Vol. 8/1 (2008) 109 - 114

JOURNAL OF NATURAL REMEDIES

Anti-convulsant activity of roots and barks of *Calotropis gigantea* Linn.

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Abstract

Objective: *Calotropis gigantea* Linn. (Asclepiadaceae) a widely growing plant has been traditionally used for all kinds of fits, epilepsy, convulsions in children and paralysis complaints. The present study has been undertaken to evaluate scientifically the anti-convulsant effects of roots and barks of *Calotropis gigantea* Linn. using maximal electroshock seizures (MES) and Pentylenetetrazole (PTZ) induced seizure models. Methods: The effect of different extracts of roots and barks of *Calotropis gigantea* Linn. were evaluated for their anti-convulsant profile in MES test and PTZ test using albino Wistar rats of either sex. The ED_{50} dose of Phenytoin (25 mg/kg) and Diazepam (4 mg/kg) was used for comparison. Results: The methanolic extract at 400 mg/kg (Root) and 200 mg/kg (Bark) significantly ($P<0.001$) inhibited the hind limb tonic extension (HLTE) induced by MES and onset of clonic convulsion induced by PTZ respectively. Conclusions: The results show that the methanolic extract of the roots and barks of *Calotropis gigantea* Linn. produce better anti-convulsant activity compared to other extracts. So, on the basis of the present findings it can be observed that, the methanolic extracts of barks of *C. gigantea* possess potential anti-convulsant activity than methanolic extracts of roots of *C. gigantea*.

Key Words: *Calotropis gigantea*, Anti-convulsant activity, Maximum Electroshock (MES), Pentylenetetrazole (PTZ), Hind Limb Tonic Extension (HLTE).

1. Introduction

Calotropis gigantea Linn. (Asclepiadaceae) a widely growing plant has been reported to possess number of medicinal properties [1]. In the traditional system of medicine the roots and barks of *Calotropis gigantea* are used as anti-cancer [2], anti-fertility [3], antidote for snakebite, anti-scabetic [4], cardiovascular diseases [5] and various skin diseases. Leaves are used in asthma, skin diseases like eczema [6], 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 and paralysis

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complaints [6]. 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 (1980) had isolated, crystallized and studied the properties of calotropins D_1 and D_2 from *Calotropis gigantea* [9]. The plant is considered crude drugs of Bangladesh [10] and medicinal plant of Indonesia [4].

The new oxiopregnane-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 [3]. The cytotoxic principle of "akondmul" (Roots of *Calotropis gigantea*), cardinolide glycoside, calotropin, frugoside and 4-O-β-D- glucopyronosyl frugoside were obtained as cytotoxic principles [10]. Chitme H.R. *et al*. (2004) have proved *Calotropis gigantea* is having a significant anti-diarrhoeal activity against castor oil induced diarrhoea [11].

The objective of the present study is to investigate anti-convulsant activity of different extracts of roots of *Calotropis gigantea* Linn. against seizures induced by MES, PTZ models using albino Wistar rats of either sex.

2. Materials and methods

2.1. Plant Material

The roots and barks of *Calotropis gigantea* were collected around Belgaum city in May 2004. A voucher specimen (No.BSI/WC/Tech/2004/556) has been deposited at the Botanical Survey of India, Pune and the Dept. of Pharmacognosy and Phytochemistry, K.L.E.S's College of Pharmacy, Belgaum, India.

2.2. Preparation of Extracts

The roots and barks were dried under shade (1.0 kg), crushed to coarse powder and extracted in soxhlet assembly successively with petroleum ether (40-60°), benzene, chloroform and methanol. Finally, the marc was macerated with chloroform water. Each time before extracting with the next solvent the marc 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 tween 80 just before use.

2.3. Animals

Albino Wistar 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 ± 1°C and relative humidity of 45-55%. A 12 : 12 dark : light cycle was followed during the experiments. Animals had free access to food and water. However, food was withdrawn 8 h 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 J.N. Medical College, Belgaum (India).

2.4. Drugs

Pentylenetetrazole (PTZ) (Sigma, USA), Diazepam (Ranbaxy, India) and Phenytoin (Parke 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 to the albino Wistar rats of either sex.

2.5. Toxicity Studies

The acute toxicity was tested according to the method of OECD guidelines [12]. The five extracts were administered orally with tween 80 (suspension) in five groups (n=3) of animals. The animals were continuously observed for mortality and behavioural responses for 24 h and thereafter once daily for 14 days after administration to calculate the approximate LD_{50} values.

2.6. Assessment of anticonvulsant activity

2.6.1. Anti-convulsant activity against MES in albino Wistar rats

Animals were randomly divided in to Seven Groups of six animals each (n=6). Group One served as control, received equivalent amount of the respective vehicle, Group Two received Phenytoin (25 mg/kg i.p.) served as reference standard and Group Three, Four, Five, Six and Seven received the crude extracts of roots and barks (separately) of pet. ether, benzene, chloroform, methanol and aqueous p.o. respectively (shown in Table 1 and 2) [13]. The rats were subjected to MES at 150 mA, 60 Hz for 0.2 sec through pinnal electrodes at 60 min after vehicle/drug administration. In all, electrically induced convulsions the rats are manually restrained and released immediately.

After stimulation, the seizure observed through out its entire course. MES results in Hind Limb Tonic Extension (HLTE) and the duration was measured in sec. The severity of convulsions was assessed by duration of tonic flexion, tonic extensor, clonus and stupor phase for each animal. The duration of each phase for each animal (in sec) was measured by using stopwatch. Rats were pre tested 24 h 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).

Table 1. Effect of different extracts of Roots of *Calotropis gigantea* on MES induced seizures in albino Wistar rats.

	Time (in sec) in Various Phases of Convulsions				
Treatment (mg/kg b.w.)	**Flexon**	**Extensor**	**Clonus**	**Stupor**	**% Incidence of Convulsion**
Control (Saline1ml/rat)	9.50 ± 0.76	24.83 ± 0.54	2.33 ± 0.42	121.00 ± 1.18	100
Standard Phenytoin (25)	0.00 ± 0.00***	0.00 ± 0.00***	0.00 ± 0.00***	1.50 ± 0.80	0
Pet. Ether Extract (400)	8.83 ± 0.13	13.33 ± 0.66*	10.16 ± 0.60	116.66 ± 1.33	56
Benzene Extract (400)	15.66 ± 1.14	10.83 ± 1.01**	16.16 ± 1.19	130.55 ± 1.38	44
Chloroform Extract (400)	10.16 ± 1.08	13.33 ± 1.02*	16.66 ± 1.91	123.83 ± 1.44	56
Methanol Extract (400)	6.83 ± 0.79	8.0 ± 1.54***	16.33 ± 0.91	120.66 ± 1.05	32
Aqueous Extract (400)	10.5 ± 0.99	11.83 ± 0.94**	1.33 ± 0.88	119.16 ± 2.84	48

Note: All the rats under study recovered, there were no deaths.
All values are mean ± SD; *P<0.05, **P<0.01, ***P<0.001 vs. Control n = 6 (n = number of animals)

Table 2. Effect of different extracts of Barks of *Calotropis gigantea* on MES induced seizures in albino Wistar rats.

	Time (in Sec) in Various Phases of Convulsions				
Treatment (mg/kg b.w.)	**Flexon**	**Extensor**	**Clonus**	**Stupor**	**% Incidence of Convulsion**
Control (Saline1ml/rat)	9.50 ± 0.76	24.83 ± 0.54	2.33 ± 0.42	121.0 ± 1.18	100
Standard Phenytoin (25)	0.00 ± 0.00***	0.00 ± 0.00***	0.00 ± 0.00***	1.8 ± 0.80***	0
Pet. Ether Extract (200)	4.33 ± 0.42	12.66 ± 0.55**	6.83 ± 0.30	112.33 ± 1.26	52
Benzene Extract (200)	12.16 ± 0.87	9.5 ± 0.67**	14.16 ± 1.24	126.33 ± 1.45	40
Chloroform Extract (200)	7.83 ± 1.24	14.33 ± 0.88*	15.66 ± 1.49	118.83 ± 1.40	60
Methanol Extract (200)	3.66 ± 0.47	4.0 ± 0.58***	12.5 ± 0.78	109.66 ± 2.43	16
Aqueous Extract (200)	7.83 ± 0.60	8.83 ± 0.70**	9.33 ± 0.80	118.66 ± 3.12	36

Note: All the rats under study recovered, there were no deaths.
All values are mean ± SD; *P<0.05, **P<0.01, ***P<0.001 vs. Control n = 6 (n = number of animals)

Table 3. Effect of different extracts of Roots of *Calotropis gigantea* on PentyleneTetrazole (PTZ) induced seizures in albino Wistar rats.

Group	**Treatment (mg/kg b.w.) (Dose)**	**Onset of Action in Seconds**	**% Incidence of Convulsions**
1.	Control (PTZ) [80 mg]	496.66 ± 16.66	100
2.	Diazepam + PTZ [4 + 80]	0.00 ± 0.00***	0
3.	Pet. Ether Extract + PTZ [400 + 80]	274.33 ± 8.67**	55.2
4.	Benzene Extract + PTZ [400 + 80]	387.83 ± 7.07*	78
5.	Chloroform Extract + PTZ [400 + 80]	246.66 ± 7.71**	50
6.	Methanol Extract + PTZ [400 + 80]	235.11 ± 6.11***	47
7.	Aqueous Extract + PTZ [400 + 80]	246.5 ± 7.93***	49

All values are mean ± SD; *P<0.05, **P<0.01, ***P<0.001 vs. Control n = 6 (n = number of animals)

Table 4. Effect of different extracts of Barks of *Calotropis gigantea* on Pentylene Tetrazole (PTZ) induced seizures in albino Wistar rats.

Group	**Treatment (mg/kg b.w.)**	**Onset of Action in Seconds**	**Percentage (%) Incidence of Convulsions**
1.	Control (PTZ) [80 mg]	496.66?16.66	100
2.	Diazepam + PTZ [4 + 80]	0.00?0.00***	0
3.	Pet. Ether Extract + PTZ [200 + 80]	363.83?5.81*	73.23
4.	Benzene Extract + PTZ [200 + 80]	249.66?6.62**	50
5.	Chloroform Extract + PTZ [200 + 80]	247.83?6.11**	50
6.	Methanol Extract + PTZ [200 + 80]	203.33?7.15***	41
7.	Aqueous Extract + PTZ [200 + 80]	220.9?72.74***	45

All values are mean ± SD; *P<0.05, **P<0.01, ***P<0.001 vs. Control n = 6 (n = number of animals)

2.6.2. Anti-convulsant activity against Chemo shock (PTZ) induced seizures in rats

Seizures were induced in rats with PTZ at 80 mg/kg i.p. which is the convulsive dose in 97% of the animals (14). PTZ was dissolved in 0.9% saline and injected i.p. in rats at 0.2 ml/100 gm.

Animals were randomly divided in to Seven Groups of six animals each (n=6). Group One served as control, received equivalent amount of the respective vehicle, Group Two received Diazepam (4 mg/kg i.p.) served as reference standard and Group Three, Four, Five, Six and Seven received the crude extracts of roots and barks (separately) of pet. ether, benzene, chloroform, methanol and aqueous p.o. respectively [16]. All the crude extracts and standard drugs are administered 60 min before the administration of PTZ and the rats were observed for the clonic convulsions/onset of action. Values are expressed in terms of Mean ± SD (Time in sec).

2.7. Statistical analysis

The experimental results are represented as Mean ± SD (Standard Deviation), the one way ANOVA was used to compare the HLTE data of the various groups. The HLTE data obtained in the MES test were analysed using appropriate models of analysis of variance and $P<0.05$, $P<0.01$ and $P<0.001$ are taken to be significant, more significant and highly significant respectively.

3. Results

3.1. Toxicity studies

The extracts of pet. ether, benzene, chloroform, methanolic and aqueous were found to have a LD_{50} of 4000 mg/kg b.w. (Roots) and 2000 mg/kg b.w. (Barks). Doses of the test compounds were fixed on the bases of their LD_{50} values. Doses of the standard drugs were fixed based on the human therapeutic equivalent dose.

3.2. Assessment of Anti convulsant activity

3.2.1. Maximum electroshock test (MES)

All the extracts (p.o.) and standard drug (i.p.) were administered 60 min before application of electroshock. Values are expressed as Mean ± SD from the experiment. The duration of the Hind Limb Tonic Extension (HLTE) in rats treated with vehicle (control) was 24.83 ± 0.54 sec. The Methanolic extract at a dose of 400 mg/kg (Root) and 200 mg/kg (Bark) protected the animals from the seizures and duration of HLTE. The dose of 400 mg/kg and 200 mg/kg (Other extracts) had less protection from seizures compared to the methanolic extract of roots and barks. The rats treated with the different extracts exhibited the HLTE are illustrated in Table 1 and Table 2.

3.2.2. Pentylene Tetrazole Induced Seizures (PTZ)

In animals treated with vehicle the onset of action appeared at 496.66 ± 16.66 sec, after PTZ administration one animal died in chloroform extract of the bark and one in methanolic extract of the root after seizures. The methanolic extract of the roots and barks significantly inhibit the onset of convulsions compared to the control group. Diazepam (4 mg/kg) inhibited the seizures completely and the results are shown in Table 3 and Table 4.

4. Discussions and conclusions

The results obtained in the MES test in rats suggest that the standard drug as well as the different extracts of roots and barks of C. gigantea protected against MES induced seizures. In the PTZ test all the extracts of roots and barks of *C. gigantea* shows anti-convulsant activity. In the MES test since, inhibition of the MES test predicts the activity of drugs against generalised tonicclonic and cortical focal seizures. Hence, it is suggests that the methanolic extract of the roots and

barks of *C. gigantea* may be useful in suppressing generalised tonic clonic seizures. Laboratory studies are currently fortifying the tenets of ancient wisdom derived from herbal remedies. 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 [14]. Thus in conclusion methanolic extracts of the barks of *Calotropis gigantea* possess anti-convulsant property against the MES and PTZ induced seizures in albino Wistar rats, compared to the methanolic extracts of roots of *C. gigantea*. However, the further research is needed to isolate the compound responsible for the activity.

5. Acknowledgement

We want to thank Dr. F.V. Manvi, Principal, K.L.E.S's College of Pharmacy, Belgaum, India for providing all the facilities to carry out the work.

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