



Anticonvulsant effect of *Cyperus rotundus* Linn rhizomes in rats

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Abstract

Objective: To evaluate the anticonvulsant effect of *Cyperus rotundus* Linn rhizomes against maximal electroshock (MES) and pentylenetetrazole (PTZ) induced tonic seizures in albino rats. **Materials and methods:** Ethanol extract of *Cyperus rotundus* Linn rhizomes were obtained by continuous hot soxhlet extraction. The extract was assessed for anticonvulsant activity by measuring (sec) the duration of tonic flexion, tonic extensor, clonus, stupor and recovery phase in rats. **Results:** Phytochemical investigation of ethanol extract revealed the presence of flavonoids, vitamins and carbohydrates. The ethanol extract (100 mg / kg, p.o.) reduced hind limb extension and duration of convulsion significantly, ($P < 0.001$) which was comparable to standard drug phenytoin (25 mg / kg, i.p.) and diazepam (4 mg /kg, i.p.) respectively. **Conclusion:** The ethanol extract of *Cyperus rotundus* rhizomes is worthwhile to develop the potent phytoconstituent for treatment of epilepsy and the flavonoids present in ethanol extract could be attributed for anticonvulsant activity.

Keywords: Anticonvulsant, *Cyperus rotundus*, rhizomes, Maximal electroshock, Pentylenetetrazole.

1. Introduction

Epilepsy is a group of seizures characterized by excessive or over-synchronized discharge of cerebral neurons, which requires continuous medication to keep the patients free of chronic seizures [1]. The present synthetic potent anti-epileptic drugs prevail with complex side effects, which elicit the approach towards herbal drugs that retain therapeutic efficacy

and are devoid of side effects. *Cyperus rotundus* Linn (Cyperaceae), commonly known as Nut grass, is a perennial sedge distributed throughout India. The roots and rhizomes of this plant are used in different diseases like chronic diarrhea, inflammation, skin rashes, and excess bleeding. It also possesses antiestrogenic, antimicrobial,

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anthelmintic, antihistaminic, antiemetic, antipyretic, and antidiabetic activities [2, 3, 4, 5]. Rhizomes of *C. rotundus* on preliminary chemical analysis, revealed to contain β -sitosterol, cyperene, cyperol, flavonoids, sesquiterpenoids, vitamins and polyphenols [6, 7]. Recently, a great deal of interest has been directed towards the bioactivity of flavonoids, vitamins and polyphenols, as a source of anticonvulsant [8]. Hence, it is of timely interest, to search for new anticonvulsant agent from the rhizomes of *C. rotundus* Linn.

However, rhizomes of *Cyperus rotundus* plant for anticonvulsant activity have not been reported. In the present study, we aimed at utilizing the easily available plant to evaluate its potent bioactive constituents for anticonvulsant activity.

2. Materials and Method

2.1 Plant Material and Extraction

Rhizomes of *Cyperus rotundus* were collected from surrounding fields of Gadag district, Karnataka and were authenticated from Dept. of Botany, J.T College, Gadag, Karnataka. A voucher specimen (CG No. 11) has been deposited at the departmental herbarium. Rhizomes were shade dried and powdered to particle size (#) 40. About 300 gm of dried powder was subjected to continuous hot soxhlet exhaustive extraction with ethanol (95%). The total ethanol extract was filtered and evaporated to dryness at 40°C under reduced pressure in a rota evaporator [9]. The yield of ethanol extract was found to be 35 gm (12 % w/w). The extract was kept in dessicator till the experiment.

The concentrated ethanol extract was evaluated for anticonvulsant activity against MES and PTZ induced seizures in albino rats. Activity was compared with control (Saline) and standard drugs phenytoin and diazepam respectively.

2.2 Animals

Healthy albino rats of either sex weighing between 150 to 220 g (8 to 12 weeks old) were used for the study. The animals were housed in polypropylene cages and fed on standard laboratory diet (Lipton India Ltd) and water *ad libitum*, maintained at an ambient temperatures of $25 \pm 2^\circ\text{C}$ and exposing them to 12 h light/dark cycle. The ethical clearance was obtained by institutional animal ethics committee (Registration No. 221/CPCSEA) before the experiment.

2.3 Drugs

Pentylentetrazole (PTZ; Sigma Poole UK), phenytoin sodium (Epsoline injection, Zydu Neurosciences, India) and diazepam (Calmose injection, Ranbaxy, India) were used after appropriate dilution with distilled water.

2.4 Toxicity assessment

The acute toxicity of ethanol extract was evaluated in mice. The animals were fasted overnight prior to the experimental procedure. The Up and Down or 'Staircase' method was adopted, and accordingly dose of ethanol extract was fixed to 100 mg/kg, p.o. [10].

2.5 Anticonvulsant activity against Maximal Electroshock Seizure (MES)

The experimental animals were divided into three groups of six rats each. Group I (control) received normal saline (1ml / rat, p.o), Group II received standard drug Phenytoin (25 mg / kg, i.p) and Group III received 100 mg / kg, p.o of ethanol extract, 1 h prior to the induction of convulsions respectively. Maximal electroshock of 150 mA current for 0.2 seconds was administered through ear electrodes to induce convulsion in all the experimental animals [11,12].The severity of convulsions was evaluated by measuring (sec) the duration of tonic flexion, tonic extensor, clonus, stupor and recovery phase in all the grouped animals and compared with standard.

Table 1. Effect of *Cyperus rotundus* rhizomes against MES-induced convulsions in rats.

Group		Treatment Time (sec) in Various Phases of Convulsions (Mean±SEM)				
		Flexon	Extensor	Clonus	Stupor	Death/Recovery
1.	Control (Saline 1ml/rat, p.o)	3.03 ± 0.09	12.70 ± 1.24	2.80 ± 0.10	169.0 ± 5.23	Recovery
2.	Phenytoin (25 mg / kg, i.p.)	0.12 ± 0.06*	0.05 ± 0.03 *	0.10 ± 0.05 *	24.20 ± 5.32 *	Recovery
3.	Ethanol Extract (100mg /kg, p.o.)	1.35 ± 0.14 *	4.12 ± 0.24 *	1.50 ± 0.09 *	96.16 ± 3.42 *	Recovery

Each value represents Mean ± SEM (n = 6), * P < 0.001 v/s Control Group (One-way ANOVA followed by Post-hoc Dunnett's *t*-test)

Table 2. Effect of *Cyperus rotundus* rhizomes against pentylenetetrazole (PTZ) induced seizures in rats.

Group	Treatment	Duration of Convulsions in seconds (Mean ± SEM)	% of Mortality
1.	Control (Saline 1 ml/rat)	560 ± 42.89	100
2.	Diazepam (4 mg/kg, i.p.)	0.00 ± 0.00 *	0
3.	Ethanol Extract (100 mg/kg, p.o.)	306 ± 14.29 *	50

Each value represents Mean ± SEM (n = 6), * P < 0.001 v/s Control Group (One-way ANOVA followed by Post-hoc Dunnett's *t*-test)

2.6 Anticonvulsant activity against PTZ induced seizures

The animals were divided into three groups of six animals each. Group I (control) received normal saline (1ml/rat, p.o), Group II received standard drug diazepam (4 mg/kg, i.p) and Group III received ethanol extract (100 mg/kg, p.o.). Pentylenetetrazole (80 mg/kg) was administered intraperitoneally to induce convulsions to all the grouped animals at 1 h post treatment of saline (vehicle), standard drug and ethanol extract [8, 13]. The anticonvulsant effect was assessed by measuring the time in sec for the test drugs to delay the onset of action/protection against PTZ (chemoshock) induced convulsions and mortality time was recorded.

2.7 Statistical analysis

All the results were expressed as Mean ± SEM. The statistical significance was analyzed by performing one-way ANOVA followed by Post-hoc Dunnett's *t*-test. P < 0.001 implies significance [14].

3. Results and discussion

The effect of ethanol extract of *Cyperus rotundus* rhizomes against both MES and PTZ induced seizures are represented in Table 1 and 2 respectively. In MES induced seizures the ethanol extract (100 mg/kg, p.o.) exhibited significant decrease in the duration of hind limb extension as compared with control. Similarly, in PTZ (chemo shock) induced seizures the ethanol extract has showed the significant

reduction in the duration of convulsion as compared with control. The acute toxicity evaluation of ethanol extract of *Cyperus rotundus* rhizomes revealed no mortality when administered orally up to a maximum dose of 1 g/kg. At this dose there was no gross behavioral change. The 1/10th of lethal dose was taken as the screening dose. The phytochemical investigation of ethanol extract has revealed to contain carbohydrates, glycosides, flavonoids and vitamins.

In MES induced seizures the Standard drug Phenytoin (25 mg/kg, i.p.) reduces the hind limb tonic extension by inhibiting voltage dependent Na⁺ channels. On the other hand, Diazepam (4 mg/kg, i.p.) prevents the convulsions induced by PTZ by enhancing gamma amino butyric acid type A (GABA_A) receptor mediated inhibitory neurotransmission [15, 16, 17]. The ethanol extract of *Cyperus rotundus* rhizomes has exhibited significant anticonvulsant activity

against both MES and PTZ induced convulsions revealing the multiple mechanism of action which could, possibly, be due to inhibition of voltage dependent Na⁺ channels by blocking glutaminergic excitation mediated by N-Methyl-D-aspartate (NMDA) receptor, by reducing Ca²⁺ channels or by enhancing gamma amino butyric acid type A (GABA_A) receptors mediated inhibitory neurotransmission.

Phytochemical investigation of ethanol extract revealed the presence of flavonoids, vitamins and carbohydrates. The flavonoids are known to possess action on central nervous system [18]. Hence, the presence of flavonoids in ethanol extract could be attributed for the observed significant anticonvulsant activity. However, research work is under progress in our laboratory to confirm the exact mechanism of action and to elucidate the structure of bioactive principle for the claimed anticonvulsant activity.

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