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EVALUATION OF ANTI EPILEPTIC ACTIVITY OF *ALLIUM CEPHA* BULBS EXTRACT IN MICE

G. Prasanna Ramakrishna*[1], G. Kiran[1], Tulasi uma rani[1], Syed Ayesha begum[1], Y.Anuradha[2], Chedella Swetha[1]

[1*]Department of Pharmacology, A.M. Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh, India.

[2]Department of Pharmaceutical Analysis, A.S.N College of Pharmacy, Tenali, Guntur, Andhra Pradesh, India.

ABSTRACT

The epilepsy is one of the most common neurological conditions that show a prevalence rate in 1–2% of the world population. Although a considerable number of antiepileptic drugs are available for the management / treatment of epilepsy there is still an urgent need for development of new drugs as alternatives. *Allium Cepha* [family: Liliaceae] has been used, as anti-epileptic agent in traditional system of medicine in India. However, there are no reports about its scientific validation for the claimed activity. Hence, the present study was aimed to explore the possible antiepileptic activity of bulbs extract of *Allium Cepha in* experimental animal models. For assessing of antiepileptic activity Pentylenetetrazole (PTZ) and Maximal Electro Shock **(**MES) induced convulsive models were used. Diazepam was used as a standard reference for all models. In PTZ and MES induced convulsion models, high dose, medium dose and low dose of *Allium Cepha* extract showed significant anti-epileptic activity by delaying the onset of convulsions and by prolong the onset of clonus and tonic-extensor convulsion. Thus, the result recorded with above experimental models confirms that hydroalcoholic extract of *Allium Cepha* bulbs possesses antiepileptic activity.

KEYWORDS

Allium Cepha, Pentylenetetrazole, Maximal Electro Shock, Hydroalcoholic Extract and Anti-epileptic.

Author for Correspondence:
G. Prasanna Ramakrishna,
Department of Pharmacology,
A.M. Reddy Memorial College of Pharmacy,
Narasaraopet, Guntur, Andhra Pradesh, India.
Email: gprkchowdary@gmail.com

INTRODUCTION

Epilepsy is a collective term for a 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

and sometimes with autonomic hyperactivity[1,2].

Incidence[3]

Studies in developed countries suggest an annual incidence of epilepsy of approximately 50 per 100,000 of the general population. However, studies in developing countries suggest that this figure is nearly double that at 100 per 100,000. One of the main reasons for the higher incidence of epilepsy in developing countries is the higher risk of experiencing a condition which can lead to permanent brain damage. These conditions include neurocysticercosis, meningitis, malaria, pre and perinatal complications and malnutrition.

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. However, there are hardly few drugs in the market which overcomes from epilepsies without any side effects. 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, carminative, stomachic, anodyne and tonic. They are useful in haemorrhoids, dysentery, flatulence, dyspepsia, colic, jaundice, splenopathy, hepatopathy, tumours, pneumonopathy, bronchitis, otalgia, pharyngodynia, lumbago, epilepsy, wounds, paralysid, arthralgia, leucoderma and skin diseases[4].

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.

MATERIAL AND METHODS

Materials used during the experiment are Diazepam [Ranbaxy Laboratories Ltd, Mumbai, India], Pentylenetetrazole [Sigma-Aldrich, St.Louis, MO 63103 USA], Ethyl Alcohol [Ranbaxy Laboratories Ltd, Mumbai, India], Methyl Paraben [Ranbaxy Laboratories Ltd, Mumbai, India].

Collection of Plant Material

Bulbs of *Allium cepha* were collected in the month of Jan 2011 from D.C.R.M. Pharmacy collge, Inkollu. The specimen was identified and authenticated by the renowned botanist Prof.Vijay Kumar, Department of Botany, Hindu College, Machilipatnam. 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 Extracts [5]

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 based on that, further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50ºC to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container till used.

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 [6]

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.

Principle of the Test

Animals are dosed, one at a time, at 24 hour intervals. The first animal receives a dose at the level of the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the next animal is adjusted up or down. If an animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased. After reaching the reversal of the initial outcome, i.e. the point where an increasing (or decreasing) dose pattern is reversed by giving a smaller (or a higher) dose, four additional animals are dosed following the same UDP. The LD_{50} is calculated using the method of maximum likelihood.

Procedure

The systemic acute oral toxicity (LD_{50}) profile of the extract was evaluated in female wistar albino rats according to OECD 425 guidelines. In brief, this method was carried out in three steps, the initial investigation in which nine animals were used, three animals per treatment group. The animals used were fasted overnight, note down the fasted body weights and calculate the doses; the dose volume should not be exceeded 1ml/100gm. The different doses selected were 500, 1000, 2000 mg/kg of the extract per body weight. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter for a total of 14 days. However, the duration of the observation period should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary.

Determination of anticonvulsant activity Ptz (pentylenetetrazole) induced convulsions[7,8]

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)

GroupE-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. Each animal was then placed into individual plastic cages and were observed initially for 30min and later up to 24 hrs. 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

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's-t -test to calculate the significance difference if any among the groups. $p<0.05$ was considered as statistically significant. The results are compiled in Table No.5.

Maximal Electro Shock (Mes) Induced Convulsions[9]

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. For recording various parameters, mice were placed in clear rectangular plastic cages with an open top, permitting full view of the animal's motor responses to seizure. In the pilot study various phases of convulsions, viz., tonic flexion, extension, clonus, stupor and mortality due to convulsions were selected as the parameters. Phenytoin (25mg/kg p.o.) used as standard drug.

The following parameters were recorded during 1hr test session.

Tonic flexion
Tonic extension
Clonus convulsions
Percent protection

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's-t-test to calculate the significance difference if any among the groups. $p<0.05$ was considered as statistically significant. The results were shown in Table No.8.

RESULTS

Acute Oral Toxicity Study

For the LD_{50} dose determination, hydro alcoholic extract of *Allium cepha* bulbs were administered up to dose 2000 mg/kg body weight and extract did not produce any mortality, thus $1/5^{th}$, $1/10^{th}$, $1/20^{th}$ of maximum dose tested were selected for the present study . LD_{50} of Hydro alcoholic extract of *Allium cepha* bulbs were found to be - 2000 mg/kg.

Assessment of Anti-Convulsant Activity of Hydro Alcoholic Extract of *Allium Cepha* Bulbs

Ptz (Pentylenetetrazole) Induced Convulsions

Allium cepha bulbs were screened for anticonvulsant activity using PTZ induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of *Allium cepha* bulbs (100, 200 & 400mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that *Allium cepha* low, medium and high doses (100, 200 & 400mg/kg respectively) exhibited a very significant anti convulsant effect by increasing onset time of seizures and reducing the duration of tonic-colonic seizures. The lower, medium and higher doses of Allium cepha bulbs offered a protective effect of 66.66%, 83.33% and 83.33% up to 1hr interval respectively. The standard drug diazepam (5mg/kg) exhibited a significant anti-convulsant activity and offered 100% protection.

MES Induced Convulsions

Allium cepha bulbs were screened for anticonvulsant activity using MES induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of *Allium cepha* bulbs (100, 200 & 400mg/kg) respectively. The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that *Allium cepha* bulbs low, medium and high doses (100, 200 & 400mg/kg respectively) exhibited a highly significant anticonvulsant effect by reduced the duration of tonic extensor phase and increases the onset of convulsions (but in low dose produces significant effect in onset of convulsions). The lower, medium and higher doses of *Allium cepha* bulbs offered a protective effect of 33.33%, 66.66% and 83.33% up to 1hr interval respectively. The standard drug diazepam (5mg/kg p.o.) exhibited a highly significant anticonvulsant activity and offered 100% protection.

Table No.1: Nature and Percentage Yield of the Extract

S.No	Name of the Extract	Nature	Colour	%Yield(% w/w) in g.
1.	Hydro alcoholic extract	Sticky	Reddish	24.50

Table No.2 : Phytochemical Constituents présent in *Allium Cepha* Bulbs Extract

S.No	Test	Hydro alcoholic extract
1	**Carbohydrates**	
	Benedicts test	+
	Fehling's test	+
2	**Proteins**	
	Biuret test	–
	Millons test	–
3	**Amino acids**	
	Ninhydrin test	+
	Tyrosine test	+
4	**Alkaloids**	
	Mayers test	+
	Dragendroffs test	+
5	**Glycosides**	
	Borntragers test	+
6	**Flavonoids**	
	Lead acetate test	+
7	**Phytosterols**	
	Salkowski test	+
8	**Fats and oils**	
	Solubility test	–
	Stain test	–
9	**Phenolics and tannins**	
	Lead acetate test	–
	Acetic acid test	–
10	**Volatile oils**	
	Solubility test	+

(+) Indicates positive, (–) Indicates negative result.

Table No.3: (a) 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)						MEAN ± SEM	ANIMAL STATUS AFTER 1hr Death/Recovery
	No. of animals in the group							
	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 by Dunnett's't' test.

Where, **represents highly significant at p<0.01

ACE: *Allium cepha* Extract

PTZ: Pentylenetetrazole.

Table No.4: 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)						MEAN±SEM
	No. of animals in the group						
	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.

Table No.5: Effect of hydro alcoholic extract of *Allium cepha* bulbs on PTZ (60mg/kg) induced convulsion in mice

TREATEMENT	AVG.WT.	AVG.VOL. OF DOSE (ml)	ONSET OF SEIZURE (Sec)	TONIC CLONIC SEIZURE	STATUS OF ANIMAL (No. of animals Alive)	% PROTECTION (1hr)
Control (2% Gum acacia p.o.)	21	0.21	364±4.163	70±2.436	0	0
Diazepam (5mg/kg p.o.)	24.3	0.24	1082.6±3.783 **	10.6±1.626 **	ALL	100
ACE (100mg/kg p.o.)	24.33	0.25	530.1±8.619**	46±3.941 **	2	66.66
ACE (200mg/kg p.o.)	21.3	0.22	1526±930.85 **	47±2.477 **	5	83.33
ACE (400mg/kg p.o.)	21.00	0.21	733±8.359 **	40.5±1.057 **	5	83.33

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

Table No.6: 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)						MEAN ± SEM
	No. of animals in the group						
	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.

Table No.7: 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)						MEAN ± SEM	STATUS OF ANIMALS Death/Recovery
	No. of animals in the group							
	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.

Table No.8: Effect of hydro alcoholic extract of *Allium cepha* bulbs on MES induced convulsions in mice

S.No	TREATEMENT	AVG.WT. (g)	AVG.VOL.OF DOSE (ml)	ONSET OF CLONUS (Sec) Mean ± SEM	DURATION OF TONIC EXTENSOR (Sec) Mean ± SEM	% PROTECTION (1hr)
1	**Control (2% Gum acacia p.o.)**	24.6	0.25	11.8±1.400	54.3 ±2.883	33.33
2	**Diazepam (5mg/kg p.o.)**	23.8	0.23	25 ±0.577 **	10.5±1.945 **	100
3	**ACE (100mg/kg p.o.)**	23.3	0.23	17.8 ±1.046 *	39.6 ±4.529 **	33.33
4	**ACE (200mg/kg p.o.)**	22.4	0.22	19.3 ±1.961 **	33.8 ±1.352 **	66.66
5	**ACE (400mg/kg p.o.)**	24	0.24	21.8 ±1.327 **	28 ±1.342 **	83.33

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.

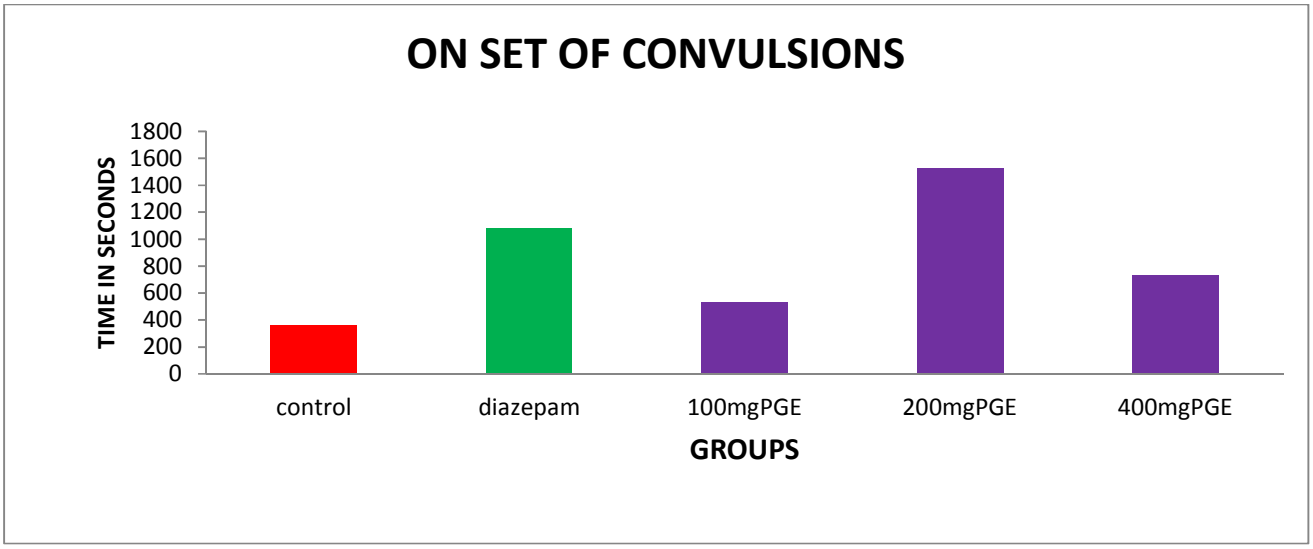

Figure No.1: Effect of hydro alcoholic extract of *Allium cepha* bulbs on PTZ (60mg/kg S.C.) induced convulsions (onset of seizure) in mice

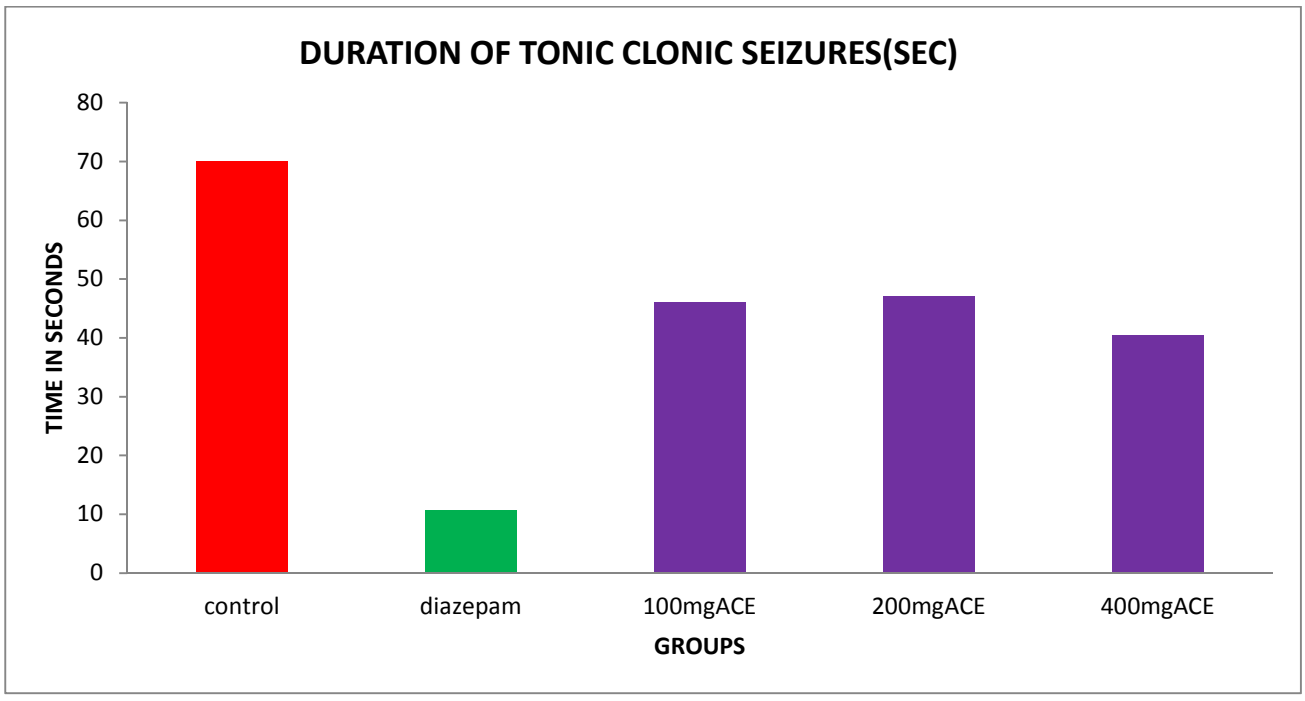

Figure No.2: Effect of hydro alcoholic extract of *Allium cepha* bulbs on PTZ (60mg/kg) induced convulsions (Duration of tonic-colonic seizure) in mice

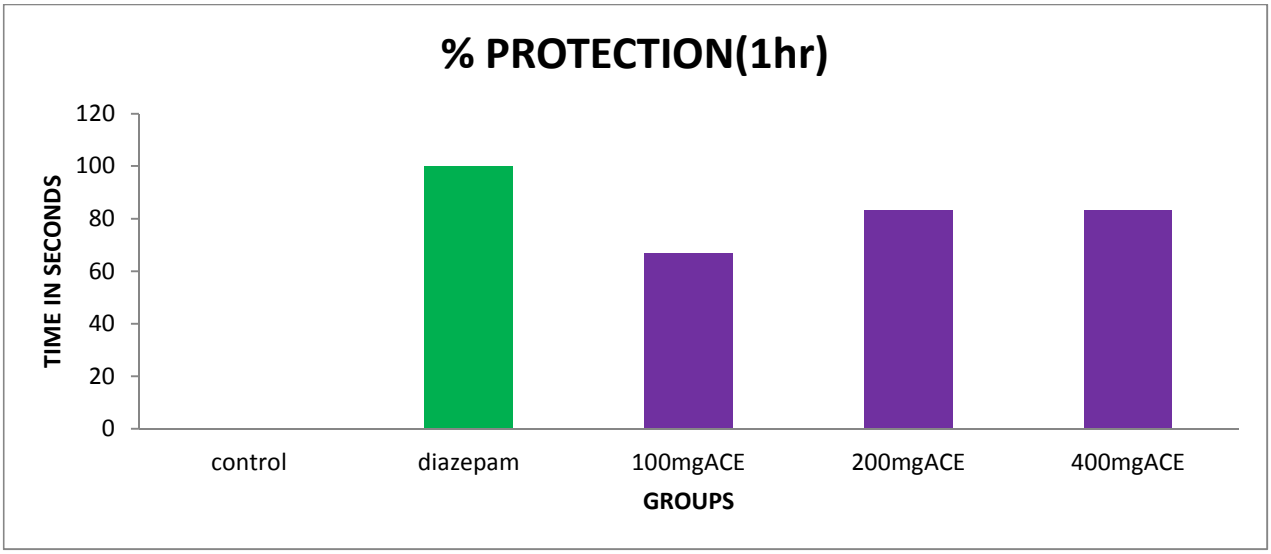

Figure No.3: Effect of hydro alcoholic extract of *Allium cepha* bulbs on PTZ (60mg/kg) induced convulsion in mice

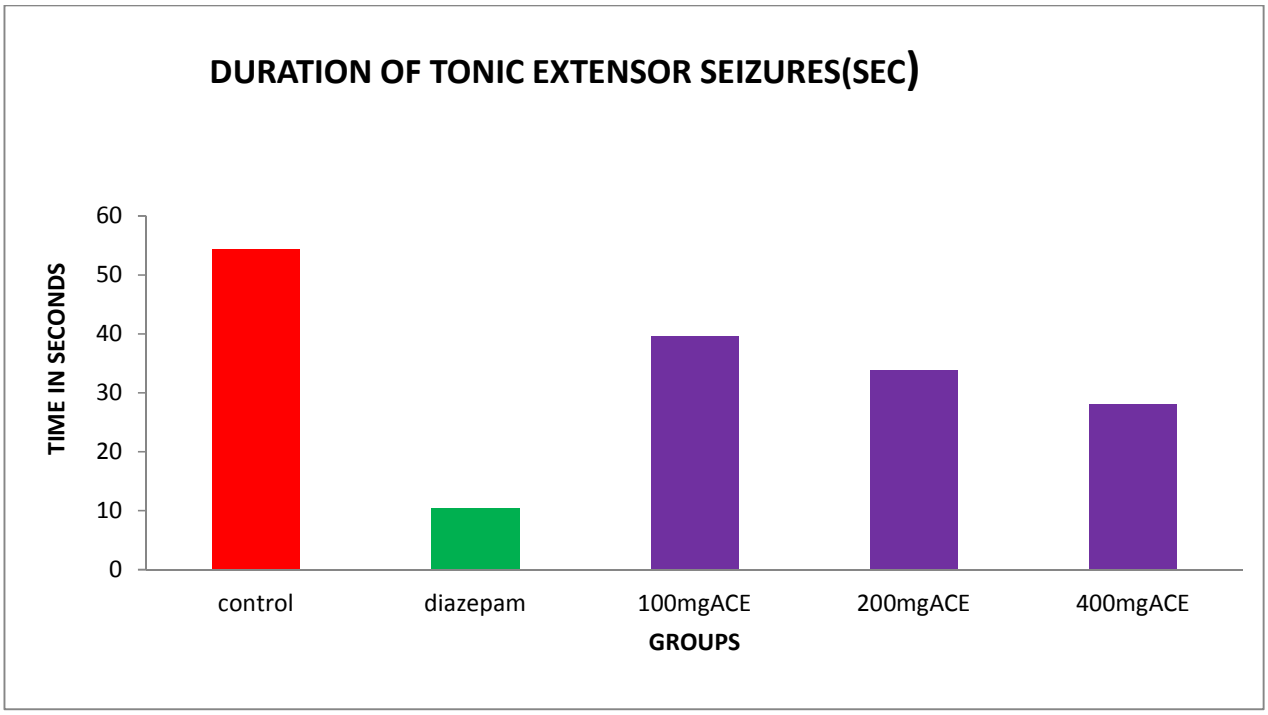

Figure No.4: Effect of hydro alcoholic extract of *Allium cepha* bulbs on MES induced convulsions (Duration of tonic extensor seizure) in mice

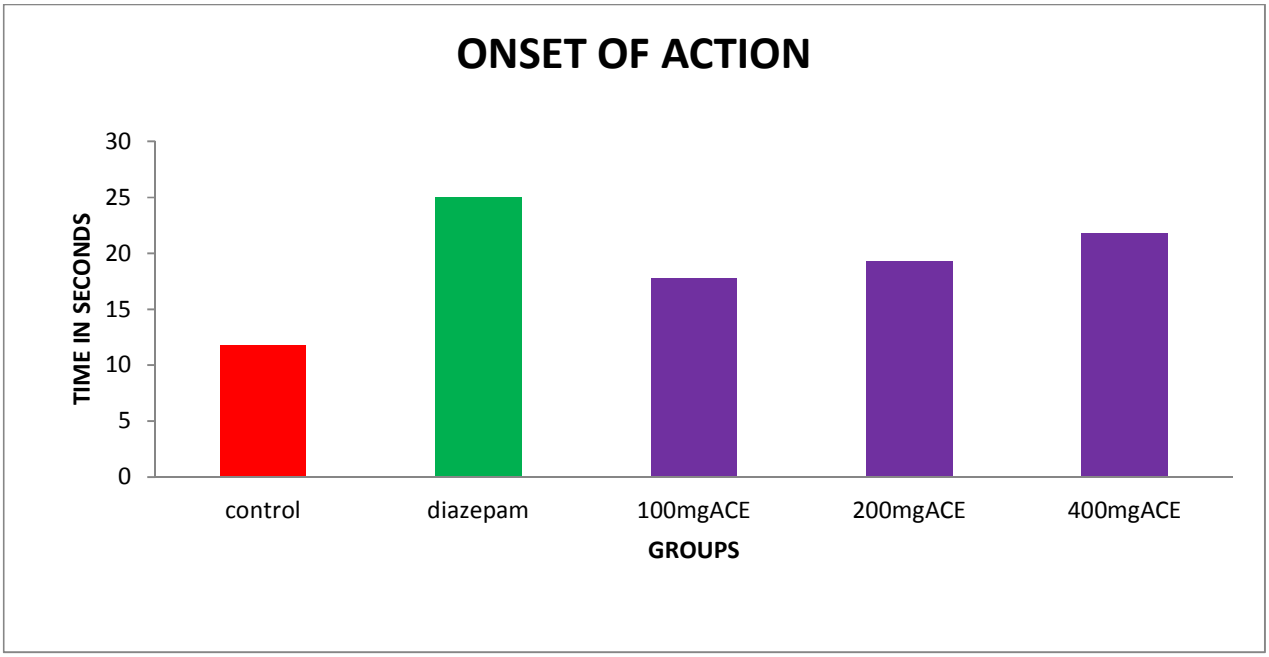

Figure No.5: Effect of hydro alcoholic extract of *Allium cepha* bulbs on MES induced convulsions (Onset of clonic seizure) in mice

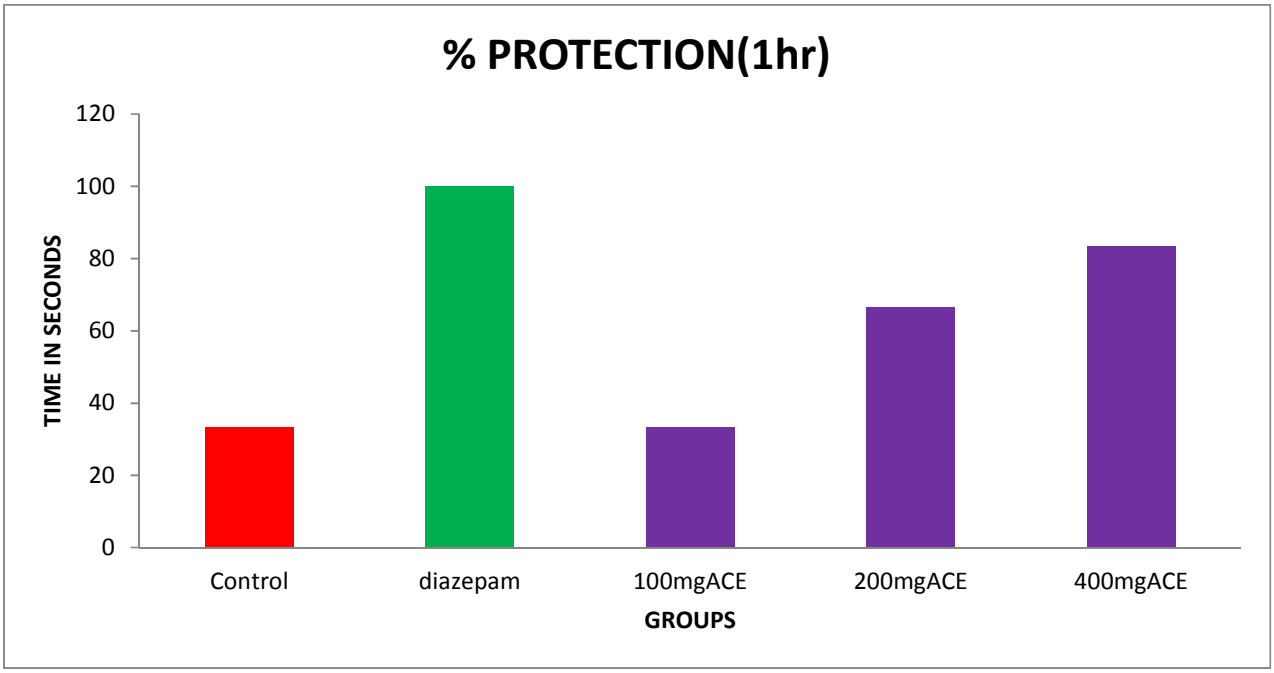

Figure No.6: Effect of hydro alcoholic extract of *Allium cepha* bulbs on MES induced convulsions (Onset of clonic seizure) in mice

CONCLUSION

The present study was carried out to find out, evaluation of anti-epileptic activity on *Allium cepha* bulbs extract in mice. From the results obtained, we conclude that the *Allium cepha* bulbs extract at higher and medium doses produces highly significant and sustained increase in the delay of onset of convulsions and decrease in the of convulsions. This activity may be due to the presence of different phytoconstituents viz, flavanoids and saponins in the extract. However, long term studies in different animals and epileptic subjects may further substantial our study result.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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