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EFFECT OF PARNAYAVANI (COLEUS AMBOINICUS LOUR.) ON AUDIOGENIC & PENTYLENETETRAZOLE (PTZ) INDUCED EPILEPSY IN RATS

Monika Sharma[1*], Janardhan Singh[2], Arun Kumar[3], Vijay Kumar[4]

[1]Assistant Professor, Department of Dravyaguna, CBPACS, Najafgarh, New Delhi, India.
[2] Professor, Department of Pharmacology, Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India.
[3]Assistant Professor, Department of Pedodontics, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India.
[4] MDS, Department of Oral and Maxillofacial Surgery, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

ABSTRACT

Since time immortal Epilepsy is a common neurological disorders affecting people across all nationalities. It is estimated that 1% of the world population has epilepsy and it is considered as the second most common neurological disorder next to stroke. The currently used AED medication is associated with many side effects & recurrence of seizures as the therapy discontinued. So, it's high time to search remedies from the traditional treasure which may be proven as safe & effective antiepileptic agent. The present study was designed to investigate the antiepileptic potential of *Coleus amboinicus Lour.* on Audiogenic and Pentylenetetrazole (PTZ) induced seizure model in rats. Total 50 Albino rats of wistar strain of either sex weighing between100-180g were used in the study. For each model animals were divided into 5 groups each having 10 rats. Fresh juice of *Parnayavani* i.e.*Coleus amboinicus* (2ml/rat) was given in one group while the other group receives Aqueous & alcoholic extract of *Parnayavani* (500mg/bw p.o.). Phenytoin (25 mg/kg bw i.p.) served as standard drug for comparision where as control group receives distil water as vehicle. In Audiogenic model delayed in time of onset of convulsion was taken as end point & in PTZ model antiepileptic effect was evaluated by the presence or absence of clonic seizures & total duration of seizure episode. *CALJ* & CAalE was having highest efficacy as antiepileptic drug in comparison to CAaqE in Audiogenic model where as in PTZ model CAaqE *C* was more potent than its *CALJ* & CAalE. *Coleus amboinicus* leaf juice and extracts shows anticonvulsant & neuroprotective activity & thus can be effectively used for treatment of epileptic seizures.

Keywords: Epilepsy, Audiogenic, PTZ, *Parnayavani*, seizures.

INTRODUCTION

Since time immortal, herbal plants and their phytochemicals have been used as remedies for the prevention and treatment of various diseases and disorders. Epilepsy is one such common neurological disorders affecting people across all nationalities [1]. It is a chronic condition characterized by sudden, transient alterations of brain function usually with motor, sensory autonomic or psychic symptoms often accompanied by loss of, or altered consciousness. In Ayurveda it is described as Apasmara and is considered as *Maharoga* by charaka [2].

It is reported that 1% of the world population has epilepsy and it is considered as the second most common neurological disorder next to stroke [3].

There is no cure for epilepsy even in the present era. The modern antiepileptic drugs can only control the seizures in about 75-80% of patients if taken regularly for prolonged period [4]. The seizures will be under control as

Corresponding Author

Monika Sharma
Email id: monika.harmony@gmail.com

long as the medication continues. The major risk associated with discontinuing AED therapy is seizure recurrence. In children, almost half the recurrences occur within 6 months of medication withdrawal, and 60-80% occur within 1 year of stopping the drugs. More than 70% 0f recurrences occur within the first 5 years [5]. Further these drugs are associated with many side effects like osteoporosis, bone marrow depression , hyperparathyroidism G [6], chronic toxicity, teratogenicity [7] etc. Therefore even today this disease is affecting attention of researchers all over the world.

Ayurveda has documented indications of potent antiepileptic herbs but these herbs fail to gain mainstream acceptance due to lack of evidence as per the contemporary prevailing assessment parameters. A drug to be introduced into clinical practice requires to go through a series of tests commencing from experimental study on animal models. Therefore it is prudent to evaluate & re-establish the efficacy of herbs describe in Ayurveda through animal models, where ever possible & feasible. Therefore the whole world is looking towards ancient science of Ayurveda to explore safe, alternative, cost effective treatment as well as reliable cure with no or minimal side effects. *Coleus amboinicus Lour.* Synonym *C. aromaticus Benth. or Plectranthus amboinicus Lour.* belonging to family Lamiaceae is commonly known as *Parnayavani* in Sanskrit, Patta ajwain in hindi, *Karpurvalli* in south India & Country borage in English. The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhoea, epilepsy, renal & vesicle calculi & fever [8]. It has been reported to exhibit antilithotic, chemopreventive & antioxidant properties [9]. Although *Parnayavani* have been in folklore use in Epilepsy no scientific investigation is yet to be done for establishing its antiepileptic activity. Therefore the present study aimed to study the effect of Leaf juice & extracts (aqueous & alcoholic) of *Coleus amboinicus Lour*. which are known to have antiepileptic property on animal model.

MATERIALS AND METHODS

The study was conducted in the P.G. dept. of Dravyaguna, NIA, Jaipur & dept. of Pharmacology, Pt. B.D.Sharma Post Graduate Institute of Medical Sciences, Rohtak. Total 60 Rats were taken for the study.

Collection of Plant Material

The leaves of *Coleus amboinicus Lour*. were collected from the medicinal herbal garden of NIA Jaipur where it is cultivated. The Botanical identification was carried out by BSI, Jodhpur letter no. BSI/AZC/A.19014/SE-1/Estt./162 dt.23.6.2010. The leaves were dried under shade, coarsely powdered & were packed in air tight containers.

Extract Preparation

For the aqueous & alcoholic extraction of plant material the "Hot Extraction Method" i.e. Soxhelt extraction was used recommended by W.H.O. [10]. Distil water & ethanol was used as solvents for extraction.

Preparation of Leaf Juice

About 500 gms of fresh leaves of *Coleus amboinicus* were cut into small pieces and juice was prepared by crushing them into mortar and pestle by adding 30 ml distil water to it.

Experimental Animals

Total 60 Albino rats of Wistar strain of either sex weighing between100-180g were used in the study. Rats were procured from the disease free animal house, Haryana Agricultural University (HAU), Hissar. The experimental rats were housed in poly propylene cages under laboratory conditions of 28± 2˚C temp with 75% relative humidity and photoperiod of 12 hrs light & dark cycle. They were provided with a standard pellet diet supplied by Hafed, India Limited, Rohtak and water *ad libitum*, throughout the experiment. A minimum period of 7 days was allowed for the animals to get habituated to the animal house. The protocol of the study was approved by Institutional Ethical Committee (IAEC, PGIMS, Rohtak) & the experiments were carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment & Forest. Govt. of India.

Chemicals

Phenytoin (Epsolin, Cadilla, India), Sodium valproate & Pentylenetetrazole (Sigma, St.Louis, USA) were used in the study. All other chemicals used were of analytical grade. Double distilled water was used in the study where ever required.

Selection of Dose

As per OECD [11] (2002) guideline no. 425, the LD 50 of *Coleus amboinicus* was estimated to be >5000 mg/kg. Hence, 1/10 th of the LD 50, i.e. 500 mg/kg, dose was selected for the study. Fresh leaf juice was administered at a rate of 2 ml/rat [12].

Administration of test substance

The test drugs were prepared by dissolving the required extracts in distil water. The volume of administration was kept at 0.1ml/kg bw. A gastric catheter was used for oral drug administration. PTZ & Phenytoin were dissolved in normal saline before i.p. administration.

Experimental design (Study Protocol)

Seizures were induced by 2 methods. They are as follows:

Audiogenic Test Chamber

In the present study effect of leaf juice & extracts (aqueous & alcoholic) of *Coleus amboinicus Lour.* Were observed on the seizures induced by Audiogenic stimulus in

rats. For induction of seizures "Techno Audiogenic test chamber" was used as described by Plantnikoff and Green (1957) [13]. Albino rats of either sex weighing between 100-180g were used. Overnight fasted rats were taken & screened for Audiogenic stimulus. Rats showing convulsive responses were selected for further experiment. Rats were exposed (individually) to the auditory stimulus after placing them in the Test Chamber for a period of 90 seconds. Stimulus was produced by 2 door electric bells fitted in the chamber. The animals were divided into 5 groups of 10 rats each.

Group 1- Served as Control & receives normal saline (10ml/kg) as Vehicle.

Group 2- Receives leaf juice of *Coleus amboinicus (2ml/rat)*.

Group 3- Receives aqueous extract of *Coleus amboinicus* (500mg/kg bw p.o.).

Group 4- Receives alcoholic extract of *Coleus amboinicus* (500mg/kg bw p.o.).

Group 5- Receives Phenytoin (25mg/kg bw i.p.) standard drug for comparison.

Control (Initial) time in seconds at which seizures initiated was noted at the interval of 1st hr, 2nd hr, 3rd hr in drug treated groups. Control (Initial) time was compared with the time in drug treated groups statistically.

Pentylenetetrazole (PTZ) induced Seizures

The method of Prasad and Malhotra (1948) [14] was used. Seizures were produced in adult albino rats with PTZ (70mg/kg bw i.p.).Seizures began with jerks of the head and body consisting chiefly the clonic contraction. The seizures ended fatally or were followed by depression & recovery finally took place. Animals were divided into 5 groups of 10 animals each.

Group 1- Served as Control & receives normal saline (10ml/kg) as Vehicle + PTZ.

Group 2- Receives leaf juice of *Coleus amboinicus (2ml)* + PTZ.

Group 3- Receives aqueous extract of *Coleus amboinicus* (500mg/kg bw p.o.) + PTZ.

Group 4- Receives alcoholic extract of *Coleus amboinicus* (500mg/kg bw p.o.) + PTZ.

Group 5- Receives Sodium Valproate (150mg/kg bw i.p.) standard drug + PTZ.

The test drugs were given 1 hour prior to Pentylenetetrazole (PTZ) injection administration and animals were tested for convulsions if any. The antiepileptic effect was evaluated by the presence & absence of clonic seizures, time required for onset of seizures and total duration of seizures episode. The Percentage protection in different groups was also calculated.

Statistical analysis

All the results were expressed as mean ± standard error of mean (SEM). Statistical analysis of data was performed by analysis of variance (ANOVA) followed by Turkey- Kramer Multiple Comparison Test. A value of $p<0.001$ was considered to be highly significant where as $p<0.05$, $p<0.02$, $p<0.01$ were considered to be significant.

RESULTS

In Audiogenic Model

In almost all the experiments, the animal in control group took more time for the initiation of seizures than that of drug treated trial groups. All the trial drug treated groups i.e. Group 1,2,3 showed delayed convulsion i.e. the time of onset of convulsion was slightly increased after 1 hr, reached its maximum after 2 hr which was statistically highly significant and later showed gradual decline after 3 hr of drug administration. This means that trial drugs showed their effect right from the 1st hr itself, reached its peak in the 2nd hr & then gradually declined. In Phenytoin pre-treated group there is complete disappearance of Convulsion from the 1st hr after the drug administration. It showed that Phenytoin is more potent than *C.amboinicus* as an antiepileptic drug. Group 1,2,3 only showed delayed convulsion while Group 4 animals were completely protected from convulsion. Among the drug pre-treated group 1,2,3 the animal in group 1 pre-treated group showed maximum delayed Convulsion followed by group 3 & 2 i.e *C.ambonicus* leaf juice was most potent than its extracts in delaying the time of onset of convulsions which was statistically highly significant.

CALJ > CAalE > CAaqE

No mortality & morbidity has been observed during the Experiment. Result of the acute study (single dose) pre-treatment with *C. amboinicus* leaf juice & extracts showed mild effect in delaying the audiogenic seizures but both the extracts failed to show marked antiepileptic effect.

In PTZ Model

The administration of all the trial drugs showed significant increase in onset of Convulsion & highly significant decrease in duration of convulsion as compared to control group. It was maximum in Group 3 followed by group 4 & 2. While the decrease in duration of convulsion was maximum in CALJ pre-treated Group 2 followed by Group 3& 4 respectively.

Increase in onset of Convulsion

CAaqE > CAalE > CALJ .

Decrease in Duration of Convulsion

CALJ >CAaqE > CAalE

Standard drug Sodium Valproate was more potent than any of the extract treated group bcoz.90% animal from this group completely antagonize clonic convulsions induced by PTZ.CALJ showed max.i.e.70% protection of animals from PTZ while CAalE & CAaqE pre-treated groups showed 40% & 30% protection against PTZ induced

seizure. CAaqE pre-treated group proved to be better in both increasing the onset & decreasing the duration of convulsion as compared to CAalE & leaf juice. It means that CAaqE was more potent than CAalE & CALJ in PTZ induced seizure model.3 mortality were observed in control group & no mortality was observed in any of the *C. amboinicus* pre-treated group.

Table 1. Effect of *Coleus amboinicus* leaf juice (CALJ) & extracts (aqueous CAaqE & alcoholic CAalE) on Audiogenic Seizures in rats

Sr. No.	Treatment group n= 10	Dose mg/kg	Time in seconds in which seizures appeared			
			Control	After 1 hr	2hr	3 hr
1.	CALJ	2ml / rat	19.80±2.26	23.80±2.15**	30.0±2.64***	26.0±2.25**
2.	CAaqE	500mg/kg p.o.	12.00±0.66	13.90±0.604***	18.10±0.887***	12.40±0.400
3.	CAalE	500mg/kg p.o.	13.80±0.879	15.70±0.830**	22.20±0.800***	18.60±0.702***
4.	Phenytoin	25mg/kg i.p.	12.10±0.94	NIL	NIL	NIL

Data represented as mean+ SE of 10 rats. . Data compared with one way ANOVA followed by Turkey- Kramer Multiple Comparison Test.

P >0.05 non significant **P <0.01 significant *** P <0.001 highly significant

Table No. 2. Effect of *Coleus amboinicus* leaf juice (CALJ) & extracts (aqueous CAaqE & alcoholic CAalE) on Pentylenetetrazole (PTZ) induced Seizures in rats

Sr. No.	Treatment group n= 10	Dose mg/kg	Time (sec) Onset of Convulsion	Time (min) Duration of Convulsion	Mortality	% Protection
1.	Control +PTZ	70mg/kg i.p.	49±3.71	19.4±1.69	3/10	-
2.	CALJ+ PTZ	2ml+70mg/kg i.p.	16.0±9.16***	0.60±0.33***	0/10	70%
3.	CAaqE+PTZ	500mg/kg p.o. . + 70mg/kg i.p.	420±83.96***	1.4±0.400***	0/10	30%
4.	CAalE+PTZ	500mg/kg p.o. . + 70mg/kg i.p.	162±49.67	3±1.265***	0/10	40%
5.	Sodium Valproate+PTZ	150mg/kg i.p. + 70mg/kg i.p.	30±30.2***	0.1±0.100***	0/10	90%

Data represented as mean ± SE of 10 rats. Data compared with one way ANOVA followed by Turkey- Kramer Multiple Comparison Test.

P >0.05 non significant **P <0.01 significant *** P <0.001 highly significant

DISCUSSION AND CONCLUSION

Drugs effective against partial & Secondarily generalised tonic clonic seizures appears to work by either to limit the sustained repetitive firing of neurons an effect mediated by promoting the inactivated state of voltage activated Na+ Channels or by enhancing GABA mediated synaptic inhibition [15]. Inhibition of high frequency firing is thought to be mediated by reducing the ability Na+ Channels to recover from inactivation [16]. However reducing the rate of Na+ Channels from inactivation would limit the ability of a neuron to fire at frequencies an effect similar to produced by other anti epileptic drugs (Phenytoin, topiramate, Valproic acid etc) against partial seizures. Therefore, it is possible that the antiepileptic effect of *C.amboinicus* may be due to modulation of Na+ Channel function. Enhanced GABA mediated synaptic inhibition would reduce neuronal excitability & raise the seizure threshold [16]. GABA receptor activation inhibits postsynaptic cell by increasing the inflow of Cl^- ions in the cell which tends to hyperpolarize the neuron [16]. Thus *C. amboinicus* may increase GABA release/ concentration of GABA in the Brain or by reducing metabolic degradation of GABA. A reduction in inhibitory synaptic activity or enhancement of excitatory synaptic activity might be expected to trigger a seizure. GABA and glutamate are main inhibitory & excitatory neurotransmitters respectively in the brain. Pharmacological studies demonstrated that antagonists of the GABA receptor or agonists of glutamate receptor subtypes (NMDA, AMPA or Kainic acid) triggers seizures in experimental animals in vivo. Drugs that enhance GABA mediated synaptic inhibition suppress seizures in animal models and glutamate receptor antagonists also inhibit seizures [16]. Therefore beneficial effect of *C.amboinicus* in Audiogenic model of epilepsy can be explained on the basis of modulating excitatory or inhibitory neurotransmitters involved in the genesis of seizures. In addition to these mechanism we cannot exclude the role of *C.amboinicus* in modulation of voltage gated K+ current, since K+ current has been shown to effect synaptic transmission in the brain [17]. Therefore recently described effect on inward rectifying K+ channels cannot be excluded as possible target. Another possibility is that *C.amboinicus* suppresses epileptic activity by interacting with adenosine receptors or may enhanced endogenous anticonvulsants in

the brain. Since Melatonin, adenosine some prostaglandin and antioxidants have been shown to posses endogenous anticonvulsant properties. Well known antioxidant effect of *C.amboinicus* may be helpful in controlling seizures [18, 19].

From the results of the present study it is difficult to propose exact anti seizure mechanism of *C.amboinicus* but the study suggest that leaf juice & both extracts posses significant anti epileptic activity in rats against Audiogenic & PTZ models. Further studies are required to explore mode of anticonvulsant action of *C.amboinicus* at molecular level.

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