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Research Article

ANTICONVULSANT ACTIVITY OF AQUEOUS ROOT EXTRACT OF CALOTROPIS GIGANTEA IN ALBINO MICE

KALABHARATHI HL, PUSHPA VH, VAIBHAVI PS, SURESHA RN, PADMAJA SHETTY K, SATISH AM, SUSHMA NAIDU, PRAMOD SG

Department of Pharmacology, JSS Medical College, JSS University, Mysore 570015

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ABSTRACT

Objectives: To evaluate the anticonvulsant activity of aqueous root extract of Calotropis gigantea (C.gigantea) in seizure induced mice.

Methods: Albino mice (25-30g) of either sex were randomly selected and divided into 4 groups of 6 mice each, Group 1(Control) - gum acacia (vehicle) 1ml, Group 2 (Standard) - Valproic acid (40mg/kg), Group 3- aqueous root extract of C. gigantea 200mg/kg (T1) and Group 4 -valproic acid (20mg/kg) and aqueous extract of C. gigantea 100mg/kg (T2). All drugs were suspended in gum acacia and administered orally 1hr prior to induction of seizures. The anticonvulsant activity were screened using maximal electro shock (MES) model and pentylenetetrazole (PTZ) model.

Results :The aqueous root extract of Calotropis gigantea 200mg/kg showed a considerable reduction in the duration of hind limb extensor phase in MES model and also delayed the latency of seizures induced by PTZ when compared with control group.The probable mechanism of anticonvulsant action of aqueous root extract of calotropis gigantea could be due to its interference with the GABA aminergic mechanism, modulation of nicotinic and NMDA receptors which could be attributed to the phytochemical constituents present in the roots mainly theterpenoids along with cardiac glycosides, carbohydrates, proteins, amino acids and sterols.

Conclusion: Aqueous root extract of Calotropis gigantea possesses anticonvulsant activity and has a beneficial role in epilepsy.

Keywords: Anticonvulsant, Calotropis gigantea, MES, PTZ.

INTRODUCTION

Seizure refers to a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons. It is a paroxysmal event due to abnormal,excessive, hyper excitable asynchronous discharges from an aggregate of central nervous system neuron. Depending upon the distribution of discharges, this abnormal CNS activity can have various manifestations, ranging from convulsions to EEG changes.The term epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of recurrent seizures.[1]The cause of epilepsy are many ranging from idiopathic to infection, neoplasm and trauma (head injury). Epilepsy is a major public issue in many nations with its frequency increased in early childhood and adulthood.[2,3] Despite the massive scale of problem and much research, epilepsy remains poorly understood in terms of etiology and pathogenesis. Although several drugs are used in the treatment of epilepsy, the treatment for epilepsy is still far from adequate. Several attempts have been made in the past to screen anticonvulsant from plant origin and these attempts will continue till a satisfactory treatment is available.[4]

Calotropis gigantea commonly known as "Madar" belonging to family "Asclepediaceae " is traditionally being used to cure various diseases such as fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea. Calotropis gigantea also called as 'Milkweed' is a common wasteland weed distributed in tropical and subtropical region of Asia. A wide spectrum of biological activities have been reported with various constituents isolated from different parts of the plant.

The whole plant was reported for various activites such as anti-inflammatory, laxative, anti-implantation, anthelmintic, spasmolytic and the root of Calotropis gigantea have been used in leprosy, eczema, syphilis, ulceration and cough. In recent studies alcoholic extract was shown to have analgesic, anticonvulsant and anxiolytic property.[11]Thus in our study an attempt has been made to evaluate the anticonvulsant activity of aqueous root extract of Calotropis giganteain seizure induced mice in both MES and PTZ models.[5,6]

MATERIALS AND METHODS

Animals

Albino mice (25-30g) of either sex were randomly selected from central animal facility, JSS Medical college, Mysore. Animals were housed into groups of 6-8 per cage at a temperature of 25^0+ 1^0 and relative humidity of 45-55 %. Animals had free access to food and water. The Instititional Animal Ethical Committee approved the protocol of this study(IAEC:5128).

Preparation of the extract and isolation of active principle

The Calotropis gigantea along with its roots found in the vicinity of Yadavgiri, Mysore were taken to JSS Ayurvedic College for authentication of the plant and the species. After the plant and its roots were verified, the extract was prepared in JSS Pharmacy College, Mysore. Firstly the roots were kept in the hot air oven at 45^0 C for 7 days. The 50gms of dried roots were powdered and soaked in 500ml of distilled water(with chloroform for 7 days) with intermittent shaking of the beaker at regular intervals. At the end a brownish colour liquid was obtained as extract. The brownish colour extract was filtered, evaporated, shade-dried, scraped out and weighed.[7]

Drugs and chemicals

Valproic acid 40mg/kg body weight [8](Cadila Laboratories, India),pentylenetetrazole 80 mg/kg[9](Sigma, USA), aqueous root extract of calotropis gigantea 200 mg/kg body weight[10,11], gum acacia 1% and distilled water.

Methodology

Animals were divided into four groups (with 6 mice each) for both the models i.e MES induced and PTZ induced seizure models. After overnight fasting, group I received 2ml/kg gum acacia and served as the control, Group II received sodium valproate (40mg/kg orally) as standard, Groups III received aqueous root extract of calotropis gigantea (200mg/kg orally) as test and Group IV received sodium valproate (40mg/kg) and calotropis gigantea (100 mg/kg), all of which were administered orally 60 minutes prior to the test in this acute study.

Assessment of anticonvulsant activity

Maximal electro shock induced seizures (MES MODEL)

Swiss albino mice weighing 25 – 30 gms were used. The animals were pre-screened for their ability to develop full tonic extension in the maximal electro shock test and only those which showed good response were included in the test. Electrical stimulation causes seizures which passes through phases of tonic limb extension, tonic limb flexion and clonus period. Suppression of tonic hind limb extension was taken as a measure of efficacy in this test. Calotropis gigantea along with control and standard drugs were administered to respective groups of mice 30 mins before application of electro shock (50 A, 0.2 second) using ear electrodes. The duration of tonic extension of hind limbs was noted.[12]

Pentylenetetrazole induced seizures (PTZ MODEL)

Pentylenetetrazole is a central nervous system stimulant. The convulsive effect is analogous to petitmal type of convulsions in man. Seizures were induced in mice with PTZ at a dose of 80mg/kg body weight intraperitonially. The experiment was assessed by its ability to delay the onset of myoclonic spasm and clonic convulsions.The test compound and standard drug was administered to respective groups of mice groups 30 mins prior to PTZ. The animals were observed for onset of myoclonic spasm and clonic convulsions.[13,14]The onset of convulsions were observed till 30 minutes after administering PTZ.

RESULTS

MES model

The hind limb extensor phase in Calotropis gigantea treated group was 3.10+0.17 seconds and that of control group was 15.31+2.0 seconds. The aqueous root extract of Calotropis gigantea 200mg/kg showed a reduction in duration of hind limb extensor phase in MES model. It was noted that the clonus period was also reduced in Calotropis treated groups to 2.20+0.09secswhen compared to 3.72+0.10secs of control groups as shown in table-1. The combination of Calotropis gigantea and sodium valproate (T_2) reduced the duration of hind limb extensor phase when compared to both test (T_1) and control groups.

PTZ model

The delay in onset of seizures in Calotropis gigantea treated group was 92.6+4.31secs when compared to 80.03+5.95secs in control treated group. The aqueous root extract of Calotropis gigantea 200mg/kg showed a delay in the latency of seizures induced by PTZ when compared with control group as shown in Table -2 whereas the combination of Calotropis gigantea and sodium valproate (T_2) showed a delay in onset of seizures by113.6+5.94secs when compared to both the standard treated and control groups.

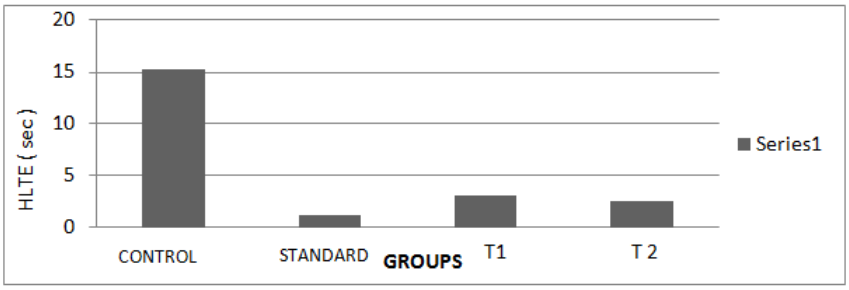
Statistical analysis

The effects of different drugs under study were calculated by taking the mean and SD of the outcome parameters. ANOVA (Analysis of Variance) was applied to compare the effects of drugs under the study. The data were analysed using ANOVA followed by Dunnett's test. Differences were considered significant at 5 % level (*p value < 0.05)

Table 1: Duration of hind limb extensor phase (sec) in MES model

Groups	Hind limb tonic flexion (sec)	Hindlimb tonic extension(sec)	Clonus
Control	2.30+0.09	15.31+2.0	3.72+0.10
Standard	1.74+0.44	1.25+0.10	0
T_1	2.74+0.12*	3.10+0.17*	2.20+0.09*
T_2	2.79+0.08*	2.62+0.10*	1.98+0.16*

*P value < 0.05 when compared with control and drug treated groups.

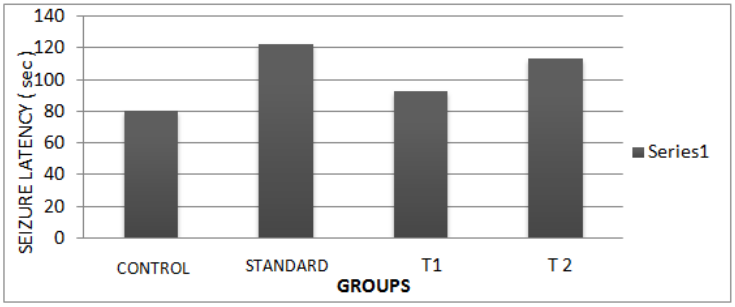


Graph 1: Duration of hind limb extensor phase (sec) in MES model

Table 2: Latency and duration of convulsions in PTZ model.

Groups	Onset of convulsions (sec)	Duration of convulsions (sec)
Control	80.03+5.95	390.93+5.14
Standard	122.65+4.66	192.91+3.72
T_1	92.6+4.31*	264.16+4.10*
T_2	113.6+5.94*	212.22+3.77*

Values are in Mean + SD and the data was analysed by one way ANOVA followed by Dunnett's test. *P value < 0.05 when compared with control and treated groups.



Graph 2: Latency and duration of convulsions in PTZ model

Table 3: % inhibition of hindlimb extensor phase and onset of convulsions

Groups	% inhibition of hind limb extensor phase	% inhibition of onset of convulsions
Control	0	0
Standard	91.8	53.25
T1	79.75	15.7
T2	82.8	41.9

DISCUSSION

Epilepsy is a very common disorder affecting 0.5-1% of world's population.Its incidence in India is around 20-50 cases per lakh population.[3] Although newer and selective agents are currently used,there is still a drawback due to their side effect profile and also few cases being refractory to conventional treatment. The current study was undertaken to evaluate the anticonvulsant activity of aqueous root extract of Calotropis gigantea (200 mg /kg) in MES and PTZ seizure induced mice.

MES predicts activity against generalised tonic clonic and cortical focal seizures while PTZ tests activity against petitmal epilepsy or absence seizures. The reduction in duration of hind limb extensor phase and delay in the latency of seizures are considered as important parameters to assess the efficacy of anticonvulsant agents. MES model is useful for screening of drugs against primary and secondary generalised tonic-clonic seizures but do not give any clue regarding the mechanism of action of the compound.Since PTZ acts as convulsant by antagonising the inhibitory GABAergic neurotransmission, any drug effective against PTZ model is said to possibly exert its anticonvulsant action through GABA receptor.[15]

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 seizure.[5]

The aqueous root extract of calotropis gigantea (ARECG)brought about a reduction in duration of hind limb extensor phase in MES modeland showed a delay in the latency of seizures induced by PTZ when compared with control group.[16,17]In this study T2 was more effective than T1 in both MES and PTZ induced seizure models when compared to control group.

The properties of Calotropis gigantea can be attributed to its phytochemical constituents like akundarin, cardiac glycosides, calotropin, uscharin, calotoxin, calactin, uscharidin,gigantin and ά – and β- calotropeols[18]. Recently four new chemical constituents including one naphthalene derivatives, named Calotropnaphthalene, two terpene derivatives, named Calotropisesquiterpenol and Calotropis-esterterpenol and an aromatic product designated as Calotrobenzofuranone along with a known compound sucrose, were isolated from the roots of the Calotropis gigantea.[19] The phytochemical studies on the roots of calotropis gigantea have resulted in the isolation of four new ursan type of triterpenoids whose structure has been established on the basis of chemical and spectroscopic techniques. [20,21]

According to earliar studies all extracts of roots of Calotropis gigantea contain phytochemical constituents like cardiac glycosides, carbohydrates, proteins, amino acids, terpinoids and sterols. The major component among these was found to be cardiac glycosides which was present maximally in methanolic extract of calotropis gigantea which showed potent anticonvulsant activity when compared to other extracts[22]. However the anticonvulsant activity of aqueous root extract of calotropis gigantea can be possibly be attributed to other phytoconstituents along with cardiac glycosides.Terpenoids are oxygenated products formally derived from C5 isoprene units and are classified by the number of C5 units in their structure. Monoterpenoids have 2 x C5 units, sesquiterpenoids 3 x C5 units, diterpenoids 4 xC5 units, and triterpenoids 6 x C5 units.Triterpenoids have been reported to possess anticonvulsant activity in some experimental seizure models like PTZ and MES.[23]Some known triterpinoids are known to negatively modulate nicotinic and NMDA receptor activity.Most sesquiterpenoids act as direct partial agonist on $GABA_A$ receptors and partial agonists of the $5\text{-}HT_{5a}$ receptor in vitro.[24]

Therefore the probable anticonvulsant action of aqueous root extract of calotropis gigantea could be due to its interference with the GABA aminergic mechanism, modulation of nicotic and NMDA receptors. And, this effect of the extract may be related mainly to the terpenoids along with cardiac glycosides, carbohydrates, proteins, amino acids and sterols.

Thus Calotropis gigantea was observed to have anti convulsant activity against MES and PTZ induced models of seizure.The study explores the complementary nature of Calotropis gigantea with conventional treatment making it comparatively safer, economical, easily available and well tolerated therapy.

CONCLUSION

We conclude from the study that aqueous root extract of Calotropis gigantea has a beneficial role as an anticonvulsant. Further studies are indicated to identify the adverse effects, optimal treatment routes and dosage.

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