

## A study of effect of acute and chronic administration of aqueous extract of *Calotropis procera* leaves on maximal electroshock induced seizures in rats

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### ABSTRACT

**Background:** The effect of acute and chronic administration of aqueous extract of leaves of *Calotropis procera* on maximal electroshock (MES) induced seizures in rats was evaluated.

**Methods:** Four groups of wistar rats were treated with distilled water 10 mL/kg (oral), phenytoin 25 mg/kg (intraperitoneal), 250mg/kg (oral) and 500mg/kg (oral) of aqueous extract of leaves of *C procera*, respectively in both acute and chronic studies. For producing maximal electroshock seizures, an electroconvulsimeter was used to deliver an electric stimulus of 150mA for 0.2 sec. The duration of hind limb extension (HLE) and seizures were noted in seconds (sec). In the acute study, the shock was given 1 h after administration of drugs. For the chronic study, all drugs were administered for 6 weeks. One hour after the last dose of drugs, the shock was administered. Data was analyzed by one way ANOVA followed by Tukey's post hoc test.

**Results:** There was a significant decrease in the duration of hind limb extension, and convulsions as compared to control following acute ( $p < 0.01$  vs control) and chronic ( $p < 0.05$  vs control) administration of aqueous extract of leaves of *Calotropis procera* in rats.

**Conclusions:** The study demonstrated that aqueous extract of *C procera* leaves resulted in significant protection against maximal electroshock induced seizures in rats.

**Keywords:** Tonic hind limb extension, Phenytoin

### INTRODUCTION

Epilepsy is characterized by seizures which is due to excessive, abnormal and asynchronous neuronal activity in the brain.<sup>1</sup> Though many antiepileptic drugs are

available, yet about 20-30% of patients are refractory to treatment.<sup>2</sup> Hence, it is important to evaluate newer agents for treatment of epilepsy. Herbal remedies have been used for treatment of various diseases including epilepsy. *Calotropis procera* is known for its use in folklore medicine for the treatment of fever, jaundice, asthma, leprosy, diarrhea and painful joints.<sup>3,4</sup>

*Calotropis procera* has been used traditionally for treatment of epilepsy. The root and bark have been used for this purpose.<sup>5,6</sup> Studies have demonstrated the anticonvulsant effect of roots of *Calotropis procera* in pentylenetetrazole and maximal electroshock induced seizures in rats.<sup>2</sup> The leaves have shown to produce beneficial effect in pentylenetetrazole induced seizures in rats.<sup>7</sup> This study was undertaken to study the acute and chronic effect of aqueous extract of leaves of *Calotropis procera* on maximal electroshock (MES) induced seizures in rats. Maximal electroshock (MES) induced seizures model is used for screening of drugs for anticonvulsant activity in generalised tonic clonic seizures.<sup>8,9</sup>

## METHODS

### Preparation of extract

The leaves of *Calotropis procera* were authenticated by a botanist following whom they were shade dried and coarsely ground. The powder was soaked in distilled water for seven days. The liquid was filtered and the filtrate was concentrated on water bath. The paste obtained was dried in a desiccator.

### Animals

Adult Sprague dawley male rats weighing 150-200g were used. They were housed individually in polypropylene cages and maintained at a temperature of 27±3°C, relative humidity of 60±10% and 12 h light/ dark cycle, standard diet and water *ad libitum*.

The maximal electroshock induced seizure model was used to study anticonvulsant activity of leaves (aqueous extract) of *Calotropis procera* following its acute and chronic administration in rats. Four groups of rats, with six rats in each group, were used for each acute and chronic study (total= 48 rats). The doses of test and standard drugs were selected based on previous studies.<sup>1,7</sup> The groups were as follows:

- Group I (Control): Distilled water, 10mL/kg oral
- Group II (Standard): Phenytoin, 25mg/kg, intraperitoneal (i.p).
- Group III (Test): Aqueous extract of *C procera* leaves, 250 mg/kg oral
- Group IV (Test): Aqueous extract of *C procera* leaves, 500mg/kg oral

### Maximal electroshock (MES) induced seizure model

Twenty four hours before the study, rats were screened to check whether they could develop full tonic extension when shock was administered. Those rats which developed full tonic extension were selected for the study. On the day of experiment, seizures were induced by an electro-convulsimeter (Technoelectronics Ltd., Lucknow, India) using a current of 150 mA, 50Hz delivered through the ear clip electrodes for 0.2 sec.<sup>8</sup> Duration of hind limb tonic extension (HLTE) and seizures was recorded. Abolition or decrease in the duration of tonic extension was considered to be indicative for antiepileptic activity.<sup>8</sup> If extension of the hind limb was not more than 90 degree angle with the plane of the body, then the tonic component was considered to be abolished.<sup>10-12</sup>

### Acute study

Four groups of rats were treated with drugs (as described above) followed by administration of electric stimulus 1 h later.<sup>13</sup>

### Chronic study

All drugs were administered to four groups of rats for 6 weeks. One hour after the last dose of drug, an electrical stimulus was administered.<sup>13</sup> After observing the parameters, rats were sacrificed, brain was dissected, homogenized and GABA was estimated.<sup>14</sup>

### Statistical analysis

Data was expressed as mean±SEM and analyzed using one way ANOVA followed by post hoc Tukey test. The level of significance was set at P <0.05.

## RESULTS

### Acute study

Following acute administration of aqueous extract of leaves of *Calotropis procera*, there was a significant decrease (p<0.01 vs control) in duration of hind limb extension and seizures as compared to control group (Table 1). There was no significant difference in effect between phenytoin and plant extract treated groups on duration of HLE and seizures.

### Chronic study

Chronic administration of high dose (500mg/kg) of aqueous extract of leaves of *Calotropis procera*, resulted in a significant decrease (p<0.05 vs control) in duration of hind limb extension and seizures as compared to control group (Table 2). There was no significant difference between effect of phenytoin and plant extract (500 mg/kg) on duration of HLE and seizures. There was

no significant change in GABA levels in brain homogenate (Table 2).

**Table 1: Effect of acute administration of aqueous extract of leaves of *Calotropis procera* on maximal electroshock induced seizures.**

Group	Drug, dose	HLE in seconds (Mean±SEM)	Total duration of convulsions in seconds (Mean±SEM)
1 (control)	Distilled water, 10mL/kg	12.33±1.86	39.66±9.85
2 (standard)	Phenytoin, 25 mg/kg	3.66±1.0*	9.33±1.86*
3 (test)	Aqueous extract of leaves of <i>Calotropis procera</i> , 250 mg/kg	7.5±1.5*	17.16±4.66*
4 (test)	Aqueous extract of leaves of <i>Calotropis procera</i> , 500 mg/kg	4.5±1.0*	12.33±4.13*

\*p≤0.01 versus control

**Table 2: Effect of chronic administration of aqueous extract of leaves of *Calotropis procera* on maximal electroshock induced seizures.**

Group	Drug, dose	HLE in seconds (Mean±SEM)	Total duration of convulsions in seconds (Mean±SEM)	GABA in µmol/g (Mean±SEM)
1 (control)	Distilled water, 10mL/kg	10±0.34	26.33±1.17	85.94±13.4
2 (standard)	Phenytoin, 25 mg/kg	5.3±0.48*	18.1±1.04*	100.8±18.5
3 (test)	Aqueous extract of leaves of <i>Calotropis procera</i> , 250 mg/kg	7.1±0.31*	21.50±1.25	89.6±11.6
4 (test)	Aqueous extract of leaves of <i>Calotropis procera</i> , 500 mg/kg	6.6±0.26*	17.33±2.31*	92.8±30.09

\*p ≤0.05 versus control

## DISCUSSION

The maximal electroshock induced seizure model helps to identify drugs which are effective against generalised tonic clonic seizures. Seizures induced in this model are indicative of grand mal epilepsy. If a drug abolishes or reduces the duration of the tonic hind-limb extensor phase or seizures in the MES model, then it has potential to be effective in grand mal epilepsy.<sup>15-18</sup> In this study, the effect of *Calotropis procera* on duration of hind limb extension and convulsions was assessed and compared with phenytoin. The standard antiepileptic drug phenytoin significantly reduced the duration of tonic hind-limb extensor phase and seizures. The high dose of plant extract in our study produced a significant decrease in both parameters both on acute and chronic administration which indicates its anticonvulsant activity. There are various mechanisms by which drugs produce their action in GTCS. Drugs like phenytoin, carbamazepine, valproate, lamotrigine etc. act by prolonging the inactivated state of sodium channels. Drugs that increase gamma amino butyric acid (GABA) have exhibited anticonvulsant activity against seizures induced by MES.<sup>17,18</sup> Brain homogenate levels of GABA was not significantly altered in rats treated with plant extract as compared to control. Free radicals have also been suggested to have a role in neuronal changes during MES.<sup>19</sup> They enhance neuronal excitability thereby promoting abnormal discharge. The leaf of *C procera* has

been found to have antioxidant activities. This could have contributed to its beneficial effect in MES.<sup>20</sup> Saponins, tannins and glycosides are important constituents of aqueous leaf extract of *C procera*.<sup>21</sup> Whether these were responsible for the beneficial effect in MES needs to be investigated. Further investigation is required to understand the mechanism of action of aqueous extract of *Calotropis procera leaves* by testing in other models of seizures.

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