



ANTI-CONVULSANT ACTIVITY OF VARIOUS EXTRACTS OF LEAVES OF *CALOTROPIS GIGANTEA* LINN AGAINST SEIZURE INDUCED MODELS

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ABSTRACT

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 anticonvulsant activity of Petroleum ether, Benzene, Chloroform, Methanol and Aqueous extract of leaves of the plant *C.gigantea* in albino Wistar rats on electrically and chemically induced seizures. The anti-convulsant activity of different extracts of *C.gigantea* leaves was studied against seizures induced by maximal electroshock seizures (MES) and pentylenetetrazole (PTZ). There was no difference between preventing of PTZ-induced death and MES-induced hind limb tonic extension (HLTE) in methanolic extract comparing to control groups. In the MES test, the methanol extract (180mg/kg.p.o) of *C.gigantea* leaves showed the most significant ($p < 0.001$) anticonvulsant effect by decreasing the duration of HLTE, clonus and also the duration of stupor phase than other extracts when compared to control group and produced 68% protection in rats. In the PTZ test, the methanol extract (180 mg/kg.p.o) showed a highly significant ($p < 0.001$) effect and produced 55% protection in rats, The ED₅₀ dose of Phenytoin (25 mg/kg.i.p) was used as standard. In conclusion it was realized that methanol extract (ED₅₀ = 180 mg/kg p.o) is more potent than other extracts in showing anti seizure effects.

Keywords: *Calotropis gigantea*, Anti-convulsant activity, MES, PTZ, HLTE

INTRODUCTION

Calotropis gigantea Linn (Asclepiadaceae) a widely growing plant has been reported to possess number of medicinal properties¹. In the traditional system of medicine the roots and barks of *C. gigantea* are used as anticancer², anti-fertility³, antidote for snakebite, antiscabetic⁴, cardiovascular diseases⁵ and various skin diseases. Leaves are used in asthma, skin diseases like eczema. Juice is used in leprosy, syphilis and idiopathic ulceration etc. Traditionally roots and barks of *C.gigantea* are used for all kinds of fits, epilepsy, convulsions in children's and paralysis complaints⁶. 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^{7,8}. 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. Pal and Sinha had isolated, crystallized and studied the properties of calotropins D1 and D2 from the plant⁹. The new oxypregnane-oligoglycosides named; calotropins A and B have been isolated from the roots of *C.gigantea* and their chemical structures have been elucidated by chemical and spectroscopy methods³. The cytotoxic principle of "akondmul" (roots of *C.gigantea*) obtained as cytotoxic principles¹⁰. Chitme and Ramesh C. have proved *C.gigantea* is having a significant anti-diarrheal activity against castor oil induced diarrhea¹¹. The objective of the present study was to investigate anti-convulsant activity of various extracts of leaves of *C.gigantea* Linn. against seizures induced by MES, PTZ model using albino Wistar rats of either sex.

EXPERIMENTAL

Plant

The fresh matured leaves of *C.gigantea* were collected locally from the suburban out fields of Bangalore, province of India. The identity of *C.gigantea* was authenticated by Dr.Siddamallayya from Regional Research Institute, Bangalore, on the basis of taxonomical characters following routine pharmacognostical studies, including organoleptic macroscopic tests and herbarium specimen was deposited in the department of herbarium. The leaves were air dried under shade and further subjected to extraction.

Preparation of extract¹²

The collected *C.gigantea* leaves were air dried under shade at room temperature and milled to a coarse powder. The obtained dried powder was subjected to successive soxhlet extraction with Pet.Ether (40-60°C), benzene, chloroform, methanol and finally the fresh drug was macerated with chloroform water. The powdered leaf material was packed in a tumble made of Whatmann's filter paper. It was subjected to extract with various non-polar to polar solvents for 40 cycles each. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50°C. The extract thus obtained was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The obtained residues were yellowish brown to dark brown colour with thick and sticky paste. The extract was stored in refrigerator (2-8°C) and reconstituted uniformly in water for injection in the presence of tween 80 just before administration to animals orally using an intragastric feeding tube.

Animals

Adult rats of Wistar strain of either sex weighing 125-175 g and 10-12 weeks old were obtained from National Institute of Mental Health and Neuro Sciences. They were fed commercial pellet diet and water *ad libitum*. The diet approximately contained: carbohydrate (55%), fat (5%), protein (24%), fiber (4%), calcium (0.6%), phosphorus (0.3%), moisture (10%) and ash (9%). Before treatment allocation and randomization rats were acclimatized to the laboratory conditions for a week. Animals were housed in polypropylene cages (38×23×10cm) with not more than four animals per cage under standard laboratory conditions (25°C ± 2°C), relative humidity 55 ± 10%, alternating 10 h dark/ 14 h light photoperiod. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained and conducted in accordance to the Indian national science academy guidelines for the use and care of experimental animals.

Drugs and chemicals

All chemicals used in present study were of analytical grade. 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 intraperitoneally (i.p.) in a volume of 1 mL/100 g of animal. Control animals received equal volume of injections of the appropriate vehicle. For dosing, the different extracts of *C.gigantea* leaves were suspended uniformly in

the presence of tween 80 (Ranbaxy Laboratories Ltd) just before administration to animals orally using an intragastric feeding tube. Fresh drug solutions were prepared on each day of the experiments.

Acute toxicity studies

The acute toxicity studies were tested according to the OECD guidelines¹³. The five extracts were administered orally with tween 80 suspension. The extracts were administered in doses of 50, 200, 500, 1000, 1800, 2000 mg/kg p.o. to different groups of rats, each containing 3 animals and mortality were observed after 24hrs. LD₅₀ cut off values for pet. Ether, benzene, chloroform and aqueous extract extracts were found to be 2000 mg/kg.b.w and for methanol extract 1800 mg/kg b.w. The 1/10th of the lethal dose was taken for effective dose (therapeutic dose) for subsequent anticonvulsant activity.

Animal experimentation and drug treatment protocol

Anti-convulsant activity against maximal electroshock seizures (MES) in albino wistar 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 Pet.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 maximal electroshock (Inco Electroconvulsimeter model #100-3). 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 produced various phases of convulsions i.e. Flexion, Extension, Clonus and Stupor (Table-1, Fig-1). The duration of HLTE 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^{14, 15, 16}

Anti-convulsant activity against pentylenetetrazole induced seizures (PTZ) in albino Wistar rats

Seizures were induced in rats with PTZ at 80 mg/kg i.p. This is the convulsive dose in 97% of the animals [14]. 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 pet. 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. Experiment is assessed by its ability to delay the onset of myoclonic spasm and clonic convulsions (onset of action). Values are expressed in terms of Mean \pm SEM (time in sec). Protection against PTZ induced convulsions and percentage of mortality was measured.

Statistical analysis

All the grouped data were statistically evaluated by using Graph pad Prism software. Data obtained from delay convulsion behavior were expressed as Mean \pm SEM and were analyzed for statistical significance using one-way ANOVA followed with Dunnett's 't' test $p < 0.001$ considered significant.

RESULTS

The effect of various extracts on MES-induced convulsion in albino Wistar rats

The methanolic extract of leaves of *C.gigantea* significantly ($p < 0.001$) increased the threshold of MES-induced convulsion in albino Wistar rats than other extracts compared with the control group. At 180 mg/kg p.o. the extract produced 32% mortality and 68% of protection against MES induced convulsions in albino Wistar rats (Table-1).

Table 1: Effect of various extracts of leaves of *C. gigantea* Linn on maximal electroshock induced seizures in albino Wistar rats

Group	Treatment (mg/kg b.w.)	Onset of convulsion in seconds (Mean \pm SEM)	Number convulsed/ number used	Mortality (%)	Protection (%)
1.	Control	24.83 \pm 0.54	6/6	100	0
2.	Phenytoin (25)	0.00 \pm 0.00***	0/6	0	100
3.	Pet. Ether extract (200)	13.33 \pm 0.66*	6/6	56	44
4.	Benzene extract (200)	10.83 \pm 1.01**	6/6	44	56
5.	Chloroform extract (200)	13.33 \pm 1.021*	6/6	56	44
6.	Methanol extract (180)	8.0 \pm 1.54***	6/6	32	68
7.	Aqueous extract (200)	11.83 \pm 0.94**	6/6	38	62

Results are expressed as Mean \pm SEM, percentage mortality and protection (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control. One-way ANOVA followed by Dunnett's 't' test.

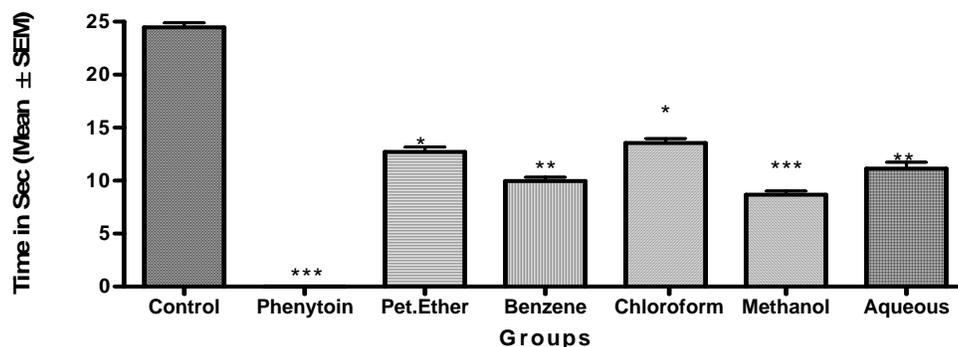


Fig. 1: Effect of various extracts of leaves of *C.gigantea* Linn on maximal electroshock induced seizures in albino Wistar rats**The effect of various extracts on PTZ-induced convulsion in rats**

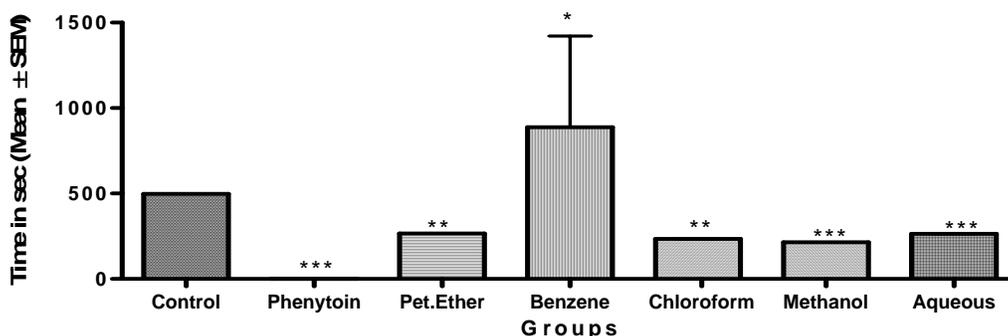
Intraperitoneal administration of PTZ induced tonic-clonic convulsions with 100% mortality in the control group. The methanolic (methanolic) extract of leaves of *C.gigantea*

(180mg/kg p.o.) significantly ($p < 0.001$) increased the onset of convulsion in rats than other extracts compared with the control group. Extract (180 mg/kg p.o.) offered 55 % protection and 45% of mortality against PTZ- induced convulsion in albino Wistar rats (Table-2).

Table 2: Effect of various extracts of leaves of *C.gigantea* Linn on PTZ induced seizures in albino Wistar rats

Group	Treatment (mg/kg b.w.)	Onset of action in seconds (Mean \pm SEM)	Number convulsed/ number used	Mortality (%)	Protection (%)
1.	Control	496.66 \pm 16.66	6/6	100	0
2.	Phenytoin + PTZ [25 + 80]	0.00 \pm 0.00***	0/6	0	100
3.	Pet Ether extract + PTZ [200 + 80]	266.21 \pm 7.351**	6/6	60.3	28.7
4.	Benzene extract + PTZ [200 + 80]	355.38 \pm 7.075*	6/6	80	20
5.	Chloroform extract + PTZ [200 + 80]	233.25 \pm 7.123**	6/6	60	40
6.	Methanol extract + PTZ [180 + 80]	213.11 \pm 6.125***	6/6	45	55
7.	Aqueous extract + PTZ [200 + 80]	264 \pm 7.822***	6/6	52	48

Results are expressed as Mean \pm SEM, percentage mortality and protection (n=6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control. One-way ANOVA followed by Dunnett's't' test.

Fig. 2: Effect of various extracts of leaves of *C. gigantea* Linn on PTZ induced seizures in albino Wistar rats**DISCUSSION**

The methanolic extract of leaves of *C.gigantea* increased the threshold of PTZ and MES induced convulsion in rats and offered protection against PTZ and MES induced convulsion. The protection offered against PTZ and MES induced convulsion in rats 55% and 68% respectively.

The results demonstrated that the *C.gigantea* has anticonvulsant activity in both PTZ and MES seizure models. Table 1-2 and figures 1-2. Indicate that, methanolic extract of *C.gigantea* showed better anti-convulsant activity than other extracts compared to control and shows the ED50 with confidence limits of phenytoin, methanol extract and other extracts in HLTE induced by MES and PTZ models. No difference was found between preventing of death and HLTE in methanolic extract and control group in both PTZ and MES models. The methanolic extract and other extracts increased the latency of convulsion parameters induced by PTZ. Among the tests used for evaluation of anticonvulsant activity, the MES and PTZ tests are of predictive relevance regarding the clinical spectrum of activity of experimental compounds, since the MES and PTZ tests are assumed to identify anticonvulsant drugs effective against human generalized tonic-clonic and absence seizures respectively^{16, 17}.

MES induced seizure can be prevented either by drugs that inhibit voltage-dependent Na⁺ channels such as phenytoin, sodium valproate, felbamate and lamotrigine, or by drugs that block

glutamatergic receptor such as felbamate. On the other hand, drugs that reduce T-type Ca⁺⁺ currents such as ethosuximide can prevent seizures induced by PTZ. Drugs that enhance gamma amino butyric acid type A (GABA_A) receptor mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital and perhaps valproate and felbamate can prevent this type of seizure¹⁸.

Since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures so activity against MES induced seizures, suggests that the methanolic extracts of leaves of *C.gigantea* was useful in suppressing generalized tonic-clonic seizures by regulating GABA mediated synaptic inhibition through an action at distinct sites of this synopsis. PTZ test predicts activity against absence seizures. Since PTZ is a GABA_A receptor antagonist, the methanolic extract may be acting by increasing GABA concentration in the brain.

In the present study, the effect of various extracts of leaves of *C. gigantea* on seizure induced by MES and PTZ in Wistar rats was evaluated and the results evidently demonstrated for the first time that, the methanolic extract of leaves of *C. gigantea* able to produce significant anti-convulsant activity in both MES and PTZ seizures than other extracts when compared to control group. In conclusion, results suggest that, the methanolic extract of leaves of *C. gigantea* will be beneficial in the management of absence and tonic-clonic seizures.

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