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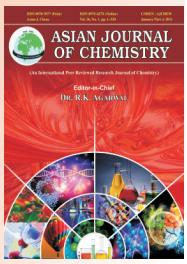

Pharmacological Activities of Leaves Extract of *Calotropis procera* (Asclepiadaceae)

SANTOSH DATTATRAYA GHULE[1,*], GALI VIDYASAGAR[2], ANIL BHANDARI[1], PRAVEEN SHARMA[3], SACHIN JAIN[3] and ABHAY JAIN[4]

[1]Jodhpur National University, Jodhpur-342 006, India
[2]Veerayatan Institute of Pharmacy, Mandvi-370 460, India
[3]College of Pharmacy, IPS Academy, Indore-452 012, India
[4]Millenium College of Pharmacy, Bhopal-462 036, India

*Corresponding author: E-mail: ghulesantosh1284@gmail.com

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Ethanolic extract of leaves of *Calotropis procera* (Asclepiadaceae) was tested for CNS activity like anticonvulsant, sedative and skeletal muscle relaxant activity. The extract of leaves of *Calotropis procera* orally in experimental animals at the dose level of 100, 200 and 500 mg/kg body weight. The anticonvulsant properties were studied on maximal electroshock test (MES) strychnine and PTZ-induced seizures model. Sedative property studied using actophotometer and skeletal muscle relaxant property studied using rotarod. *C. procera* ethanolic extract protected rats against maximal electroshock (MES) induced seizures. But had no or a moderate effect only against strychnine and PTZ-induced seizures. There was a decrease in the locomotor activity. The fall off time (motor coordination) was also decreased. No mortality was seen upto the dose of 2 g/kg. With these effects, the leaves of *C. procera* possess anticonvulsant, sedative and muscle relaxant effect that might explain its use as a traditional medicine.

Keywords: *Calotropis procera*, Maximal electroshock, Strychnine, Pentylentetrazole, Actophotometer, Muscle relaxant.

INTRODUCTION

Calotropis procera, a wild growing plant of family Asclepiadaecae is known to possess multifarious medicinal properties. Different parts of the plant have been used in Indian traditional system of medicine for the treatment of leprosy, ulcers, tumors, piles and diseases of spleen, liver and abdomen[1]. The root of the plant is used as a carminative in the treatment of dyspepsia[2]. Further, the root bark and leaves of *Calotropis procera* are used by various tribes of central India as a curative agent for jaundice[3]. The chloroform extract of the root has been shown to exhibit protective activity against carbon tetrachloride induced liver damage[4]. The milky white latex of this plant has been reported to exhibit potent antiinflammatory, analgesic and weak antipyretic activity in various experimental model's[5-7]. The latex also inhibits inflammatory response elicited by various inflammatory mediators[8]. Besides, it has also been demonstrated to possess antioxidant and anti-hyperglycemic property[9]. Recently, the aqueous extract of the latex has been shown to inhibit cellular infiltration and afford protection against development of neoplastic changes in the transgenic mouse model of hepatocellular carcinoma[10]. Ethanolic extract of *C. procera* has been shown antipyretic, analgesic, antiinflammatory and neuromuscular blocking activity[11]. The aim of the present study is to evaluate the CNS activities of the ethanolic extract of *Calotropis procera*.

EXPERIMENTAL

Strychnine was purchased from (STR, Sisco Research Lab. Mumbai), phenytoin (Dilantin, Pfizer, India), diazepam (Calmpose, Ranbaxy, India).

The leaves of *Calotropis procera* (Asclepiadaceae) were collected locally from Indore district of Madyapradesh in India and were identified at the Botanical Survey of India, Pune (India). Voucher specimens were kept in the herbarium (SAGCAP2) of the Institute for further references.

Leaves were washed with tap water, chopped into pieces and dried in shade. Dried leaves pieces were ground to coarse powder and stored in an airtight container. Leaves were Soxhlet-extracted successively with petroleum ether, chloroform and ethanol at 60-80 °C, for 24 h. Petroleum ether, chloroform and ethanol extract were stored in desiccators.

The extract thus obtained was preserved in a desiccator to prevent degradation by moisture. For pharmacological studies, the *C. procera* extract was carried by dissolved in distilled water. Preliminary phyto-chemical screening was carried out on the *C. procera* ethanolic extract to assess the presence of alkaloids, glycosides, saponins, flavanoids and steroids.

Animals: Albino mice weighing between 18-22 g and rat (150-200 g) were used. The animals were obtained from animal house, College of Pharmacy, IPS Academy, Indore. They were

placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2 °C and relative humidity of 30-70 %. A 12:12 light:day cycle was followed.

All animals were allowed to free access to water and fed with standard commercial pelleted rat/mice chaw (Trimurti feeds, Nagpur). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC (Protocol no. CPCSEA/77/2011).

Maximal electroshock seizure (MES) model: The generalized tonic-clonic seizures in rats were induced by applying current (150 mA, 0.2 s) through corneal electrodes using electroconvulsiometer (INCO, Ambala, India). Rats were divided them into five different groups each consisting of 5 rats. Group I is used as control; Group II received phenytoin (10 mg/kg); Group III, IV,V received ethanolic extract of *Calotropis procera* 100 mg, 200 mg, 500 mg/kg/p.o., respectively. Hold the animal properly, place corneal electrodes on the cornea and apply the prescribed current. Note different stages of conclusions, *i.e.* (a) tonic flexion, (b) tonic extensor phase, (c) clonic convulsions, (d) stupor, and (e) recovery of death. Note the time (SEC) spent by the animal in each phase of the convulsion. Inject phenytoin intraperitoneally to a group of 4-5 rats. Wait for 0.5 h and subject the animals to electroconvulsions as described earlier[12,13].

Strychnine-induced convulsions: Groups of 5 mice of either sex with a weight between18 and 22 g were used. They were treated orally with the test compound or the standard (*e.g.* glycine 750 mg/kg). After 1 h, the mice are injected with 2 mg/kg strychnine nitrate i.p. The time until occurrence of tonic extensor convulsions and death was noted during a 1 h period. With this dose of strychnine convulsions were observed in 80 % of the controls[14].

Pentylentetrazole induced convulsions: Mice of either sex with a body weight between 18 and 22 g were used. The test compound or the reference drug was injected i.p. to groups of 5 mice. Another group of 5 mice serveed as control. 0.5 h after i.p. injection; 80 mg/kg MTZ (metrazol) were injected intraperitonially. Each animal was placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions were recorded. At least 80 % of the animals in the control group have to show convulsions[14].

Assessment of locomotor activity: It was assessed by recording the scores for 5 min using actophotometer, after 0.5 h of dosing. Diazepam 4 mg/kg (i.p.) was used as the standard[12,15].

Assessment of skeletal muscle relaxant activity: Diazepam (2 mg/kg i.p.) served as a standard. The rats were placed individually on the rotarod (25 rpm). The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and the treated rats was recorded[12].

Statistical sgnificance: The data were expressed as mean ± SD, statistical significance was analyse using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons. $P < 0.05$ was considered as statistically significance.

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening of the extract showed that the crude extract contained alkaloids, flavonoids, saponins, glycosides, triterpenoids, tannins.

Acute toxicity study: There was no mortality amongst the graded dose groups of mice up to a dose of 2000 mg/kg for duration of 72 h. This finding probably suggests that the ethanol extract is relatively safe or non-toxic in mice at the doses used for this study.

Anticonvulsant assessment: Ethanolic extract of *C. procera* antagonized: MES-induced seizures. All three doses 100, 200 and 500 mg/kg p.o. of ethanolic extract show protective effect on rats Table-1. This effect was comparable to that of phenytoin (10 mg/kg), a standard antiepileptic drug. Moreover, treatment with leaves extract is also dose related delay of onset of convulsion caused by strychnine and even it was not be found to offer any protection against strychnine-induced convulsion.

C. procera extract administration at the tested doses did not significantly influence the latency period of duration of convulsions and mortality (Table-2). Whereas, diazepam (10 mg/kg, i.p.) treated animals failed to show any signs of convulsions and protected 80 % the mice from PTZ-induced convulsions (Table-3).

Pentylenetetrazole induced seizures in all the mice used. Pentylenetetrazole may elicit seizures by inhibiting gabaergic mechanisms[15]. Standard antiepileptic drugs, diazepam and phenobarbitone, are believed to produce their effects by enhancing γ-amino butyric acid mediated inhibition in the brain[16].

Skeletal muscle relaxant activity: The skeletal muscle relaxant activity of the drug using rota-rod showed more relaxation in extract with doses 200 and 500 mg/kg ($P < 0.05$) as compared to control but less than diazepam (Fig. 1).

Locomotor activity: In general behaviour studies, a reduction in the locomotor activity and grip strength was recorded in extract treated groups rats, which show the CNS depressant effect of the drug. This reduced locomotor activity was further assessed by actophotometer and the decrease in grip strength by rotarod, which was found to be dose dependent. Decrease in locomotion reveals depression effect on

TABLE-1
EFFECT OF THE ETHANOLIC EXTRACT OF *Calptropis procera* 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	10.40 ± 1.14	13.6 ± 1.14[b]	29.20 ± 5.12	68.80 ± 6.83	120.8 ± 9.23 [c]
Ethanolic extract	200 mg/kg	9.20 ± 1.48	9.80 ± 1.30[c]	22.40 ± 2.07	107.4 ± 10.31	153.0 ± 11.55 [c]
Ethanolic extract	500 mg/kg	6.0 ± 1.00[a]	6.80 ± 1.48[c]	14.40 ± 3.36 [c]	130.2 ± 10.03	202.6 ± 11.59[c]

Data represents mean ± SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n = 5), values are compared with control animals, $P < 0.05$. [a]$P < 0.01$, [b]$P < 0.001$, [c]$P < 0.0001$

TABLE-2
EFFECT OF THE ETHANOLIC EXTRACT OF *Calptropis procera* on STRYCHNINE-INDUCED TONIC SEIZURES IN RATS

Treatment	Dose (mg/kg)	Mean onset time of convulsion	Duration of convulsion	Percentage recovery
Control	10	55.80 ± 20.32	446.8 ± 81.25	0.0 ± 0.0
Glycine	750	NS	NS	100.0 ± 0.0[a]
Ethanolic extract	100	73.0 ± 12.35	101.8 ± 12.87[b]	00.0 ± 00.0
Ethanolic extract	200	108.4 ± 11.06[b]	71.40± 12.58[b]	20.0 ± 44.72
Ethanolic extract	500	143.8 ± 19.18[b]	41.20 ± 12.15[b]	40.0 ± 54.77

Data represents mean ± SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n = 5), values are compared with control animals, $p < 0.05$. [a]$P < 0.001$, [b]$P < 0.0001$, NS = not showed

TABLE-3
EFFECT OF THE ETHANOLIC EXTRACT OF *Calptropis procera* on PTZ-INDUCED TONIC SEIZURES IN RATS

Treatment	Dose (mg/kg)	Duration of convulsion	Percentage recovery
Control	10	232.4 ± 29.18	0.0 ± 0.0
Diazepam	10	44.60±99.73	80.0 ± 44.72
Ethanolic extract	100	192.8 ±108.0	20.0 ± 44.72
Ethanolic extract	200	133.6± 122.8	40.0 ± 44.77
Ethanolic extract	500	87.40 ± 119.8	60.0 ± 54.77

Data represents mean ± SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n = 5), values are compared with control animals, $p < 0.05$

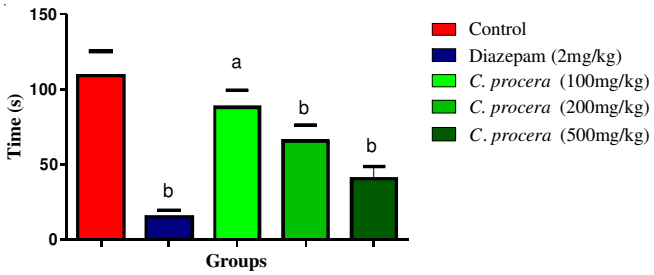

Fig. 1. Effect of the leaves extract of *Calotropis procera* on the skeletal muscle relaxant activity, Each bar represents mean ± SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n = 5), values are compared with control animals, $p < 0.05$, aP < 0.01 bP < 0.0001

CNS[17]. Fig. 2 shows the locomotor of the extract and standard drug diazepam as compared to the control. A significant decrease ($P < 0.05$) in the locomotor activity was observed at all the doses.

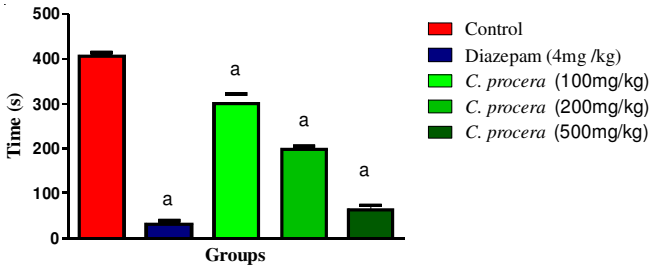

Fig. 2. Effect of the leaves extract of *Calotropis procera* on the locomotor activity, Each bar represents mean ± SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n = 5), values are compared with control animals, $p < 0.05$, aP < 0.0001

Based on the present state of knowledge of the chemical constituents of the extract, it is not possible to attribute with certainty its CNS effect to one or several active principles among those detected in the screening. However, The CNS depressant activity may be due to the increase in the concentration of γ-amino butyric acid in brain[18].

The above studies indicate that the alcoholic extract of the leaves of *Calotropis procera* possesses sedative, anticonvulsant and muscle relaxant activity. Further studies are in progress to isolate the active constituents responsible for these activities.

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