

## Research Article

## STUDIES ON ANTI-CONVULSANT ACTIVITY OF STEM BARKS OF *CALOTROPIS GIGANTEA* LINN. IN EXPERIMENTAL ANIMALS

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### ABSTRACT

The stem barks of *Calotropis gigantea* Linn. (Asclepiadaceae) a widely growing plant has been reported to possess number of medicinal properties. The purpose of the present study was to evaluate scientifically the anti-convulsant effect of barks of the plant used traditionally in Indian system of medicine, using MES and PTZ induced seizure models. The effect of different extracts of stem barks of the plant were evaluated for their anti-convulsant profile in maximal electroshock seizures (MES) test and Pentylentetrazole (PTZ) test using albino Wister rats. The ED<sub>50</sub> dose of Phenytoin (25mg/kg) was used for comparison. The methanolic extract at 180 mg/kg body weight significantly ( $p < 0.001$ ) inhibited the Hind Limb Tonic Extension (HLTE) induced by MES and onset of clonic convulsion or latency of convulsion induced by PTZ. Overall the methanolic extract of the stem barks of the plant has a faster onset of action and responses were qualitatively similar to Phenytoin. So on the basis of the present findings we can conclude that the stem bark of *Calotropis gigantea* Linn. Possess potential anticonvulsant activity.

**Keywords:** *Calotropis gigantea*; Anticonvulsant activity; MES; PTZ; HLTE.

### INTRODUCTION

*Calotropis gigantea* Linn (Asclepiadaceae) a widely growing plant has been reported to possess number of medicinal properties.<sup>1</sup> In the traditional system of medicine the roots and barks of *Calotropis gigantea*, are used as anticancer,<sup>2</sup> antifertility,<sup>3</sup> antidote for snakebite, antiscabetic,<sup>4</sup> cardiovascular diseases,<sup>5</sup> and various skin diseases. Leaves are used in asthma, skin diseases like eczema;<sup>6</sup> elephantiasis etc. juice is used in leprosy, syphilis and idiopathic ulceration etc.

Traditionally roots and barks of *Calotropis gigantea* are used for all kinds of fits, epilepsy, convulsions in children's and paralysis complaints.<sup>6</sup> Attempts to find out a common Neurochemical basis for human or experimental epilepsy have been disappointing. An imbalance between the excitatory and inhibitory neurotransmitters is responsible for seizures.<sup>7,8</sup> Many drugs that increase the brain content of GABA have exhibited anti-convulsant activity against seizure induced by MES, PTZ and lithium Pilocarpine. The MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic clonic seizures. In 1980, Pal and Sinha had isolated, crystallized and studied the properties of calotropins D<sub>1</sub> and D<sub>2</sub> from the plant.<sup>9</sup> The plant is considered crude drugs of Bangladesh<sup>10</sup> and medicinal plant of Indonesia.<sup>4</sup>

The new oxypregnane-oligoglycosides named, calotropins A and B have been isolated from the roots of *Calotropis gigantea* and their chemical structures have been elucidated by chemical and spectroscopy methods.<sup>3</sup> The cytotoxic principle of "akondmul" (Roots of *Calotropis gigantea*), obtained as cytotoxic principles.<sup>10</sup> Chitme and Ramesh Chandra have proved *Calotropis gigantea* is having a significant anti diarrheal activity against castor

oil induced diarrhea.<sup>11</sup> The objective of the present study was to investigate anti convulsant activity of different extracts of stem barks of *Calotropis gigantea* Linn. against seizures induced by MES, PTZ model using albino Wister rats of either sex.

### MATERIALS AND METHODS

**Plant Material:** The stem barks of *Calotropis gigantea* were collected around Belgaum city in May 2004. A voucher specimen has been deposited at the botanical survey of India (No.BSI/WC/Tech/2004/556), Pune and Dept of Pharmacognosy and Phytochemistry, KLE's College of Pharmacy, Belgaum, India.

**Preparation of plant extract:** The stem barks were dried under shade (1.0 kg), crushed to coarse powder and extracted in soxhlet assembly successively with petroleum ether (40-60°C), benzene, chloroform and methanol. Finally the fresh drug was macerated with chloroform water. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50°C. Each extract was concentrated by distilling off the solvent and then evaporated to dryness on water bath. The extracts were stored in a refrigerator and reconstituted in water for injection in the presence of tween 80 just before use.

**Animals:** Albino Wister rats of either sex weighing 150-200 gm were used for MES and PTZ induced seizure models. Animals were housed at a temperature of 25 °C ± 1°C and relative humidity of 45-55%, and a 12:12 dark: light cycle was followed during the experiments. Animals had free access to food and water, however, food but not water was withdrawn 8 hrs before and during the experiments. The institutional animal ethical committee approved the protocol of the study. The animals were



obtained from the central animal house of Jawaharlal Nehru. Medical College, Belgaum.

**Drugs:** Pentylenetetrazole (PTZ, Sigma, USA) and Phenytoin (Park Davis, India Ltd) were used in this study. The drugs were dissolved in water for injection and administered in a volume of 5 ml/kg body weight.

**Toxicity Studies:** The acute toxicity studies were tested according to the OECD guidelines.<sup>12</sup> The five extracts were administered orally with tween 80 suspension.

#### Assessment of anticonvulsant activity

**Anti-convulsant activity against maximal electroshock seizures (MES) in albino waster rats:** Animals were randomly divided in to seven groups of six animals each (n=6). Group 1 served as control, received equivalent amount of the respective vehicle, group 2 received Phenytoin (25 mg/kg i.p) served as reference standard and group 3, 4, 5, 6 and 7 received the crude extracts of petroleum ether (200 mg/kg), benzene (200 mg/kg), chloroform (200 mg/kg), methanol (180 mg/kg) and aqueous (200 mg/kg) p.o. respectively, before the application of electroshock. Experiments were conducted at the same time each day and the rats were subjected to MES at 150 mA, 60 Hz for 0.2sec through pinnal electrodes at 60 min after vehicle/drug administration. In all electrically induced convulsions, the rats were manually restrained and released immediately.

After stimulation to permit observation of the seizure throughout its entire course. MES results in Hind Limb Tonic Extension (HLTE), the duration being measured in seconds. Rats were pretested 24 hrs prior to drugging (baseline values) and those failing to give HLTE were rejected. The criterion for anti-convulsant activity and protection against MES induced seizures is abolishing HLTE, which is taken as the end point of the test.<sup>13, 14</sup>

**Anti-convulsant activity against chemo shock (PTZ) induced seizures in rats:** Seizures were induced in rats with PTZ at 80 mg/kg i.p. This is the convulsive dose in 97 % of the animals.<sup>13</sup> PTZ was dissolved in 0.9 % saline and injected i.p in rats at 0.2 ml/100 gms.

Animals were randomly divided in to 7 groups of each six animals (n=6). Group 1 served as control, received equivalent amount of the respective vehicle, group 2 received Phenytoin (25 mg/kg i.p) served as reference standard and group 3,4,5,6 and 7 received the crude extracts of petroleum ether (200 mg/kg), benzene (200 mg/kg), chloroform (200 mg/kg), methanol (180 mg/kg) and aqueous (200 mg/kg) p.o respectively. All the crude extracts and standard drugs were administered 60 min before the administration of PTZ. Experiments were conducted at the same time each day and the rats were observed for the clonic convulsions (onset of action) values are expressed in terms of Mean  $\pm$  SEM (time in sec).

**Statistical analysis:** The experimental results are represented as mean  $\pm$  SEM. The one way ANOVA was

used to compare the HLTE data of the various groups. The HLTE data obtained in the MES test were analyzed using appropriate models of analysis of variance and  $p < 0.001$  is accepted as a significant.

## RESULTS

### Assessment of anti convulsant activity

**Maximum electroshock test (MES):** All the extracts (p.o) and standard drug (i.p) were administered 60 mins before application of electroshock. Values are expressed as mean  $\pm$  SEM from the experiment. The duration of the Hind Limb Tonic Extension (HLTE) in rats treated with vehicle was  $24.83 \pm 0.54$  sec. The methanolic extract at a dose of 180 mg/kg has protected the animals from the seizures and duration of HLTE. The dose of 200 mg/kg (other extracts) has having less protection of animals from seizures compared to the methanolic extract, which in turn compared to the control group. The rats treated with the different extracts exhibited the HLTE for  $12.66 \pm 0.55$ ,  $9.5 \pm 0.67$ ,  $14.33 \pm 0.88$ ,  $4.0 \pm 0.577$  and  $8.83 \pm 0.703$  seconds respectively as mentioned in the Table-1.

**Pentylene Tetrazole Induced Seizures (PTZ):** In animals treated with vehicle the onset of convulsions appeared at  $496.66 \pm 16.66$  sec after PTZ administration some of the animals were died after seizures. The methanolic extract of the stem bark significantly inhibit the onset of convulsions compared to the control group. Phenytoin (25 mg/kg i.p) inhibited the seizures completely and the results are shown in the Table-2.

## DISCUSSION AND CONCLUSION

The results obtained in the MES test in rats that, the standard drug as well as the different extracts of stem barks of *Calotropis gigantea* protected against MES induced seizures. Stem barks of *Calotropis gigantea* had a slower onset of action and lesser degree of anticonvulsant activity. (Approximately one fifth the activity of Phenytoin) but the activity lasted for 24 hrs. The results obtained in these studies demonstrate unequivocally that like Phenytoin and possessed anticonvulsant activity. In the MES test since, inhibition of the MES test predicts the activity against generalized tonic clonic and cortical focal seizures. Hence it is suggests that the methanolic extract of the stem barks of the plant is useful in suppressing generalized tonic clonic seizures. Several drugs are thought to inhibit the seizures by regulating GABA mediated synaptic inhibition through an action at distinct sites of the synapse. Researchers are gaining new insight in to the traditional medicine in assisting the body to maintain its own self healing systems while preventing debilitating effects of chronic diseases, like epilepsy.<sup>13</sup>

Thus methanolic extracts of the stem barks of *Calotropis gigantea* possess anticonvulsant property against the MES and PTZ induced seizures in albino wistar rats. However the further research is in progress to isolate the compound responsible for activity.



**Table 1:** Effect of different extracts of stem barks of *Calotropis gigantea* on MES induced seizures in albino Wister rats.

Group	Treatment (mg/kg bw)	Time (Sec) in various phases of convulsions (Mean ±SEM)				
		Flexon	Extensor	Clonus	Stupor	% Incidence of convulsions
1.	Control (saline 1 ml/rat)	9.50±0.76	24.83±0.54	2.33±0.42	121.0±1.18	100%
2.	Standard Phenytoin (25)	0.00±0.00	0.00±0.00	0.00±0.00	1.8±0.80	0%
3.	Pet. Ether extract (200)	4.33±0.42	12.66±0.55	6.83±0.30	112.33±1.256	52%
4.	Benzene Extract (200)	12.16±0.87	9.5±0.67	14.16±1.24	126.33±1.452	40%
5.	Chloroform Extract (200)	7.83±1.24	14.33±0.88	15.66±1.49	118.83±1.40	60%
6.	Methanol Extract (180)	3.66±0.474	4.0±0.577	12.5±0.767	109.66±2.431	16%
7.	Aqueous Extract (200)	7.83±0.060	8.83±0.703	9.33±0.802	118.66±3.120	36%

Note: Time (in sec) in various phases of convulsions (Mean±SEM);

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with MES+Saline treated groups.

**Table 2:** Effect of different extracts of stem barks of *Calotropis gigantea* on Pentylene Tetrazole induced seizures in albino wistar rats.

Group	Treatment (mg/kg bw)	Onset of Action in Seconds (Mean ± SEM)	Percentage (%) Incidence of Convulsions
1.	Control (PTZ) 80 mg	496.66±16.66	100%
2.	Phenytoin + PTZ [4+80]	0.00±0.00	0%
3.	Benzene Extract + PTZ [200+80]	363.83±5.81	73.23%
4.	Petroleum Ether Extract + PTZ [200+80]	249.66±6.616	50%
5.	Chloroform extract + PTZ [200+80]	247.83±6.112	50%
6.	Methanol Extract + PTZ [180+80]	203.33±7.149	41%
7.	Aqueous Extract + PTZ [200+80]	220.9±72.74	45%

N=6, \*\*\*p<0.001, \*\*p<0.01, \*p<0.05 when compared to the PTZ + Vehicle treated group.

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