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Anticonvulsant activity and acute neurotoxic profile of *Achyranthes aspera* Linn.

Dinesh Y. Gawande[a], Dmitry Druzhilovsky[b], Raghbir Chand Gupta[c], Vladimir Poroikov[b], Rajesh Kumar Goel[a,*]

[a] Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, Punjab, India
[b] Institute of Biomedical Chemistry, Pogodinskaya Str., 10/8, Moscow 119121, Russia
[c] Department of Botany, Punjabi University, Patiala 147002, Punjab, India

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ABSTRACT

Ethnopharmacological relevance: Root powder of *Achyranthes aspera* Linn. (*A. aspera*) belongs to family Amaranthaceae is used in Indian traditional medicine for the management of epilepsy and its efficacy is widely acclaimed among the different rural communities.

Aim of the study: The present study was aimed to establish the possible anticonvulsant effect of *A. aspera* methanolic root extract using acute anticonvulsant models and to evaluate the acute toxicity and neurotoxic potential *A. aspera* extract.

Material and methods: *A. aspera* methanolic extract was standardized with respect to betaine using HPTLC. The maximal electroshock (MES), pentylenetetrazol (PTZ), picrotoxin and bicuculline induced seizure models were used to evaluate the anticonvulsant potential of standardized *A. aspera* root extract. The GABA content in cortex and hippocampus of extract treated mice was evaluated using HPLC. Moreover, the animals were also evaluated for acute toxicity study and neurotoxicity test.

Results: A significant enhancement in the seizure threshold was observed by *A. aspera* extract (5 and 10 mg/kg) treated mice in PTZ, picrotoxin and bicuculline models as compared to saline treated mice respectively, whereas the extract failed to show protection in MES induced seizures. Moreover, *A. aspera* treatment (5 and 10 mg/kg) significantly enhances the GABA levels in hippocampus and cortex as compared to saline treated group. *A. aspera* root extract was devoid of any sign of acute toxicity as well as neurotoxicity.

Conclusions: *A. aspera* root extract exhibits significant anticonvulsant effect by facilitation of GABAergic neurotransmission in the brain.

1. Introduction

Epilepsy is a most common and widespread serious neurological disease, affects around 70 million people worldwide and can profoundly affect many aspects of quality of life (Goel et al., 2015). At the molecular level in epilepsy, there is an imbalance between inhibitory GABA-mediated and excitatory glutamate-mediated neurotransmission (Staley, 2015). Many GABAergic agonists and GABA modulators have been known as antiepileptic agents (Greenfield, 2013), benzodiazepines have been used in termination of spontaneous seizures from many decades and diazepam is a prototype agent in this category (Raabe and Gumnit, 1977), along with benzodiazepines there are several antiepileptic drugs (AEDs) but most of these AEDs carries potential side effects (Kaur et al., 2016). Therefore epilepsy warrants new pharmacological interventions with high efficacy and minimal side effects.

Over the centuries humans have relied on phytomedicine for the management of chronic disorders, several countries across the globe are dependent on phytomedicine (Singh et al., 2014; Lagunin et al., 2014). In developing countries like India, the World Health Organization (WHO) recommended the initiation of programs designed to use medicinal plants more effectively in traditional health care systems (WHO, 2013). Several traditional medicinal plants have been known for their potential role in the management of central nervous system disorders (Kumar, 2006). Similarly, many plants used for the treatment of epilepsy in different systems of traditional medicine have shown antiepileptic effects when tested preclinically, but those plants have not yet been scientifically investigated (Muazu and Kaita, 2008; Sharma et al., 2013). *A. aspera* is widespread as a weed in many countries such as Australia, America, Africa Baluchistan, Ceylon and Tropical Asia (Saba, 2014). Phytochemically *A. aspera* is

* Corresponding author.
E-mail address: goelrkpup@gmail.com (R.K. Goel).

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well known for the presence of alkaloids, saponins, carbohydrates, glycosides, flavonoids, tannins and triterpenoids (Ali, 1993). As, it is a member of Amaranthaceae family it has been used in asthma, kidney stone, skin diseases and snake bite in traditional Indian medicine (Nadkarni, 2009). Previous studies reported the CNS depressant (Bhosale et al., 2011), anxiolytic (Barua et al., 2012) and antinociceptive (Barua et al., 2010) effects of *A. aspera* leaf extract. Tribal communities from Indian states use roots powder of *A. aspera* for the treatment of epilepsy (Sharma et al., 2013). Moreover, in Ayurveda it was reported as a brain tonic (Dwivedi et al., 2008). Therefore the present study was envisaged to investigate the anticonvulsant effect of the methanolic extract obtained from the roots of *A. aspera* against four pharmacological models viz. PTZ, picrotoxin, bicuculline and MES induced convulsions respectively.

2. Materials and methods

2.1. Plant material collection, authentication, and extract preparation

The roots of *A. aspera* were collected from the botanical garden of Punjabi university, Patiala, India from September to November 2013 and identified by Prof. R.C. Gupta (Department of Botany, Punjabi university, Patiala, India). A specimen of *A. aspera* (No.: 59208) was submitted in the herbarium (Department of Botany, Punjabi University, Patiala, India). The roots were washed and shade dried followed by oven drying at 50 ± 4 °C and coarsely powdered using a mechanical grinder. Further, plant material was extracted by a simple maceration process by using methanol for 72 h with occasional shaking and collection of menstruum after every eight hours. The menstruum was pooled and concentrated under reduced pressure at 60 °C using rotary evaporator (Buchi, Switzerland), lyophilized and kept at −4 °C in a refrigerator until use. The percentage yield of pale yellow color powdered extract was found to be 8.0% (w/w).

2.2. Phytochemical investigation and standardization of plant extract using high performance thin layer chromatography

Preliminary phytochemical screening of the crude extract was carried out qualitatively for the presence of alkaloids, glycosides, flavonoids, saponins, tannins, amino acids, proteins, fatty acids, carbohydrates and terpenoids by using standard procedures (Trease and Evans, 1997).

Betaine quantification in *A. aspera* extract was performed by high performance thin layer chromatography (HPTLC) method reported by Mehta et al. (2011) with slight modification. Briefly, the crude extract was transferred to a separating funnel and defat with n-hexane; the aqueous extract was concentrated under reduced pressure. The presence of betaine in *A. aspera* was confirmed by thin layer chromatography (TLC) using HPTLC plate coated 250 mm layers of Silica gel G60 F_{254} (Merk, Germany) as a stationary phase and mobile phase was methanol: water (9:1 v/v). Additionally the plate was sprayed with Dragendorff's reagent followed by 10% ethanolic sulphuric acid and dried at 110 °C for 5 min. Further, *A. aspera* was standardized with betaine using HPTLC. A stock solution of extract (aqueous extract) (10 mg/mL), and betaine (1 mg/mL) were made in methanol. The mobile phase and spraying reagent for developing the chromatogram was same as used for TLC. Detection was done by measurement of absorbance at 520 nm. HPTLC fingerprinting was performed using aluminium backed HPTLC plate coated 250 mm layers of silica gel G60 F_{254} (Merk, Germany), Camag-HPTLC instrumentation (Camag, Switzerland) equipped with Linomat V sample applicator, Camag TLC scanner IV and WINCATS IV software for data analysis. Standard curve was prepared with betaine and quantification was based on the standard curve of betaine. Further the validation parameters for betaine like linearity, limit of detection (LOD), limit of quantitation (LOQ) and relative standard deviation (RSD) were addressed.

2.3. Animals

Present study was carried out using Swiss Albino mice weighing (22–28 g), animals were obtained from Chaudhary Charan Singh Haryana Agricultural University, Hisar, India and housed under controlled conditions (22 ± 2 °C), under natural light/dark cycle, with free access to food and water prior to the experiments. The mice were acclimatized at lab conditions for one day before commencement of the experimental protocols. The behavioral experiments were carried out from 09:00 a.m. to 02:00 p.m. Experimental procedures were carried out according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA) and were approved by the Institutional Ethical Committee (Approval no.: 107/99/CPCSEA 2012-02).

2.4. Drugs and chemicals

Analytical grade standard chemicals were used in present study. Betaine, Pentylenetetrazol (PTZ), Picrotoxin, Bicuculline and HPLC grade Methanol were obtained from Sigma Aldrich (USA), Diazepam and Phenytoin sodium from Jackson Laboratories Ltd. (Amritsar, India). GABA was obtained from Central Drug House (Delhi, India).

2.5. In vivo studies

2.5.1. PTZ induced convulsions

Intraperitoneal injection of PTZ (60 mg/kg i.p.) was given to five groups of mice (n=6/group) pretreated 30 min prior with varying doses of extract (2.5, 5 and 10 mg/kg), saline (10 mL/kg) and diazepam (5 mg/kg). The latency to tonic-clonic convulsions was noted in all groups (Swinyard et al., 1952). Extract and diazepam treated groups were compared with a control group in order to find out the significant anticonvulsant effect.

2.5.2. Picrotoxin induced convulsions

All the groups were injected with picrotoxin (5 mg/kg) pretreated (30 min prior) with varying intraperitoneal doses of extract (2.5, 5 and 10 mg/kg), saline (10 mL/kg) and diazepam (5 mg/kg). The latency to clonic convulsions was noted in all groups (Leewanich et al., 1996; Singh and Goel, 2009). Extract and diazepam treated groups were compared with saline so as to find out the considerable anticonvulsant effects.

2.5.3. Bicuculline induced convulsions

Intraperitoneal bicuculline (4 mg/kg) was administered into five groups (n=6/group) pretreated (30 min prior) to extract treatment (2.5, 5 and 10 mg/kg i.p.), saline (10 mL/kg) and diazepam (5 mg/kg i.p.). The latency to clonic convulsions was noted in every group (Johansson et al., 1996). Extract and diazepam treated groups were compared with saline so as to find out the considerable anticonvulsant effects.

2.5.4. MES induced convulsions

MES induced convulsions model was used to determine the anticonvulsant potential of the extract. Convulsions were induced in mice by delivering transauricular electroshock of 50 mA for 0.2 s via convulsiometer (Rolex, Ambala, India), using crocodile ear clips pair. Five groups of mice (n=6/group); each group pretreated intraperitoneally with extract (2.5, 5 and 10 mg/kg), saline (10 mL/kg) and phenytoin (25 mg/kg) respectively and animals were evaluated following 30 min for MES seizure response. Tonic hind limb extension duration was recorded in all groups (White et al., 1995; Johansson et al., 1996; Singh