Original Article

Study of central nervous system depressant and behavioral activity of an ethanol extract of *Achyranthes aspera* (Agadha) in different animal models

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Abstract

Background: *Achyranthes aspera* Linn., an indigenous herb, has been reported to have antifertility, antihyperlipidemic, antidiabetic, immunomodulatory, anticarcinogenic, diuretic, cardiotonic, analgesic anti-inflammatory, hypnotic, antifungal, antibacterial, and central antinociceptive activities. **Aims:** This study was designed to evaluate depressant effects on central nervous system (CNS) and behavioral effects of ethanol extract of A. *aspera* (EEAA) and to find the phytochemical responsible for these activities. **Materials and Methods:** The pharmacological assays used to study CNS depressant effect in albino mice were rota rod and actophotometer performance test. Effects on behavioral activity were studied using open field test. The extract was given intraperitoneally (i.p.) at a dose of 400 mg/kg. Diazepam (2 mg/kg body weight i.p.) was used as standard. **Statistical Analysis Used:** Data were analyzed by using analysis of variance followed by Dunnett's test. $P < 0.05$ was considered significant. **Results:** Phytochemical screening revealed presence of triterpenoids, saponins, alkaloids (betaine, achyranthine), and steroids as major constituents. The result of this study reflected that EEAA (400 mg/kg i.p.) decreased locomotor activity, produced muscle relaxation, and showed anxiolytic activity. **Conclusions:** EEAA exhibit CNS depressant and significant anxiolytic activity comparable to diazepam.

Key words: *Achyranthes aspera*, actophotometer, open field test, rota-rod

Introduction

Achyranthes aspera Linn. (Amaranthaceae) grows as wasteland herb and is in use as folk medicine. It is known by different names such as Chirchita (Hindi), Apamarga (Sanskrit), Aghedi (Gujarati), Apang (Bengali), Nayurivi (Tamil), Kalalat (Malyalam),[1] and Agadha (Marathi) in our country. Previous studies have reported that the herb has antifungal, antifertility, antihyperlipidemic, antidiabetic, immunomodulatory, anticarcinogenic, diuretic, and cardiotonic, anti-inflammatory analgesic, and antibacterial activities.[2-7] It has been also used as brain tonic and in treatment of insomnia in folk medicine.[1,8]

Ethanol extract of *A. aspera* (EEAA) has been reported to have central antinociceptive activity in thermal-induced pain methods.[5,6] Taking these guidelines we made an attempt to study its neuropharmacological effects as per standard protocol for screening newer antinociceptive agents[9] and to further evaluate the phytochemical responsible for this neuropharmacological activity.

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Materials and Methods

This study was conducted in the Department of Pharmacology of a Medical College. The experimental protocol was approved by Institutional Animal Ethics Committee.

Plant material

The leaves of *A. aspera* were procured from Empress Garden, Koregaon Park, Pune, and were identified by Botanical Survey of India, Pune (specimen voucher no: MRZAA1 date:

16/9/09). The leaves were washed under running water, shade dried, and the dehydrate leaves were powdered to a fine texture; 100 g of the dried leaves were repeatedly extracted with 95% ethanol for 10 days at room temperature; as ethanol evaporates completely, it fulfils the requirements of an ideal solvent. EEAA was then filtered through filter paper and concentrated by evaporation. The dried extract was stored in refrigerator. The crude extract was weighed and percentage yield was calculated. Phytochemical study, acute toxicity study, and neuropharmacological study were performed using EEAA [Figure 1].

Phytochemical study

Freshly prepared EEAA was evaporated, and to this residue dilute hydrochloric was added, shake well, and filtered. With filtrate, tests were performed for the detection of various constituents using conventional protocol like Mayer's, Wagner, Hagner's, and Dragendorff's test for alkaloids; foam and hemolytic tests for saponins; Salkowski's test and Lieberman–Buchard test for steroids/triterpenoids; gelatin test, ferric chloride test for tannins; Shinoda test for flavonoids; and Molisch's test for carbohydrates.[10]

Experimental animals

Wistar albino mice of either sex weighing 35–40 g, bred in Central Animal House facility of the institute, were used for this study. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle, and had free access to food and water. They were acclimatized to laboratory conditions before the experiment. Each animal was used once in every experiment, and all experiments were carried out in daylight.

Acute toxicity study

Acute toxicity study was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines No. 423. Three animals were used for each step. The dose level to be used as the starting dose was selected from one of four fixed levels, i.e., 5, 50, 300, and 2000 mg/kg body weight p.o. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, recommended starting dose is 300 mg/kg body weight. Hence, we have used 300 mg/kg body weight as starting dose.

Neuropharmacological study

Six animals were used in each group for each experiment separately. Animals treated with 5% gum acacia suspension (0.1 ml p.o.) served as control. Diazepam (2 mg/kg i.p.) served as standards, and animals in test group were treated with EEAA (400 mg/kg i.p.), respectively. Each animal was treated with respective drug 30 min before experiment. Tests performed were as follows:

Rota-rod performance

Four animals at a time were placed on rod rotating at 20–25 rpm speed. Only the mice that demonstrated their ability to remain on the revolving rod (20–25 rpm) for 5 min after training sessions during pretest screening were selected for studies. The fall off time was recorded in all the groups before and 30 min after drug administration. Decrease in fall off time is suggestive of depression of the central nervous system (CNS).[11]

Actophotometer test

The animal locomotor behavior was monitored using actophotometer. Animals were placed in actophotometer individually, and basal activity score was recorded over the period of 5 min. Each animal was treated with respective drug, and activity score was recorded after 30 min and 1 h. Decreased activity score was taken as index of CNS depression.[12-14]

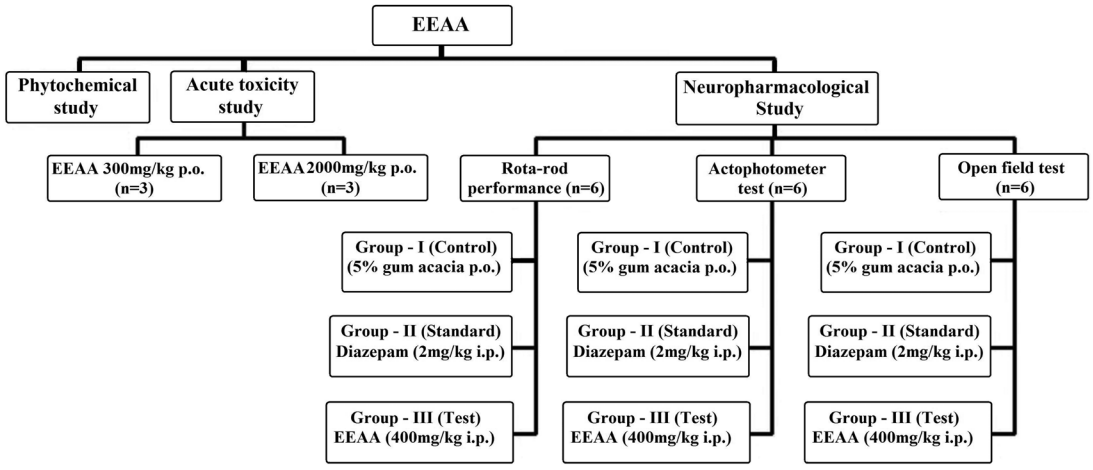

Figure 1: Protocol flow chart

Open field test

Open field apparatus was designed as described by Gray and Lalji (1971) with few modifications. Dimensions were 50 × 50 × 40 cm made up of plywood open from top and bottom kept on white table top; surface was divided into 25 equal squares, i.e., 9 central and 16 peripheral. The animals were pretreated with samples (5% gum acacia suspension, EEAA, and diazepam) 1 h before. During 5-min session of observation, each animal was placed in the corner of open field apparatus, and behavior of animal as determined by ambulation (number of squares entered with both forelimbs), rearing, preening, and defecation was recorded.[15]

Statistical analysis

Data were analyzed by analysis of variance test followed by Dunnett's test. All the results were expressed as mean ± SEM. $P < 0.05$ was considered significant. Percent reduction in activity score and fall off time were calculated with reference to respective basal recordings.

Results

Phytochemical analysis

Total yield of extract was 6.53% (w/w). The leaves yielded triterpenoids, saponins, alkaloids (e.g., betaine, achyranthine), and steroids as major constituents, while flavonoids and tannins were found absent [Table 1].

Acute toxicity study

The results of acute toxicity study showed no clinical signs of toxicity and mortality in the EEAA treated animals even after administration of 2000 mg/kg dose. Hence, as per OECD guidelines lethal dose was assigned to be more than 2000 mg/kg. One-fifth of this lethal dose (400 mg/kg) was taken as effective dose for the study.

Rota-rod method and actophotometer test

Diazepam (2 mg/kg i.p.) and EEAA (400 mg/kg i.p.) treated groups showed significant CNS depressant activity when compared with control; however; this depression was less with EEAA treated group than diazepam-treated group [Table 2] [Figure 2, 3].

Open field test

Diazepam (2 mg/kg i.p.) and EEAA (400 mg/kg i.p.) significantly ($P < 0.001$) exhibited anxiolysis; as evident from increased ambulation, rearing and preening; and decreased defecations compared with control [Table 3] [Figure 4].

Discussion

Anxiety and hypnosedation are principally mediated in the CNS by the $GABA_A$ receptor complex, which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, Parkinson syndrome, and Alzheimer's disease.[16] Diverse drugs that are used in various psychological

Table 1: Phytochemical screening of ethanol extract of *Achyranthes aspera*

Chemical constituents	Test	Result
Test for alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	–
	Dragendroff's test	+
Test for carbohydrates	Molisch's test	+
Test for saponins	Foam test	+
	Haemolytic test	–
Test for triterpenoids/steroids	Salkowski's test	+
	Liebermann-Burchard test	+
Test for flavonoids	Shinoda test	–
Test for tannins	Ferric chloride test	–
	Gelatin test	–

+ Present; – absent

Table 2: Activity score in actophotometer method and mean fall off time in rota-rod method

Drugs ($n = 6$)	Group I (5% gum acacia p.o.)	Group II (diazepam 2 mg/kg i.p.)	Group III (EEAA 400 mg/kg i.p.)
Mean score in 5 min (% reduction)			
Basal	350 ± 12.5	372 ± 57.7	344.3 ± 9.8
30 min	341.7 ± 12.1	244.2 ± 36.8*	244.8 ± 15*
60 min	344.8 ± 12.8	108.8 ± 19.7 (70.8%)*	258.8 ± 9.9 (24.8%)*
Mean fall off time in s (% reduction)			
Basal	223.3 ± 18.9	229.7 ± 22.9	230 ± 18.5
30 min	226.7 ± 22.3	86.3 ± 2.4 (62.4%)*	139.3 ± 20.1 (39.4%)*

Values indicate mean ± SEM. (analysis of variance test followed by Dunnett's t-test). Significant variation against control at $*P < 0.001$; Percent reduction in parenthesis calculated with reference to basal score

Table 3: Mean score in open field performance method

Drugs ($n = 6$)	Group I (5% gum acacia p.o.)	Group II (diazepam 2 mg/kg i.p.)	Group III (EEAA 400 mg/kg i.p.)
	Mean scores and SEM		
Ambulation (numbers)			
Peripheral squares	25.2 ± 3.2	101.7 ± 31**	97 ± 9.5**
Central squares	0.5 ± 0.34	9 ± 4.3*	35.2 ± 5.6**
Rearing (counts)	5.7 ± 1.4	33.2 ± 8.6**	31.7 ± 3.8**
Preening (counts)	1.3 ± 0.80	9 ± 0.82**	3.7 ± 0.42**
Defecation(counts)	2.8 ± 0.31	1.3 ± 0.49**	1 ± 0.52**

Values indicate mean ± SEM. (analysis of variance test followed by Dunnett's t-test). Significant variation against control at $*P < 0.05$, $**P < 0.001$

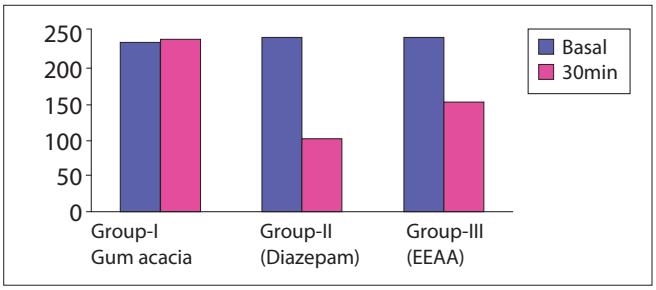

Figure 2: Mean fall off time in rota-rod method

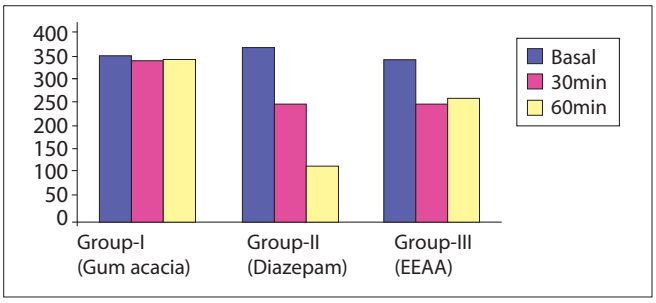

Figure 3: Activity score in actophotometer

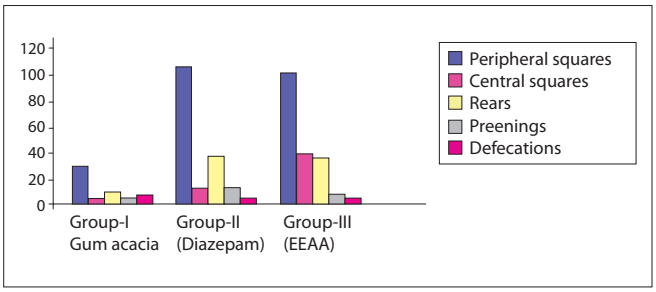

Figure 4: Mean score in open field performance method

and neurological disorders might modify the GABA system at the level of the synthesis of GABA, induce anxiolysis or hypnosis in animals by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors,[17] and thirdly by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents like barbiturates.[18]

In this study, CNS depressant activity of EEAA was evaluated by rota rod test, which has clearly demonstrated the CNS depressant activity evidenced by decreased fall off time. Another important step in evaluating CNS drug action is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS, and decreased activity results from CNS depression.[19] The extract significantly decreased the locomotor activity as observed in the results of the actophotometer test. [Table 2] [Figure 2, 3]

Moreover, anxiolysis was studied by measuring external signs like ambulation, rearing, preening, and defecation in open field test. It is used for evaluating the effect of drugs on gross general behavior and is used to measure the level of nervous excitability when the animals are exposed to a novel environment.[20] Exploration in a new environment is an essential part of normal behavior in animals; lower ambulation, exploration, and reduction in normal rearing and preening behavior with increased defecation in new environment are due to anxiety and fear. However, disinhibitory actions of anxiolytics increase these behavioral activities in new environment by releasing novelty-induced suppression of behavior.[20,21]

As mentioned in results, EEAA possesses various phytochemical substances such as triterpenoid saponins (A and B) possessing oleanolic acid as aglycone, alkaloid achyranthine, water-soluble base betaine, and steroids. CNS depressant and anxiolytic activity of EEAA was supposed to be attributed to these phytochemicals found in the extract. Several plants have been reported to have CNS depressant and anxiolytic activity due to the presence of triterpenoids,[22] saponins,[23] and flavonoids.[22,23] Phytochemical analysis of EEAA also revealed presence of triterpenoid saponins[5,6]; however, flavonoids were found absent. Triterpenoid saponins are reported to have agonistic/facilitatory activities at $GABA_A$ receptor complex,[24,25] which led to the hypothesis that they act as benzodiazepine-like molecules. This is supported by their behavioral effects in animal models of CNS depression and anxiety.[22,23]

From the results we can conclude that EEAA possesses considerable CNS depressant and anxiolytic activity which is comparable with the standard. Triterpenoid saponins may be the phytochemicals responsible for this activity. Central depressant and anxiolytic activity along with strong analgesic effect as reported in earlier studies may complement to each other and thus may be used in variety of painful and excitatory conditions.

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