Protective effect of *Cyperus scariosus* R. Br. root ethanol extract against pentylenetetrazole and isoniazid induced seizures in mice

Sumitra Singh, Nidhi Sharma and Shailendra Kumar Singh

Abstract

The objective of the present study was to evaluate the protective effect of the ethanol extract of *Cyperus scariosus* R. Br. roots in pentylenetetrazole and isoniazid induced seizures using Swiss albino mice. Animals were treated with *Cyperus scariosus* R. Br. root ethanol extract at doses 50 mg/kg and 100 mg/kg p.o. Seizures were induced by pentylenetetrazole and isoniazid. *Cyperus scariosus* R. Br. root ethanol extract showed significant reduction of pentylenetetrazole and isoniazid induced seizure. Latency of seizure was increased and the duration of seizure was found to be lowered in the extract treated animals as compared to control group in both models. Protection against seizure was also indicated by increased GABA level in ethanol extract treated mice. Thus the results suggest that the ethanol extract of *Cyperus scariosus* R. Br. roots possess significant protection against seizures.

Keywords: *Cyperus scariosus*, pentylenetetrazole, isoniazid, seizures

### 1. Introduction

Epilepsy is one of the major neurological disorders, characterized by recurrent and unpredictable interruptions of normal brain function, called epileptic seizure [1, 2]. Seizure refers to a transient alteration of behaviour due to disordered, synchronous and rhythmic firing of populations of brain neurons [3]. A deficiency in the gamma-aminobutyric acid (GABA) concentration or overexcitation of glutamate may result in many pathological alterations in central nervous system (CNS) that can further implicate in epilepsy [4]. Traditional plants have significantly contributed in the discovery of modern drugs and can be used as an alternative source for the discovery of antiepileptic drugs [5]. *Cyperus scariosus* R.Br. is a traditional plant, found as a perennial, delicate slender sedge belonging to family Cyperaceae, is commonly known as “nagarmotha” or “nutgrass.” It is widely distributed and found in various parts of India, especially in damp or marshy areas like around rivers and waterfalls. Nagarmotha is also found in South Africa, China and Pacific Islands. The plant is well known for its uses in the traditional systems of medicine [6].

The plant has been proved for various pharmacological activities such as antimicrobial [7, 8], antinociceptive, hypoglycaemic [9], hepatoprotective [10], hypolipidemic [11], anti depressant [12], hypotensive and spasmolytic activities [13]. The plant has been been used as a folk medicine to treat epileptic seizures. Therefore, the present study was undertaken to evaluate the protective effect of antiepileptic activity of *Cyperus scariosus* roots against seizures.

### 2. Material and methods

#### 2.1 Plant collection and authentication

*Cyperus Scariosus* R. Br. roots were procured by Nature and Nurture Healthcare Pvt. Ltd. (New Delhi) in March, 2015. The roots of this plant have been selected for present work. The plant was authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, CSIR-National National Institute of Science Communication and Information Resources, New Delhi, vide reference no, NISCAIR/RHMD/Consult/2015/2835/28 dated 16.06.2015. The plant was identified as *Cyperus scariosus* R. Br.

#### 2.2 Preparation of extracts

The powdered roots of *Cyperus scariosus* R. Br. were defatted by extracting with petroleum ether and then extracted with ethanol in soxhlet extractor. The extract obtained was concentrated by distilling off the solvent and recovering the same. The extract was then evaporated to dryness to obtain dried extract and kept in dessicator. The ethanol extract obtained was further used for evaluation of anti-seizure effect.
2.3 Drugs and chemicals
Pentylenetetrazole, Sodium valproate (MP Biomedicals, USA), isoniazid (SD Fine-Chem Limited, Mumbai, India) were used in the present study. All other chemicals used for biochemical estimation were of analytical grade.

2.4 Experimental Animals
Swiss albino mice (20-25 g) were purchased from Disease free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana) and were used for biological activity as per the experimental protocol approved by Institutional Animal Ethical committee (Guru Jambheshwar University of Science and Technology, Hisar, Haryana), Registration number 0436, dated 10th August 2015. The animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.5 Experimental protocol
2.5.1 Groups for pentylenetetrazole (PTZ)-induced seizures
The animals were grouped into four major groups:
Group 1: Control group treated with vehicle only.
Group 2: Standard group treated with sodium valproate (SV) 200 mg/kg p.o.
Group 3: Test group treated with Cyperus scariosus root ethanol extract (CE; 50 mg/kg p.o.).
Group 4: Test group treated with Cyperus scariosus root ethanol extract (CE; 100 mg/kg p.o.).

The animals were treated for 14 successive days. On 14th day, PTZ (80 mg/kg, i.p.) was injected after 1 hour of treatment [14]. Each animal was placed into an individual plastic cage for observation of seizures. The latency of seizures, duration of seizures and percentage protection were recorded.

2.5.2 Groups for Isoniazid (INH) induced seizures
The animals were grouped into four major groups:
Group 5: Control group treated with vehicle only.
Group 6: Standard group treated with sodium valproate (SV) 200 mg/kg p.o.
Group 7: Test group treated with Cyperus scariosus root ethanol extract (CE; 50 mg/kg p.o.).
Group 8: Test group treated with Cyperus scariosus root ethanol extract (CE; 100 mg/kg p.o.).

The animals were treated for 14 successive days. On 14th day, INH (300 mg/kg, s.c.) was injected after 1 hour of treatment [15]. Each animal was placed into an individual plastic cage for observation of seizures. The latency of seizures and duration of seizures were recorded. The number of animals recovered from seizures was represented as percentage protection [15].

2.6 Biochemical estimation
2.6.1 Isolation of brain and homogenate preparation for estimation of gamma- amino butyric acid (GABA) levels
Animals of groups 1 to 4 were sacrificed by decapitation after performing behavioural tests; brain was isolated, rinsed with saline and weighed. Homogenates were prepared in HCl-Butanol (0.1 M HCl in butanol) in cold environment for the estimation of GABA level.

2.6.2 Estimation of GABA level
Lowe, 1958 method was utilized for the estimation of brain GABA level. 0.1ml of above homogenate was mixed with 0.2ml of 0.14 M ninhydrin solution in 0.5M carbonate bicarbonate buffer (pH 9.95), kept for 30 minute on water bath at 60 °C, cooled and then treated with 5ml of copper tartrate reagent (0.03% copper sulphate, 0.16% sodium carbonate and 0.0329% tartaric acid). After 10 minute, readings were noted at 377/455nm using spectrofluorimeter (Model 152, Systronic, Gujarat, India) [16].

2.7 Statistical analysis
All the results are expressed as mean ± S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by Dunnett’s test.

3. Results
3.1 Effect of Cyperus scariosus R. Br. root ethanol extract on pentylenetetrazole-induced seizures
PTZ (80 mg/kg, i.p.) administration significantly produced severe seizures characterized by quick onset of jerks, severe straub tail, myoclonic seizure, extensor phase and mortality. However, treatment with Cyperus scariosus ethanol extract (50 and 100 mg/kg, p.o.) significantly delayed latency of seizures (p<0.05 and p<0.01, respectively). Duration of seizures was also reduced significantly (p<0.01) as shown in Figure 1. Percentage protection was also increased in ethanol extract treated mice as compared to PTZ treated control mice (Table 1).

![Figure 1](image)

Fig 1: Effect of Cyperus scariosus R. Br. root ethanol extract on pentylenetetrazole-induced seizures

Values are Mean ± SEM (n = 6). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test. a denotes p<0.01 and b denotes p<0.05 as compared to latency of seizures in group treated with PTZ only. * denotes p<0.01 as compared to duration of seizures in group treated with PTZ only.

3.2 Effect of Cyperus scariosus R. Br. root ethanol extract on INH induced seizures
Tonic clonic seizures are elicited in mice by INH (300 mg/kg, s.c.) administration. However, treatment with Cyperus scariosus ethanol extract (50 and 100 mg/kg, p.o.) significantly delayed latency of seizures (p<0.01). Duration of seizures was also reduced significantly (p<0.01) as shown in Figure 2. Percentage protection of ethanol extract treated mice was also increased as compared to INH treated control mice Table 1.

Table 1.
Table 1: Percentage protection of *Cyperus scariosus* R. Br. root ethanol extract against PTZ and INH induced seizures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Protection</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>INH only</td>
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<td>SV + INH</td>
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</tr>
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Fig. 2: Effect of *Cyperus scariosus* R. Br. root ethanol extract on isoniazid induced seizures. Values are Mean ± SEM (n = 6). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test.

*denotes *p* <0.01 as compared to latency of seizures in group treated with INH only
a denotes *p* <0.01 as compared to duration of seizures in group treated with INH only

3.3 Effect of *Cyperus scariosus* R. Br. ethanol extract on brain GABA level

GABA level was significantly diminished with the treatment of PTZ. However, pre-treatment with *Cyperus scariosus* ethanol extract (50 and 100mg/kg, p.o.) significantly increased GABA level in epileptic animals (Figure 3).

Fig 3: Effect of *Cyperus scariosus* R. Br. ethanol extract on brain GABA level

Values are Mean ± SEM (n = 6). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test. *denotes *p*<0.01 as compared to latency of seizures in group treated with PTZ only.
4. Discussion
Pentylenetetrazole (PTZ) is commonly used to induce seizures for screening of antiepileptic drugs. PTZ induces seizures by its inhibitory effect on GABA mediated chloride ion influx through an allosteric interaction in the chloride ion channel [17]. Isoniazid (INH) induces seizures by causing GABA deficiency. INH is converted to its hydrazones which block pyridoxine phosphokinase and prevents conversion of pyridoxine to pyridoxal 5’ phosphate, which is crucial for the synthesis of gamma amino-butyric acid from glutamic acid. Thus, resulting GABA deficiency and glutamic acid accumulation lead to central nervous system excitation and seizures [18, 19].

In the present study, administration of *Cyperus scariosus* R. Br. root ethanol extract (CE) at doses of 50 mg/kg and 100 mg/kg p.o. for 14 successive days significantly ameliorated the epileptic seizures induced by PTZ and INH in Swiss albino mice as indicated by delay in onset of seizures, decreased duration of seizures and increased percentage protection. The plant roots were more effective against PTZ induced seizures.

5. Conclusion
The results showed that *Cyperus scariosus* R. Br. root ethanol extract has significant protective effect against pentylenetetrazole and isoniazid induced seizures. This effect was possibly due to increased brain GABA levels. Thus, the plant roots can be used for the management of epileptic seizures.

6. Acknowledgement
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7. References
4. DOI: 10.1036/0071443436