by increasing the PTZ seizure threshold for the onset of tonic extension phase in mice. EEAP (100mg/kg and 200mg/kg) has showed the decreased percentage of convulsions. So our drug (AP) has anticonvulsant activity by GABA mediated mechanism[11]. It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking voltage-dependent Na^+ channels. In the present study EEAP does not showed significant results in MES model, so, our drug might not have inhibitory action on voltage dependent Na+ channels nor excitatory neurotransmitter mediated mechanism.

CONCLUSION

In the present study, the ethanolic extract of *Anacyclus pyrethrum* was studied for anticonvulsant effect against PTZ and MES models.AP at a dose of 100mg/kg has produced significant protective effect against PTZ model. The chemical constituent present in *Anacyclus pyrethrum* has to be explored to find the lead molecule which can be used for the treatment of petitmal type of epilepsy. AP root consists of pellitorine which is a sialogogue and helps to prevent nervous debility[12.]

Table I: Effect of *EEAP* on anticonvulsant activity (MES model)

S.No	Treatment	Time spent in sec			
		flexion	Extension	clonus	Stupor
1	Control(vehicle,p.o)	2.618±0-66	11.5±1.37	38.33±0.02	87.83±3.65
2	*EEAP*(100mg/kg p.o)	1.85± 0.68	9.66±1.63^{NS}	45.66±2.16	60.33±2.80
3	*EEAP* (200mg/kg p.o)	4.153. ±0.59	6.46±1.87^{NS}	35.11±2.05	60.50±2.88
4	Standard (phenytoin25mg/kg i.p)	3.00±1.89	0.33±0.81	16.16±1.16	80.33±7.36

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6)
Values are mean ± SEM of 6 animals per group
Comparison was made between control and drug treated groups
*P≤0.05
**P≤0.01
NS- No significant
EEAP- ethanolic extract of *Anacyclus pyrethrum*

Table II: Effect of *EEAP* on anticonvulsant activity (PTZ Model)

S.No	Treatment	No of convulsed: No used	Convulsions (%)
1.	Control(vehicle,p.o)	6/6	100
2.	*EEAP* (100mg/kg p.o)	1/6	16.6**
3.	*EEAP* (200mg/kg p.o)	2/6	33.3
4.	Standard (Diazepam4mg/kg i.p)	0/6	0

Data represent percentage of tonic seizures (n =6)
Comparison was made between control and drug treated groups
*P≤0.05
**P≤0.01
NS- No significant
EEAP-Ethonolic extract of *Anacyclus pyrethrum*

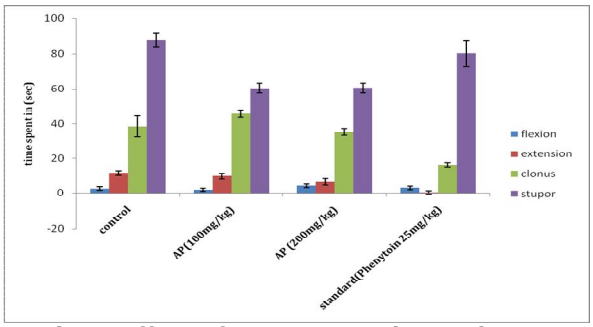

Fig.1: Effect of *EEAP* on anticonvulsant activity (MES model)

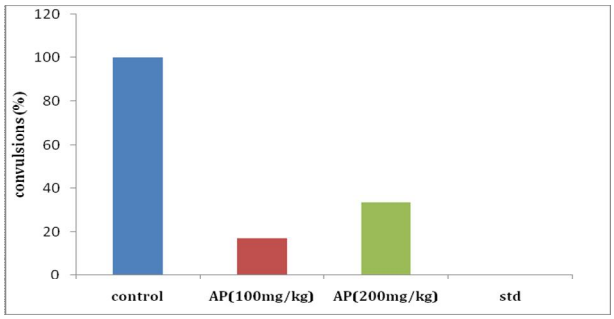

Fig. 2: Effect of *Ap* on anticonvulsant activity (PTZ Model)

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