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Research Article

EVALUATION THE EFFECT OF *ALLIUM CEPHA* BULBS EXTRACT ON ANTI-EPILEPTIC ACTIVITY IN MICE

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ABSTRACT

This study demonstrates the antiepileptic activity of hydroalcoholic extract of *AlliumCepha* bulbs. (Family: Liliaceae) in mice. Epilepsy is acollective term describing of chronic seizure disorder having in common, sudden and transient episodes(seizure) of lossor distrubance of consiousness, usually but not always with a characteristic body movement(convulsions). For assessing of antiepileptic activity Pentylenetetrazole(PTZ), Maximal electro shock(MES) models were used. Diazepam was used as a standard reference for all models. Shade dried bulbs of *AlliumCepha* were powedered and subjected to soxhelet apparatususing alcohol and water. Hydroalcoholic extract was administerd to the animals as suspension using 2% of gum acacia for experimental purpose. Preliminary phytochemical investigation of the hydroalcoholic extract of *AlliumCepha* bulbs reveals the presence of carbohydrates, proteins, alkaloids, flavonoids, phytosterols, fats and oils and voltile oils.Hydroalcoholic extract of *AlliumCepha* bulbs either upto the dose level of 2000mg/kg did not produce any sort of mortality. In pentylenetetrazole(PTZ), Maximal electro shock(MES), high dose, medium dose and low dose of *AlliumCepha* extract showed significant antiepileptic activity by delaying the onset of convulsionsand by prolong the onset of clonus and tonic extensor convulsion.In percentage protection, increased protection of animals in groups treated with diazepam, high dose, medium dose and low dose of *AlliumCepha* extract in comparision with control models.

Keywords: *AlliumCepha,* Pentylenetetrazole, antiepileptic, Maximal electro shock.

INTRODUCTION

Epilepsy is group of chronic seizure disorder having in common, sudden and transient episodes (seizure) of loss or disturbance of consciousness , usually but not always with a characteristic body movements (convulsions).[1,2]
Seizures are discrete, time limited alterations in brain function including changes in motor activity result from excessive electrical discharge of a group of neurons with in brain. [1, 2]
The incidence of a disorder is the number of new cases at a given time. Studies in developed countries suggest an annual incidence of epilepsy of approximately 50 per 100,000 of the general population.

Different types of synthetic drugs and natural drugs are available in the market for the treatment of different types of epilepsies. Synthetic drugs like Hydantoin derivatives, Barbiturates, Iminostillbines, Succinamides etc., are used for treatment of epilepsy, but these drugs are also have fatal side effects like sedation, skin rashes, megaloblastic anemia, osteomalacia, hypersensitivity reactions, hyperglycemia, ataxia, vertigo, diplopia, drowsiness, behavioral alterations, confusion, hallucination, nausea, vomiting, fall in B.P and cardiac arrhythmia.

Hence, there is an increasing demand for the alternative therapies particularly herbal therapies that are believed to be effective, safe and economical. *Allium cepha* belongs to the family-Liliaceae, is a bulbs bearing small tree cultivated in many parts of India. The bulb useful in malaria, opthalmia, disease of spleen, vomiting, asthma, scabies, earache, piles; enriches the blood of women; apply to the eyes in night-blindness. The bulbs are ant periodic, antibacterial, aphrodisiac, emmenagogue, emollient, expectorant, bronchitis, otalgia, pharyngodynia, lumbago, epilepsy, wounds, paralysid, arthralgia, leucoderma and skin diseases. [3]

However, there is no authentic scientific data reported regarding anti-epileptic activity of *Allium cepha* bulbs. In the context, in the present study an attempt is proposed to evaluate the effect *of Allium cepha* bulbs extract on electro shock induced convulsions and Pentylenetetrazole induced convulsions in rats or mice.

MATERIALS AND METHODS

Collection of plant material

Bulbs of *Allium cepha* were collected in the month of Jan 2013 from Vikas.Pharmacy collge, Visannapeta. The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation of hydro alcoholic extract[4]

The powder of *Allium cepha* bulbs was charged in to the thimble of a soxhlet apparatus and extracted using 70% ethanol and 30% water for 18 hrs. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and further extraction was terminated.

Experimental animals

Albino mice of either sex weighing between 20-30g were procured from central animal house of Bangalore for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were kept in well ventilated animal house conditions with free access to pelleted food and ad libitium water throughout the experiment.

Acute oral toxicity study by using OECD 425 guidelines [5]

This test procedure is used here because to minimize the number of animals required estimating the acute oral toxicity of chemicals, drugs and also in estimating a median lethal dose. The median lethal dose allows for comparison with historical data. In addition to the observation of mortality, it allows the observation of signs of toxicity.

DETERMINATION OF ANTICONVULSANT ACTIVITY

1. PTZ (Pentylenetetrazole) INDUCED CONVULSIONS[6,7]

Albino mice of either sex weighing between 22-25g were randomly selected and segregated in to five groups, each group consisting of six animals.

Group A - Normal control (2%w/v Gum acacia p.o.)
Group B - Standard (Diazepam 5mg/kg p.o)
Group C - Bulb extract of *Allium cepha* (100mg/kg p.o)
Group D - Bulb extract of *Allium cepha* (200 mg/kg p.o)
Group E - Bulb extract of *Allium cepha* (400 mg/kg p.o)

Experimental procedure

Albino mice of either sex with body weights between 22-25g were divided into five groups of 6 animals in each. Group A served as normal control and was administered with 2%w/v Gum acacia suspension orally, Group B with diazepam (5mg/kg p.o.) and served as standard, Groups C, D and E with three different doses of bulb extracts (low, medium and high respectively) hydro alcoholic of *Allium cepha* for seven consecutive days. On the eighth day one hour after the oral administration of either acacia suspension/standard drug/extracts respectively to different groups, PTZ 60 mg/kg was administered subcutaneously. The following parameters were recorded during test session of initial 30min and up to 24 hrs respectively:

► Latency (onset of clonus)
► Onset of tonic-clonic convulsions
► Status of animal after 1hr

► Status of animal after 24 hrs
► Percent protection

2. MAXIMAL ELECTRO SHOCK (MES) INDUCED CONVULSIONS[8]

Albino mice of either sex weighing between 22-25g were divided into five groups each group was consisting of six animals.

Group A - Normal control (2%w/v Gum acacia p.o.)
Group B - Standard (Diazepam 5mg/kg p.o)
Group C - Bulb extract of *Allium cepha* (100mg/kg p.o)
Group D - Bulb extract of *Allium cepha* (200 mg/kg p.o)
Group E - Bulb extract of *Allium cepha* (400 mg/kg p.o)

Experimental procedure

Albino mice of either sex with a body weight between 22-25g were divided into five groups of 6 animals in each. Group A served as control and was administered with 2% gum acacia suspension, Group B with phenytoin (25mg/kg p.o.) and served as standard. Group C, D and E with three different doses of hydro alcoholic extracts of *Allium cepha* (low, medium and high respectively) for seven consecutive days. On the eighth day one hour after oral administration of acacia suspension/standard drug/different extracts to respective groups, MES seizures were induced by electroconvulsometer. A 60 mA current was delivered transauricularly for 0.2sec in mice. This current intensity elicited complete tonic extension of the hind limbs in control mice.

The following parameters were recorded during 1hr test session.

- Tonic flexion
- Tonic extension
- Clonus convulsions
- Percent protection

RESULTS

Table 1: Effect of hydro alcoholic extract of *Allium cepha* bulbs on PTZ (60mg/kg s.c.) induced convulsions (onset of seizure) in mice.

TREATEMENT	ONSET OF CONVULSIONS (seconds) No. of animals in the group						MEAN ± SEM	STATUS OF ANIMAL AFTER 1hr Death/Recovery
	1	2	3	4	5	6		
Control (2% Gum acacia p.o.)	352	358	360	372	380	362	364±4.163	6/0
Diazepam (5mg/kg p.o.)	1072	1098	1080	1076	1082	1088	1082.6±3.783 **	0/6
ACE (100mg/kg p.o.)	522	538	510	545	560	506	530.1±8.619**	2/4
ACE (200mg/kg p.o.)	567	588	684	601	618	636	1526±930.85 **	1/5
ACE (400mg/kg p.o.)	738	740	762	705	714	739	733±8.359**	1/5

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed byDunnett's't' test. Where, **represents highly significant at p<0.01
ACE: Allium cepha Extract PTZ: Pentylenetetrazole.

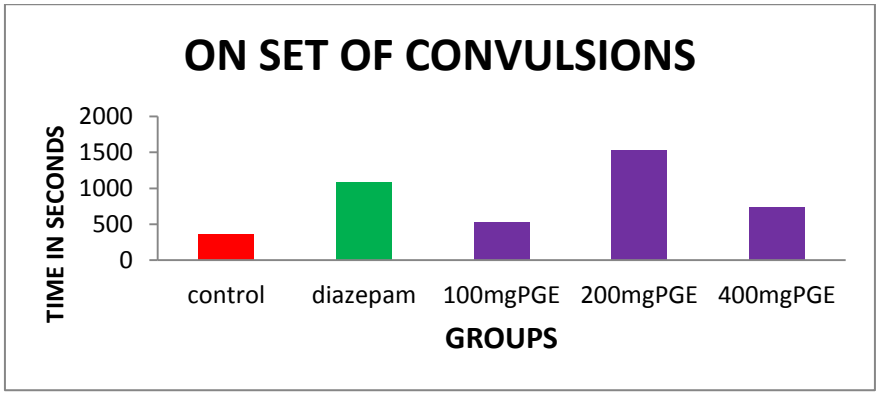

Fig. 1: Effect of hydro alcoholic extract of Allium cepha bulbs on PTZ (60mg/kg S.C.) induced convulsions (onset of seizure) in mice.

Table 2: Effect of hydro alcoholic extract of Allium *cepha* bulbs On PTZ (60mg/kg) induced convulsions (Duration of tonic-colonic seizure) in mice.

TREATEMENT	DURATION OF TONIC CLONIC SEIZURES (seconds) No. of animals in the group						MEAN±SEM
	1	2	3	4	5	6	
Control (2% Gum acacia p.o.)	70	66	74	62	79	69	70±2.436
Diazepam (5mg/kg p.o.)	12	16	7	9	14	6	10.6±1.626 **
ACE (100mg/kg p.o.)	58	38	40	57	36	47	46±3.941 **
ACE (200mg/kg p.o.)	44	48	40	53	55	42	47±2.477 **
ACE (400mg/kg p.o.)	38	44	39	38	43	41	40.5±1.057 **

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's't'test.
Where, **represents highly significant at p<0.01 ACE: Allium cepha Extract PTZ: Pentylenetetrazole.

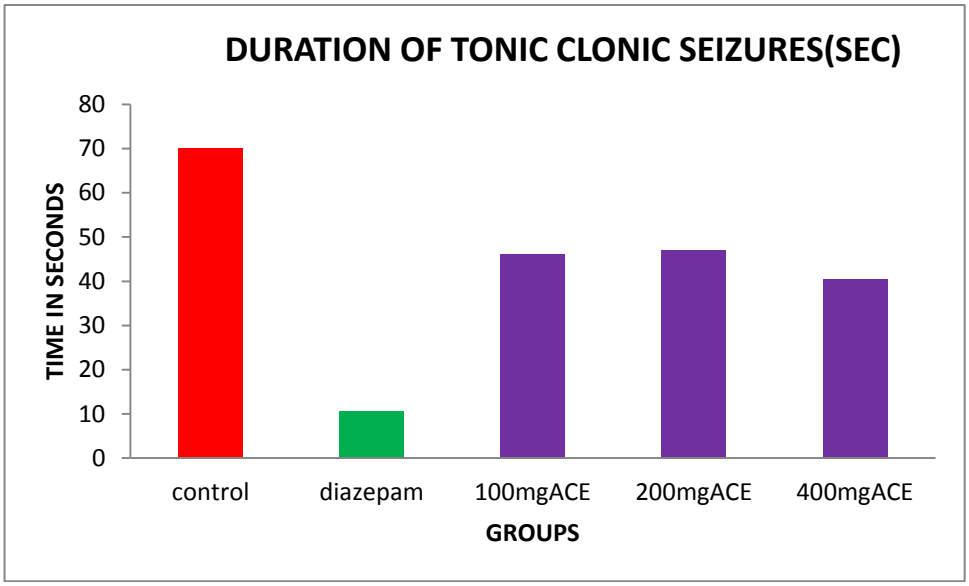

Fig. 2: Effect of hydro alcoholic extract of Allium *cepha* bulbs on PTZ (60mg/kg) induced convulsions (Duration of tonic-colonic seizure) in mice.

Table 3: Effect of hydro alcoholic extract of *Allium cepha* bulbs on MES induced convulsions (Duration of tonic extensor seizure) in mice.

TREATEMENT	DURATION OF TONIC EXTENSOR SEIZURES (seconds) No. of animals in the group						MEAN ± SEM
	1	2	3	4	5	6	
Control (2% Gum acacia p.o.)	46	66	53	49	54	58	54.3 **±2.883**
Diazepam (5mg/kg p.o.)	8	12	7	13	5	11	**10.5±1.945 ****
ACE (100mg/kg p.o.)	38	35	32	61	41	31	**39.6 ±4.529 ****
ACE (200mg/kg p.o.)	30	31	34	39	36	33	**33.8 ±1.352 ****
ACE (400mg/kg p.o.)	30	27	26	30	23	32	**28 ±1.342 ****

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's't'test.
Where, **represents highly significant at p<0.01 ACE: *Allium cepha* Extract MES: Maximal electro shock.

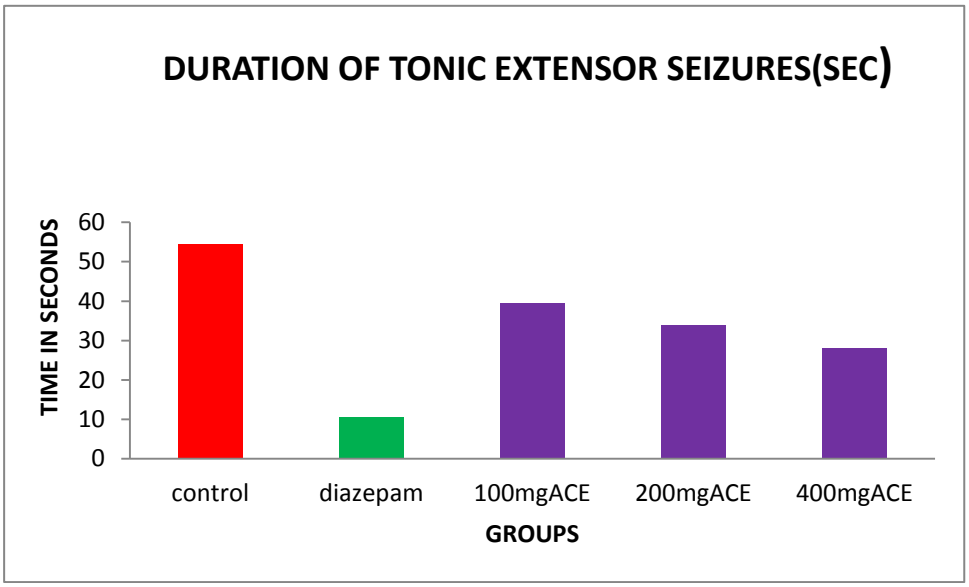

Fig. 3: Effect of hydro alcoholic extract of Allium *cepha* bulbs on MES induced convulsions (Duration of tonic extensor seizure) in mice.

Table 4: Effect of hydro alcoholic extract of Allium *cepha* bulbs On MES induced convulsions (Onset of clonic seizure) in mice

TREATEMENT	ONSET OF CLONIC SEIZURES (seconds) No. of animals in the group						MEAN ± SEM	STATUS OF ANIMALS Death/Recovery
	1	2	3	4	5	6		
Control (2% Gum acacia p.o.)	8	14	9	17	10	13	11.8±1.400	4/2
Diazepam (5mg/kg p.o.)	23	26	25	24	27	25	25 ±0.577 **	0/6
ACE (100mg/kg p.o.)	14	16	18	18	21	20	17.8 ±1.046 *	4/2
ACE (200mg/kg p.o.)	17	19	15	23	27	15	19.3 ±1.961 **	2/4
ACE (400mg/kg p.o.)	23	23	27	19	18	21	21.8 ±1.327 **	1/5

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's't'test.
Where, *represents highly significant at p<0.05,**represents highly significant at p<0.01
ACE: *Allium cepha* Extract; MES: Maximal electro shock.

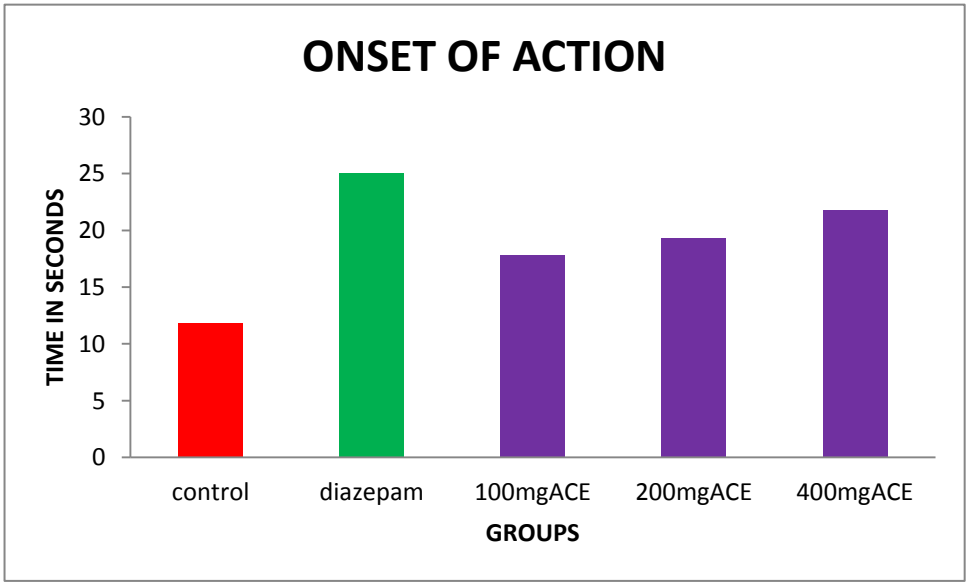

Fig. 4: Effect of hydro alcoholic extract of Allium *cepha* bulbs on MES induced convulsions (Onset of clonic seizure) in mice.

DISCUSSION

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and treatment of individuals with epilepsy, but also possess many toxic adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.

In folklore medicine *Allium cepha* is used in the treatment of convulsions. Based on its folklore application[3], the anticonvulsant activity of bulb extracts of *Allium cepha* was studied in different experimental models employing in rats.

Allium cepha at low, medium and high doses (100, 200 and 400mg/kg respectively) produced highly significantly increased the threshold for clonic and tonic convulsions and the percentage protection against convulsions were 66.66%, 83.33% and 83.33% respectively as compared to control group standard drug diazepam (5mg/kg) had abolished the clonic and tonic seizures with injection of PTZ (80mg/kg s.c.) and offered 100% protection.

MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures i.e. hind limb tonic extensor (HLTE) and clonic convulsions.

Allium cepha at low, medium and high doses (100, 200 and 400mg/kg respectively) produced highly significant increased the duration of tonic extensor phase and onset of clonus, onset of clonic seizures as compared to control and thus exhibited anticonvulsant effect and the percent protection was 66.66%, 83.33% and 33.33% respectively. Standard drug (Diazepam 5mg/kg) had abolished the tonic extensor phase and showed 100% anticonvulsant effect by preventing seizure spread. The percentage protection (Anticonvulsant effect) was found to be increased dose dependent.

Allium cepha at medium and high doses (200 and 400mg/kg respectively) had high significantly delayed the latency onset of convulsions and latency onset of tonic-extensor convulsion but Low dose(100mg/kg) had significant effect in onset of convulsions and offered 66.66%, 83.33% and 33.33% protection respectively. The anticonvulsant effect (increased percent protection) was found to be dose dependent i.e. from low dose to high dose. Standard drug diazepam (5mg/kg) had abolished the tonic convulsions and offered 100% protection.

Further, there are reports indicating that, flavonoids and saponins[9,10] containing plants substance generally exhibit anticonvulsant activity. Hence with respect to the present case it may be true, since the hydro alcoholic extract of Allium *cepha* exhibited good anticonvulsant activity since which had shown the presence of flavonoids and saponins.

Hence it is concluded that, the *Allium cepha* bulbs possesses significant anticonvulsant activity against pentylenetetrazole and MES induced convulsions

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