

## Evaluation of effect of aqueous extract of leaves of *Calotropis procera* in pentylenetetrazole induced seizures in rats

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**Received:** 16 May 2016

**Accepted:** 21 May 2016

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### ABSTRACT

**Background:** The study was carried out to evaluate the effect of aqueous extract of leaves of *Calotropis procera* in wistar rats.

**Methods:** An aqueous extract of leaves of *Calotropis procera* was prepared. The effect of acute and chronic administration of the extract was tested in pentylenetetrazole (PTZ) induced seizures in wistar rats. Four groups, each containing 6 rats, were used to evaluate acute and chronic effects of the extract. The four groups were treated with distilled water 10 mL/kg (control group), Valproic acid 200 mg/kg (standard), aqueous extract of *C. procera* 250 mg/kg and 500 mg/kg respectively. In acute study, PTZ (60 mg/kg, intraperitoneally) was given 1 h after drugs were administered. In chronic study, all drugs were given for 6 weeks following which PTZ was given 1 h after last dose of each drug. The time taken for the onset of myoclonic jerk, seizures and duration of seizures was recorded. GABA levels were estimated in the brain homogenate. Data was analysed by one way analysis of variance followed by Tukey's test.

**Results:** Acute and chronic administration of the extract significantly increased the time to onset of first clonus and seizures and decreased the total duration of seizures. There was no significant change in GABA levels.

**Conclusions:** Both acute and chronic administration of aqueous extract of leaves of *Calotropis procera* in Wistar rats inhibited pentylenetetrazole induced seizures in rats.

**Keywords:** *Calotropis procera*, Seizures, Clonus, Valproic acid, Anticonvulsant

### INTRODUCTION

*Calotropis procera* Linn. is a plant with important medicinal properties and is used in Ayurveda. Whole plant is used to treat fever, eczema, diarrhoea, boils and jaundice. In addition, leaves, fresh or dried, stem, flowers, roots and root bark are also commonly used. The root has been used for the treatment of eczema, leprosy, elephantiasis, rheumatism, epilepsy and diarrhoea. The stem is used for the treatment of skin diseases, worms, leprosy and leukoderma. The latex and leaves are used to reduce pain and swelling of joints. Flowers have been found to be useful in cough and anorexia.<sup>1-7</sup>

Previous preclinical studies revealed the medicinal properties of this plant. The leaves have shown antifungal, antimicrobial, cytotoxic and anthelmintic activities.<sup>8-10</sup> Antioxidant activity of the plant has been demonstrated.<sup>11</sup> The stem bark has anti-inflammatory and gastroprotective effects.<sup>12</sup> The latex exerts antinociceptive, antipyretic and anti-diarrheal effects.<sup>13-15</sup> The root extracts of *Calotropis procera* exerted antihyperglycemic and hypolipidemic activities in streptozotocin induced diabetic rats.<sup>16</sup> It also has anti-inflammatory and anticonvulsant actions.<sup>17,18</sup> The root extract inhibited both pentylenetetrazole and maximal electroshock seizures. Hepatoprotective effect has been exhibited by flowers.<sup>19</sup>

Saponins are considered to have potential for anticonvulsant actions.<sup>20</sup> Saponin containing plant extracts have inhibited pentylenetetrazole induced seizures.<sup>21</sup> Phytochemical analysis of water extract of leaves of this plant has shown presence of saponins.<sup>22</sup> Hence, the aim of this study was to evaluate anticonvulsant property of aqueous extract of leaves of *Calotropis procera* in pentylenetetrazole induced seizures in rats.

## METHODS

The study was carried out after obtaining clearance from Institutional Animal Ethics Committee, Manipal.

### Drugs and chemicals

Pentylenetetrazole (PTZ; Sigma, USA) and valproic acid (Sun pharma, Mumbai) were used in this study. Analytical grade chemicals were used in the study.

### Preparation of extract

The leaves of *Calotropis procera* were procured from a local ayurvedic shop in Udupi. The leaves were authenticated by a botanist. They were shade dried and coarsely ground with a grinder. The coarse powder was immersed in distilled water in a flask for seven days. This was filtered using Whatman filter paper. The filtrate was concentrated on water bath to get a viscous paste. It was finally dried in a desiccator.

### Animals

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

### Procedure

Pentylenetetrazole (PTZ) induced seizures model was used to evaluate anticonvulsant action of aqueous extract of *Calotropis procera*. The acute and chronic effect of the extract on PTZ induced seizures was assessed. A total of 48 rats were used for the study-they were randomly allotted for acute and chronic study. For each study, 24 rats were used.

### Acute study

Four groups of rats with 6 animals in each were administered drugs as follows;

Group I (Control): distilled water (10 mL/kg)  
Group II (Standard): Valproic acid (200 mg/kg)  
Group III (Test): Aqueous extract of *C. procera* (250 mg/kg)  
Group IV (Test): Aqueous extract of *C. procera*-500 mg/kg

All drugs were administered 1 hour prior to administration of PTZ (60 mg/kg, i.p.).<sup>23</sup> The dose of the plant extract was calculated from earlier acute toxicity studies.<sup>24</sup> Drugs were administered orally. Each animal was placed in an individual cage for observation lasting 30 min. The time taken for the onset of myoclonic jerk, seizures and duration of seizures was recorded.<sup>23,25</sup>

### Chronic study

Four groups of rats with 6 animals in each were used. All drugs (as mentioned in acute study) were administered once a day between 15:00 and 17:00 hours for 6 weeks prior to the administration of PTZ (60 mg/kg, i.p.) which was administered 1h after last dose of test drug.<sup>26</sup> Each animal was placed in an individual cage for observation lasting 30 min. The parameters recorded was same as that of acute study.

### Estimation of GABA in the brain

Rats were sacrificed, brain dissected out and homogenized in phosphate buffer. GABA was estimated by simple spectroscopic method.<sup>27</sup>

### Statistical analysis

The data was expressed as mean±SEM. The data was analysed by one way analysis of variance (ANOVA) followed by Tukey's test. A p-value <0.05 was considered statistically significant.

## RESULTS

### Acute study

Aqueous extract of leaves of *Calotropis procera* in both doses produced anticonvulsant effect in PTZ induced seizures. The time to onset of first clonus and seizures was significantly increased. The total duration of seizures was significantly decreased (Table 1). There was no significant change in GABA levels (Table 2).

**Table 1: Effect of acute administration of aqueous extract of leaves of *Calotropis procera* on PTZ induced seizures in rats.**

Groups	Latency of first clonus	Latency of seizures	Total duration of convulsions
	(Seconds) Mean±SEM		
1	57.33 ± 1.58	70.66 ± 1.28	151 ± 9.86
2	134.6 ± 24.0*	263.0 ± 35.32*	60.0 ± 6.49*
3	138.8 ± 8.78*	195.8 ± 59.99*	89.1 ± 14.6**
4	120.6 ± 4.63**	154.6 ± 10.58*	96.83 ± 3.59**

\*p≤0.001 vs control; \*\*p≤0.034 vs control; ANOVA followed by Tukey's test.

**Table 2: Effect of acute administration of aqueous extract of leaves of *Calotropis procera* on levels of GABA in the brain.**

Groups	GABA in $\mu\text{mol/g}$ (Mean $\pm$ SEM)
1	90.7 $\pm$ 9.90
2	120.8 $\pm$ 18.57
3	106.05 $\pm$ 8.63
4	95.5 $\pm$ 10.10

ANOVA followed by Tukey's test.

### Chronic study

Chronic administration of the extract significantly protected against PTZ induced seizures (Table 3). There was no significant difference between the effects produced by the extract and valproate. There was no significant alteration in GABA levels (Table 4).

**Table 3: Effect of chronic administration of aqueous extract of leaves of *Calotropis procera* on PTZ induced seizures in rats.**

Groups	Latency of first clonus	Latency of seizures	Total duration of convulsions
	(Seconds) Mean $\pm$ SEM		
1	52.16 $\pm$ 3.94	59.66 $\pm$ 3.79	151 $\pm$ 9.86
2	166.3 $\pm$ 19.11*	278.21 $\pm$ 70.12*	60 $\pm$ 6.49*
3	158.3 $\pm$ 65.51*	194.86 $\pm$ 129.7*	91.3 $\pm$ 25.55**
4	163.6 $\pm$ 47.76*	200.63 $\pm$ 145.4*	86.6 $\pm$ 15.77**

\*p $\leq$ 0.001 vs control; \*\*p $\leq$ 0.05 vs control; ANOVA followed by Tukey's test.

**Table 4: Effect of chronic administration of aqueous extract of leaves of *Calotropis procera* on levels of GABA in the brain.**

Groups	GABA in $\mu\text{mol/g}$ (Mean $\pm$ SEM)
1	117.3 $\pm$ 11.42
2	135.5 $\pm$ 23.35
3	113.5 $\pm$ 8.39
4	115 $\pm$ 10.27

ANOVA followed by Tukey's test.

### DISCUSSION

Despite availability of new drugs in modern medicine, some cases of epilepsy are refractory to treatment. In addition, side effects of these drugs affect patient compliance. This has led to search for treatment from alternate system of medicine.

Pentylenetetrazole (PTZ), a tetrazol derivative, is commonly used as a chemoconvulsant to screen antiepileptic drugs.<sup>28</sup> It can produce myoclonic jerk which can become generalized tonic clonic seizures. Drugs which suppress PTZ induced seizures are potentially useful in absence seizures. In this study, aqueous extract of leaves of *Calotropis procera* produced anticonvulsant effect which was evident by the

significant delay in the onset of myoclonic jerk and clonic convulsions and reduced duration of seizures. The effect was observed following both acute and chronic administration of the plant extract.

The mechanism of anticonvulsant action of pentylenetetrazole is not clearly known. It probably acts on GABA receptor and blocks the picrotoxin sensitive site.<sup>29</sup> It blocks GABA transmission in the brain.<sup>30</sup> GABA is an inhibitory neurotransmitter in the CNS. It binds to its receptor, causes influx of chloride ion, resulting in hyperpolarization of neuronal membranes. Disturbance in GABAergic transmission plays a role in epilepsy. It has been shown that drugs enhancing GABA transmission can prevent seizures induced by pentylenetetrazole.<sup>31</sup> PTZ can also activate glutamate receptors (NMDA) to produce seizures.<sup>32</sup> Seizures induced by PTZ can also be blocked by reducing T-type Ca<sup>2+</sup> currents.<sup>33</sup> Valproic acid inhibits PTZ induced seizures. It increases GABA levels by promoting its synthesis and decreasing degradation. It also blocks calcium mediated T current.<sup>34</sup> The extract of *Calotropis procera* leaves did not show significant increase in GABA levels in the brain homogenate. So, it could have acted directly on GABA receptor to enhance neurotransmission or it could have blocked T-currents or interfered with glutaminergic transmission.

Phytochemical analysis of leaf of *Calotropis procera* in previous studies have shown the presence of tannins, glycosides, phenols, terpenoids, alkaloids and saponins.<sup>22</sup> Saponin containing plant extracts have shown potential in the treatment of convulsions.<sup>35</sup> Terpenoids isolated from plants have inhibited seizures in animal models.<sup>36</sup> These phyto-constituents could have contributed to the anticonvulsant effect of leaves of *Calotropis procera*.

### CONCLUSION

Aqueous extract of leaves of *Calotropis procera* produced anticonvulsant effect against pentylenetetrazole induced seizures. Future direction involves studies to standardize it and elucidate its exact mechanism of action.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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**Cite this article as:** Ramachandra VH, Shenoy S, Madhyastha M, Desai R, Ghosh S. Evaluation of effect of aqueous extract of leaves of *Calotropis procera* in pentylenetetrazole induced seizures in rats. *Int J Basic Clin Pharmacol* 2016;5:643-6.