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Ameliorative effect of *Asparagus racemosus* root extract against pentylenetetrazol-induced kindling and associated depression and memory deficit

Priyanka Pahwa, Rajesh Kumar Goel *

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India

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ABSTRACT

Asparagus racemosus (*A. racemosus*) roots are extensively used in traditional medicine for the management of epilepsy. The aim of the present study was to investigate the ameliorative effect of *A. racemosus* root extract (ARE) against pentylenetetrazol-induced kindling and associated depression and memory deficit. Kindling was successfully induced by repeated administration of a subconvulsant dose of PTZ (35 mg/kg; i.p.) at an interval of 48 ± 2 h in 43 days (21 injections). Pretreatment with valproate (300 mg/kg; i.p.), a major antiepileptic drug as well as ARE significantly suppressed the progression of kindling. Moreover, ARE also ameliorated the kindling-associated depression and memory deficit as indicated by decreased immobility time and increased step-down latency, respectively, as compared to vehicle control animals. Further, these behavioral observations were complemented with analogous neurochemical changes. In conclusion, the results of the present study showed that ARE treatment has an ameliorative effect against PTZ-induced kindling and associated behavioral comorbidities.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent epileptic seizures, affecting 50 million of the world's total population [1,2]. Despite the availability of a number of antiepileptic drugs (AEDs), seizures cannot be satisfactorily controlled in approximately 40% of patients with epilepsy [3,4]. Further, the majority of patients with epilepsy have also been reported to have one or more behavioral comorbidities such as depression and memory deficit [5]. The most commonly prescribed drugs used to manage epilepsy are not satisfactory in terms of drug-associated behavioral comorbidities. Instead, some of them may potentiate the associated behavioral comorbidities. Furthermore, add-on therapy for the management of epilepsy-associated behavioral comorbidity may further worsen the seizures [6,7]. Therefore, there is a necessity to develop newer antiepileptic drugs for better management of epilepsy and associated depression and memory deficit. Medicinal plants could be considered as a better source for the treatment of epilepsy and its debilitating behavioral comorbidities, as some of the plants have claimed to be ethnomedicinally effective in the management of epilepsy along with associated depression and memory deficit [8]. Moreover, in the past few years, some studies have been carried out in our laboratory investigating the therapeutic potential of some traditional plants, viz., *Passiflora incarnate* [8], *Ficus religiosa* [9], and *Boerhaavia diffusa* [10] for the treatment of epilepsy and associated behavioral comorbidities, and the results were encouraging. Thus, the roots of *Asparagus racemosus* were selected for the present study.

A. racemosus (Liliaceae), commonly known as shatavari, has been used in traditional medicine for the management of epilepsy [11,12]. Pertaining to the central nervous system, several studies have demonstrated *A. racemosus* roots in animal models to be anticonvulsant [13,14], antidepressant [15], nootropic [16], antistress [17], and cerebroprotective [18]. Also, previous studies demonstrated that *A. racemosus* root protects against acute models of convulsions [13,14]. However, there is no report showing an antiepileptogenic effect of *A. racemosus* root extract in any chronic model of epilepsy. Thus, an exploration of this plant in chronic animal models of convulsions that mimic human epilepsy is warranted. Interestingly, the earlier studies evidenced that *A. racemosus* had an antidepressant and memory-enhancing potential [15,16]. In line with that, this plant has not been explored for antidepressant and memory restoration properties in epileptic animals. With this background, *A. racemosus* might be helpful in the management of epilepsy and associated depression and memory deficit.

Therefore, we aimed to determine the scientific basis for the traditional use of *A. racemosus* root extract in epilepsy and associated behavioral comorbidities.

* Corresponding author at: Pharmacology Division, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, Punjab, India. Tel.: +91 9417881189.
E-mail address: goelrkpup@gmail.com (R.K. Goel).

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2. Material and methods

2.1. Plant collection

The roots of *A. racemosus* were procured from "Chaudhary Charan Singh Haryana Agriculture University" (CCS-HAU), Hisar (Haryana), India. The botanical identification of the plant material was done by Prof. V.K. Singla, and the voucher specimen was conserved at the Herbarium, Department of Botany, Punjabi University, Patiala, Punjab under reference number 58965/08-05-2014.

2.2. Preparation of extract

The hydroethanolic extraction of dried *A. racemosus* roots was carried out using the percolation method. The extract was concentrated on a rotary evaporator, lyophilized, and stored at 4 °C for further use. The percentage yield of the extract was found to be 55.7% w/w.

2.3. Preliminary qualitative phytochemical analysis

Preliminary phytochemical screening of the crude extract was carried out qualitatively for the presence of alkaloids, glycosides, flavonoids, saponins, sterols, tannins, phenolic compounds, amino acids, proteins, fatty acids, carbohydrates, and terpenes by standard tests [19].

2.4. Drugs and chemicals

Pentylenetetrazol (PTZ), serotonin (5-HT), dopamine (DA), Griess reagent, and high performance liquid chromatography-grade methanol were procured from Sigma-Aldrich (St. Louis, MO, U.S.A.); perchloric acid was procured from Spectrochem (Mumbai, India); o-phthalaldehyde (OPA) and heptanesulfonic acid were procured from Loba Chemie (Mumbai, India); and ethylenediaminetetraacetic acid (EDTA) was procured from S.D. Fine Chemicals Ltd. (Mumbai, India). Norepinephrine (NE) and valproate were obtained as a gift sample from Troikaa Pharmaceuticals, Dehradun, Uttarakhand, India and Q. P. Pharmachem, Derabassi, India, respectively.

2.5. Animals

Swiss albino mice of either sex, weighing 20–30 g, were purchased from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The animals were housed with a 12-h light/dark cycle under controlled temperature (22 ± 2 °C) and humidity (50 ± 10%). They were allowed to acclimatize for 1 week with free access to food and water ad libitum. Animal experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India and approved by the Institutional Animal Ethical Committee (IAEC) (107/99/CPCSEA-2012-01).

2.6. Induction of kindling

Induction of kindling was performed by the method previously validated in our laboratory [20]. Briefly, pentylenetetrazol (dissolved in warm saline) was injected into the animals with a subconvulsant dose (35 mg/kg; i.p.) at an interval of 48 ± 2 h for 6 weeks or until all the animals in the vehicle control group showed tonic–clonic convulsions after two consecutive pentylenetetrazol injections [21]. After each injection, the animals were placed individually into Plexiglas cages (20 × 20 × 30 cm) and observed for 30 min. The intensity of the convulsions was registered according to the modified Racine's scale [22]: stage 0 — no response; stage 1 — hyperactivity, restlessness, and vibrissae twitching; stage 2 — head nodding, head clonus, and myoclonic jerks; stage 3 — unilateral or bilateral limb clonus; stage 4 — forelimb clonic seizures; stage 5 — generalized tonic–clonic seizures with falling; stage 6 — hind limb extensor; and stage 7 — death. The transition of the convulsion intensity from the 4th to the 5th degree reflected the generalization of the convulsive activity, manifested by the tonic–clonic convulsions.

2.7. Experimental protocol

Animals were randomly divided into six groups of six animals each. Group I (naïve group) received saline intraperitoneally while groups II to VI were administered PTZ (35 mg/kg; i.p.) dissolved in saline, every other day (48 ± 2 h). Group II (vehicle control group) received saline pretreatment whereas group III (valproate treatment group) was administered with valproate (300 mg/kg) intraperitoneally. Groups IV to VI (ARE treatment group) received ARE pretreatment in doses of 200, 400, and 800 mg/kg intraperitoneally, respectively. In these groups, valproate and ARE were given 30 min before PTZ. Pentylenetetrazol was administered up to 43 days or until stage 5 seizures on two consecutive trials were achieved. Depressive behavior was evaluated using the tail suspension test (TST) on day 43 after the last administration of PTZ while memory impairment was evaluated using step-down passive shock avoidance paradigm on day 44 followed by retrieval after 24 h. After behavioral assessments on day 45, all the animals were sacrificed for neurochemical analysis.

2.8. Behavioral assessment

2.8.1. Tail suspension test

The tail suspension test was conducted as previously described [23,24], with some modifications on day 43. Briefly, mice were individually suspended their tail with a clamp (1 cm from the tip of the end). Testing was carried out in a noise-free room. A mouse was suspended for a total of 6 min, and the duration of immobility was recorded during the final 4-min interval of the test. Mice were considered immobile only when they hung passively and completely motionless. The test session was recorded by a video camera and scored by a blinded observer.

2.8.2. Passive shock avoidance paradigm

For the evaluation of contextual fear memory, a modified passive shock avoidance paradigm previously standardized in our laboratory was used [25,26]. On day 44, the animals were trained to stay in the shock-free zone (SFZ) for at least 120 s, and the number of trials required was recorded. Further retrieval of the learned task was evaluated by recording the changes in the number of mistakes and step-down latency on day 45.

2.9. Neurochemical estimations

After behavioral evaluation on day 45, all the animals were scarified by cervical dislocation, and their brains were dissected to isolate the different brain regions (cortex and hippocampus). Isolated brain parts were weighed and subdivided into two equal portions. One portion was homogenized in ice cold 10% w/v (0.1 M) perchloric acid and centrifuged at 14,500 *g* for 30 min at 4 °C (REMI C-24 BL, Cooling Centrifuge, REMI, India), and clear supernatants were used for estimation of monoamines (noradrenaline, dopamine, and serotonin) using HPLC-ECD [27]. The second portion was homogenized in ice cold 10% w/v (0.05 M, pH 7.4) phosphate buffer and centrifuged at 6000 *g* for 20 min at 4 °C, and a clear supernatant was utilized for estimation of total nitrite level and acetylcholinesterase activity using the microplate reader method previously standardized in our laboratory [20,28,29].

2.10. Statistical analysis

The statistical analysis was performed by using GraphPad Prism® version 5 (GraphPad Software Inc., San Diego, CA, U.S.A.). Statistical significance was calculated using one-way ANOVA followed by Student–