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CNS activity of leaves extract of *Calotropis gigantea*

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ABSTRACT

Objective: To study central nervous system activity of ethanolic extract of leaves of *Calotropis gigantea* (*C. gigantea*) (Asclepiadaceae), such as anticonvulsant, sedative and muscle relaxation activity.

Methods: The ethanolic extract of *C. gigantea* administered orally in experimental animals at different doses 100, 200 and 500 mg/kg body weight. The anticonvulsant properties were studied on maximal electroshock test and strychnine-induced convulsions model. Sedative property studied using actophotometer and skeletal muscle relaxant property studied using rota rod.

Results: This extract protected rats against maximal electroshock induced seizures, but had no or a moderate effect only against strychnine-induced seizures. Locomotor activity in mice found to be decreased and motor coordination was also decreased. The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg.

Conclusions: With these effects, the leaves of *C. gigantea* possess anticonvulsant sedative and muscle relaxant effect that might explain its use as a traditional medicine.

1. Introduction

Since time immemorial, nature has played a cardinal role in discovery of modern drugs, including centrally acting medicines. Several researchers have demonstrated benefits of herbal remedies in refractory patients of anxiety, depression and epilepsy[1-8].

Calotropis gigantea (*C. gigantea*) or sweet akand belonging to family Asclepiadaceae, is native to India and grows well in lower hills at 900 m altitude[9,10].

Different parts of *C. gigantea* are reported to be used for treatment of toothache and earache, sprain, anxiety, pain, epilepsy and in mental disorders, diarrhoea, analgesic activity and pregnancy interceptive properties[11-14]. The stem bark of *C. gigantea* yields resin and wax.

The active constituent isolated from plant include b-amyrin and its isovalerate, a and b-calotropeols, mixture of tetracyclic triterpene, traces of sterols, C31 and C33 hydrocarbons, fatty acids, giganteol, cardiac glycosides, calotropin, uscharin, calotoxin, uscharidin and gigantin[15].

The leaves of *C. gigantea* are reported to contain taraxasteryl acetate, pinoresinol, medioresinol, uzarigenin, calotropin, calactin, calacitnic acid, calacitnic acid methyl ester, 19-carboxyl-calacitnic methyl ester, drummondol, 15b-hydroxycalotrin, the C11 bicylic lactone norisopenoid, the rare diphenyl furfuran lignan, salicifoliol and 19-nor- and 18,20-epoxy-cardenolides[16].

Thus in review of potential use of plant in folklore for the treatment of central nervous system (CNS) diseases and isolation of centrally active substances, it was that important to systematically evaluate the CNS activity of the ethanolic extract of *C. gigantea*.

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2. Materials and methods

2.1. Plant material

The plant was obtained locally from sub-urban hills of Indore. It was authenticated by the department of Botanical Survey of India, Pune (India) and was give a specimen no. (SAGCAG3) .

Leaves were carefully and mechanically separated washed with water. After drying in shade, they were powered and stored. The leaf powder was extracted using soxhelation successively with ether, chloroform and ethanol for 24 h. The extract obtained was stored in airtight container in desiccator. Preliminary phyto-chemical analysis was carried out on the ethanolic extract of *C. gigantea* to assess the presence of alkaloids, glycosides, saponins, flavanoids and steroids.

2.2. Drugs and chemicals

Strychnine was purchased from (STR, Sisco Research Lab. Mumbai), phenytoin and diazepam were purchased from local market.

2.3. Animals

Wistar rats weight 150-200 g and albino mice weight 18-22 g were used. The animals were obtained from animal house, College of Pharmacy, IPS Academy, Indore. Animals were randomized and allocated to different treatment groups (five per group). Animals were kept at a temperature of (24±2) °C and relative humidity of 30%-70%. A day with 12:12 light:dark cycle with free access to rodent chow and tap water. An Institutional Animal Ethics Committee approved all procedure and guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals were followed (Protocol No. CPCSEA/77/2011).

2.4. Maximal electroshock seizure (MES) model

Electroconvulsions were produced by an alternating current (0.2 seconds stimulus duration, 50 Hz) delivered via standard auricular electrodes (INCO, Ambala, India). Rats were divided them into five different groups each consisting of five animals. Group I: control; Group II: phenytoin (10 mg/kg); Groups III-V will be used as test received ethanolic extract of *C. gigantea* 100, 200, 500 mg/kg *p.o.* respectively. Hold the animal properly, placed corneal electrodes on the cornea and applied the prescribed current. The convulsion were recorded and duration of each phase (tonic flexion, tonic extensor, clonic convulsions, stupor and recovery or death) was determined. Phenytoin was injected intraperitoneally to a group of four to five rats. After 30 min, the animals were subjected to electroconvulsions as described earlier[17,18].

2.5. Strychnine-induced convulsions

Swiss albino mice (18-25 g) were divided into different groups each containing five animals and treated with distilled water (10 mL/kg) as control group, ethanolic extract of *C. gigantea* (100, 200, and 500 mg/kg, *p.o.*) as test group or glycine (750 mg/kg, *i.p.*) as standard group. After drug/vehicle administration for 30 min, seizures were induced by intraperitoneal administration of 2 mg/kg strychnine nitrate. The time until occurrence of tonic extensor, convulsions and death was noted during 1 h period[19].

2.6. Assessment of locomotor activity

Locomotor activity was assed by using actophotometer. Groups of mice were treated with *C. gigantea* extract (100, 200, 500 mg/kg, *p.o.*) or diazepam (4 mg/kg, *i.p.*) and were subjected to locomotor test session of 5 min in an actophotometer. The count was used as index of CNS depressant[17,20].

2.7. Muscle relaxant activity

Motor co-ordination activity was investigated using rota rod apparatus. Diazepam (2 mg/kg *i.p.*) served as a standard. The test compound (100, 200 and 500 mg/kg, *p.o.*) was administered. After administration for 30 min, the mice were placed on the rotating rod (25 r/min). The time duration spent by each animal on rod was reported[17,20].

2.8. Statistical significance

The data were analysed by using One-way ANOVA and Tukey's test was used for *post-hoc* analysis. $P<0.05$ was considered as statistically significant.

3. Results

3.1. Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins in the *C.*

gigantea extract.

3.2. Acute toxicity study

The acute toxicity study revealed safely of the extract up to a dose of 2 000 mg/kg dose level in mice.

3.3. Anticonvulsant assessment

A single maximal electroshock seizure produced an immediate tonic hind-limb extension of 5-10 seconds duration followed by clonic seizures, lasting up to 20 seconds. A single dose administration of *C. gigantea* (100, 200 and 500 mg/kg, *p.o.*) significantly reduced hindlimb extension, as compared to vehicle control ($^{a}P<0.05$, $^{b}P<0.001$, $^{c}P<0.000\,1$). This effect was comparable to that of phenytoin (10 mg/kg), a standard antiepileptic drug (Table 1).

Table 1
Effect of the ethanolic extract of *C. gigantea* on MES-induced tonic seizures in rats.

Treatment	Dose	Flexion	Extensor	Clonus	Stupor	Recovery time
Control	10 mL/kg	8.40±0.89	17.40±1.95	30.20±4.55	44.00±7.52	294.00±47.81
Phenytoin	10 mg/kg	2.40±0.55^{c}	3.00±1.87^{c}	8.20±6.83^{c}	11.80±5.89	102.8±11.56^{c}
Ethanolic extract	100 mg/kg	12.40±1.82^{b}	9.00±1.00^{c}	28.00±5.70	90.00±11.73	153.2±6.08^{c}
	200 mg/kg	6.80±1.92	7.40±1.14^{c}	19.00±2.24^{a}	126.4±9.45	230.4±9.66^{b}
	500 mg/kg	4.40±1.82^{b}	5.00±3.61^{c}	5.80±7.76^{c}	91.00±25.59	172.0±25.29^{c}

Data are represented as mean±SD, n=5. One-way of analysis of variance, ANOVA followed by Tukey's multiple comparison test, values are compared with control animals, $^{a}P<0.05$, $^{b}P<0.001$, $^{c}P<0.000\,1$.

C. gigantea leave extract showed dose dependent delay of onset of convulsion caused by strychnine and did not show any protection against strychnine-induced convulsion (Table 2).

Table 2
Effect of the ethanolic extract of *C. gigantea* on strychnine-induced tonic seizures in rats.

Treatment	Dose	Mean onset time of convulsion	Duration of convulsion	Percentage recovery
Control	10 mL/kg	55.80±20.32	446.80±81.25	0.00±0.00
Glycine	750 mg/kg	NS	NS	100.00±0.00^{b}
Ethanolic extract	100 mg/kg	104.00±13.40^{a}	48.20±14.62^{c}	20.00±14.72
	200 mg/kg	121.40±13.41^{b}	41.40±10.60^{c}	40.00±54.77
	500 mg/kg	135.00±39.69^{b}	32.33±18.01^{c}	60.00±54.07

Data are represented as mean±SD, n=5. One-way of analysis of variance, ANOVA followed by Tukey's multiple comparison test, values are compared with control animals, $^{a}P<0.05$, $^{b}P<0.001$, $^{c}P<0.000\,1$, NS=not showed.

3.4. Locomotor activity

One-way ANOVA revealed a significant effect of all treatment groups on the locomotor activity. The *post-hoc* analysis by Tukey's test revealed significant effect of all doses of *C. gigantea* ($P<0.001$) on locomotor activity in mice (Figure 1).

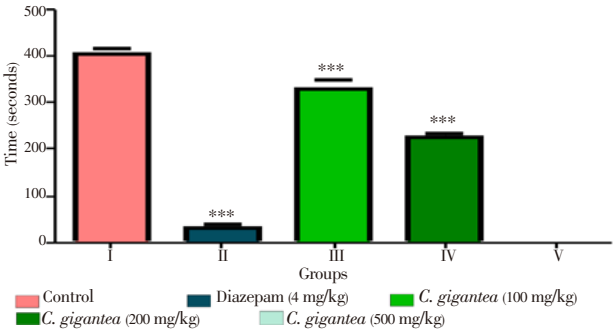

Figure 1. Effect of the leaves extract of *C. gigantea* on the locomotor activity.

3.5. Skeletal muscle relaxant activity

One-way ANOVA revealed a significant effect of 200 and 500 mg/kg treatment groups on the muscle relaxant activity. The *post-hoc* analysis by Tukey's test revealed significant effect of 200 and 500 mg/kg doses of *C. gigantea* ($P<0.001$) on muscle relaxing activity in mice (Figure 2).

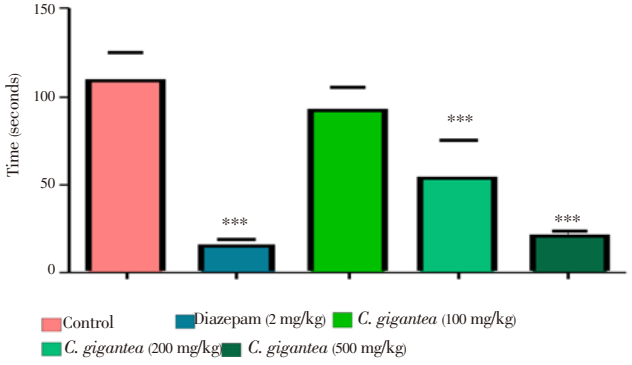

Figure 2. Effect of the leaves extract of *C. gigantea* on the skeletal muscle relaxant activity.

4. Discussion

Maximal electroshock inducing seizure is the most commonly used animal model in epilepsy, due to its simplicity and authenticity[21,22]. The MES identifies agents with activity against generalized tonic clonic seizures using clinically established antiepileptic drugs[21].

In addition to identifying drug activity against generalized tonic-clonic seizures, it has often been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures. Hence, this model was chosen for confirming the effects of *C. gigantea* on seizures. Results presented herein indicate that ethanolic extract enhanced the protective action of against the maximal electroshock in rats.

Strychnine has been demonstrated to have a well defined mechanism of convulsant action reported to be directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes[22]. *C. gigantea* extract fails to inhibit strychnine

induced seizures. This shows its lack of effect on the glycine receptors in the spinal cord. Further studies are needed for identifying the constituent responsible for the anticonvulsant effect.

Reduction in the motor co-ordination and muscle relaxation show dose dependent on the CNS depressant effect of the extract of *C. gigantea*. Decrease in locomotion reveals depression effect on CNS[23]. The CNS depressant activity may be due to the increase in the concentration of GABA in brain[24].

The above studies revealed that the ethanolic extract of the leaves of *C. gigantea* possesses anticonvulsant, depressant and skeletal muscle relaxant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Bhutada P, Mundhada Y, Bansod K, Dixit P, Umathe S, Mundhada D. Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice. *Epilepsy Behav* 2010; **3**: 207-210.

[2] Bum EN, Taiwe GS, Nkainsa LA, Moto FC, Seke Etet PF, Hiana IR, et al. Validation of anticonvulsant and sedative activity of six medicinal plants. *Epilepsy Behav* 2009; **14**: 454-458.

[3] Carlini EA. Plants and the central nervous system. *Pharmacol Biochem Behav* 2003; **3**: 501-512.

[4] Chen Y, Wang HD, Xia X, Kung HF, Pan Y, Kong LD. Behavioral and biochemical studies of total furocoumarins from seeds of *Psoralea corylifolia* in the chronic mild stress model of depression in mice. *Phytomedicine* 2007; **14**: 523-529.

[5] Li YC, Wang FM, Pan Y, Qiang LQ, Cheng G, Zhang WY, et al. Antidepressant-like effects of curcumin on serotonergic receptor-coupled AC-cAMP pathway in chronic unpredictable mild stress of rats. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 435-449.

[6] Liu P, Hu Y, Guo DH, Wang DX, Tu HH, Ma L, et al. Potential antidepressant properties of Radix Polygalae (Yuan Zhi). *Phytomedicine* 2010; **17**(10): 794-799.

[7] Pan Y, Wang FM, Qiang LQ, Zhang DM, Kong LD. Icariin attenuates chronic mild stress-induced dysregulation of the LHPA stress circuit in rats. *Psychoneuroendocrinology* 2010; **35**: 272-283.

[8] Yi LT, Xu Q, Li YC, Yang L, Kong LD. Antidepressant-like synergism of extracts from magnolia bark and ginger rhizome alone and in combination in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 616-624.

[9] Anonymous. *The wealth of India*. New Delhi, India: National Institute of Scientific and Industrial Research; 1998.

[10] Kirtikar K, Basu BD. *Indian medicinal plants*. 7th ed. DehraDun: International Book Distributors; 2001.

[11] Pathak K, Argal A. CNS activity of *Calotropis gigantea* roots. *J Ethnopharmacol* 2006; **106**: 142-145.

[12] Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrhoeal activity of *Calotropis gigantea*n r.br. in experimental animals. *J Pharm Pharmaceut Sci* 2004; **7**: 70-75.

[13] Pathak AK, Argal A. Analgesic activity of *Calotropis gigantea* flower. *Fitoterapia* 2007; **78**: 40-42.

[14] Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of *Calotropis gigantea* Linn. in rats. *Contraception* 2007; **75**: 318-322.

[15] Council of Scientific and Industrial Research. *The wealth of India: a dictionary of indian raw materials and industrial products*. New Delhi: Council of Scientific and Industrial Research Publication; 1992.

[16] Lhinhatrakool T, Sutthivaiyakit S. 19-nor- and 18, 20-epoxycardenolides from the leaves of *Calotropis gigantea*. *J Nat Prod* 2006; **69**: 1249-1251.

[17] Kulkarni SK. *Handbook of experimental pharmacology*. 3rd ed. Reprint. Chandigarh: Vallabh Prakashan; 2010.

[18] Choudhary N, Reddy KV, Kalia AN. Antiepileptic potential of flavonoids fraction from the leaves of *Anisomeles malabarica*. *J Ethnopharmacol* 2011; **135**: 238-242.

[19] Vogel HG. *Drug discovery and evaluation*. 2nd ed. Berlin Heidelberg: Springer-Verlag; 2002.

[20] Turner RA. *Screening methods in pharmacology*. 1st ed. New York: Academic Press; 2009.

[21] White HS. Clinical significance of animal seizure models and mechanism of action, studies of potential antiepileptic drugs. *Epilepsia* 1997; **38**(Suppl 1): S9-S17.

[22] Loscher W. Animal models of epilepsy for the development of antileptogenic and disease modifying drugs: a comparison of pharmacology of kindling and poststatus epilepticus models of temporal lobe epilepsy. *Epilepsy Res* 1999; **50**: 105-123.

[23] Leewanich P, Tohda M, Matsumoto K, Subhadhirsakul S, Takayama H, Aimi N, et al. Behavioural studies on alkaloids extracted from leaves of *Hunteria zeylanica*. *Biol Pharm Bull* 1996; **19**: 394-399.

[24] Nagarjun NS, Soundari PG, Kumaresan PT. CNS depressant activity of *Dalbergia malaberica*. *Indian Drugs* 2003; **40**: 716-717.