

Study of CNS depressant and behavioral activity of an ethanol extract of *Achyranthes Aspera* (Chirchita) in mouse model

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KEY WORDS

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ABSTRACT

Background: *Achyranthes Aspera* Linn., known as Chirchira (Hindi), Agadha (Marathi) is an indigenous herb found in India. The herb has been reported to have variety of activities like antifertility, antihyperlipidemic, antidiabetic, immunomodulatory, anticarcinogenic, diuretic and cardiotoxic, analgesic, anti-inflammatory, hypnotic, antifungal and antibacterial activity. It has been also reported to have central anti-nociceptive activity in thermal induced pain methods in our earlier studies. We wanted to study its neuropharmacological effects, which may throw light on understanding the underlying mechanism for its central activity. **Purpose:** The present study was designed to evaluate CNS depressant and behavioral effects of *A. Aspera* extract and to study the phytochemical responsible for these activities with possible mode of action. **Methods:** The effects on behavioral activity was studied using open field test (OFT). The extract was given intraperitoneally at a dose of 400 mg/kg. Diazepam (2mg/kg body weight i.p.) was used as standard. Data was analyzed by ANOVA test followed by Dunnett's test. All the results were expressed as Mean (\pm SEM). $P < 0.05$ was considered significant. **Results:** Phytochemical screening revealed presence of triterpenoids, saponins, alkaloids (betaine, achyranthine) and steroids as major constituents. The result of the study demonstrated that ethanol extract of *A. Aspera* (400 mg/kg i.p.) decreased locomotor activity, produced muscle relaxation and showed antianxiety activity. **Conclusions:** Ethanol extract of *A. Aspera* exhibit CNS depressant action and significant anxiolytic activity comparable to diazepam.

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Introduction

Achyranthes Aspera Linn. (Amaranthaceae) grows as wasteland herb. Since time immemorial, it is in use as folk medicine. It is well known as medicinal herb in different systems of medicine in India. 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 antifungal², antifertility, antihyperlipidemic, antidiabetic, immunomodulatory, anticarcinogenic, diuretic, cardiotoxic,³ anti-inflammatory-analgesic,⁴⁻⁶ and antibacterial⁷ activities. It has been also used as brain tonic and in treatment of insomnia in folk medicine.⁸

In our earlier studies ethanol extract of *Achyranthes Aspera* (EEAA) has been reported to have central antinociceptive activity in thermal induced pain methods.^{5,6} We therefore, made an attempt to study its neuropharmacological effects as per standard protocol for screening newer antinociceptive agents⁹ for understanding the underlying mechanism for its neuropharmacological activity and to further evaluate the phytochemical responsible for this neuropharmacological activity.

Methods

This study was conducted in the Department of Pharmacology at Smt. Kashibai Navale Medical College, Pune. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC).

Plant Material

The leaves of *Achyranthes Aspera* were procured from Empress Garden, Koregaon Park Pune and were identified by Botanical Survey of India (BSI), Pune (specimen voucher no: MRZAA1 date:16/9/09). The leaves were washed under running water,

shade dried and the dehydrate leaves powdered to a fine texture and 100 g of the dried leaves was repeatedly extracted with 95% ethanol for 10 days at room temperature. The extract (EEAA) was then filtered through Whatman 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

Freshly prepared ethanol extract of *Achyranthes Aspera* was evaporated and to this residue dilute HCl was added, shaken well and filtered. With filtrate, following tests were performed for the detection of various constituents using conventional protocols, namely:

A) The Mayer's, Wagner, Hagner's and Dragendorff's test for alkaloids B) Foam and hemolytic tests for saponins C) Salkowski's test and Lieberman-Buchard test for steroids/triterpenoids D) Gelatin test, ferric chloride test for tannins E) Shinoda test for flavonoids and F) Molisch's test for carbohydrates.¹⁰

Experimental animals

Wistar albino mice of either sex weighing 35-40gms, bred in Central Animal House (CAH) facility of the SKN Medical College, Pune were used for the 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 OECD (Organization for Economic Co-Operation and Development) Guidelines No. 423.

Test methods

Animals were divided into various groups such that 6 animals were in each group. Animals treated with 5% Gum Acacia Suspension (0.1 ml p.o.) served as control, Diazepam (2mg/kg i.p.)¹¹ served as standards and animals in test group were treated with *A. Aspera* ethanolic extract (400 mg/ kg i.p.) respectively. Each animal was treated with respective drug 30 min before experimentation. Following are the details of experiments performed:

1. Rota-rod performance: Dunham and Miya (1957) suggested that neurological depression in mice/rats could be evaluated by testing their ability to remain on a Rota-rod. Rota-rod apparatus (Dolphin make) is a four panel techno device with timer. Animals (4 at a time) were placed on rod rotating at 20-25 rpm speed. Only the mice, which 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 CNS depression.^{12,13}
2. Actophotometer test: The animal locomotor behavior was monitored using Actophotometer, described by Dews P.B. (1953). Actophotometer (Dolphin make) provided with a digital counter, photocell and a light source were used to measure locomotor activity (horizontal movement) of animals. Each animal was placed in Actophotometer for 5 minutes and basal activity score was recorded for all animals. Each animal was treated with respective drug and activity score was recorded after 30 min and 1hr. Decreased activity score was taken as index of CNS depression.¹⁴⁻¹⁶
3. Open field test (OFT): Open field apparatus was designed as described by Gray and Lalji (1971) with few modifications. Dimensions were 50cm x 50cm x 40cm made up of plywood open from top and bottom kept on white table top; sur-

face 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) 1hr before the trial. 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.¹⁷

Statistical Analysis

Data was analyzed by ANOVA test followed by Dunnett's test. All the results were expressed as Mean (\pm SEM). $P < 0.05$ was considered significant. Percent reduction in activity score and fall off time calculated with reference to respective basal recordings.

Results

- 1) *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)
- 2) *Acute toxicity study*: The results of acute toxicity study showed no clinical signs of toxicity and mortality in the *A.Aspera* treated animals. Lethal dose was calculated and was found to be more than 2000 mg/kg. 1/5th of this lethal dose (400 mg/kg) was taken as effective dose for the study.
- 3) *Rota-rod method and Actophotometer test*: Diazepam (2mg/kg) and EEAA (400mg/kg) treated groups showed significant CNS depressant activity when compared to control however this depression was less with EEAA treated group than diazepam treated group. (Table-2)
- 4) *Open field Test*: Diazepam (2mg/kg) and EEAA (400 mg/kg i.p.) significantly ($p < 0.001$) exhibited anxiolysis evidenced by increased ambulation, rearing and preening at the same time decreased defecations compared to control. (Table-3)

Table 1: Phytochemical screening of extract (EEAA)

Sr. No.	Chemical Constituents	Test	EEAA
1.	Test for alkaloids	Mayer's test	+
		Wagner's test	+
		Hager's test	--
		Dragendroff's test	+
2.	Test for carbohydrates	Molisch's test	+
3.	Test for saponins	Foam test	+
		Haemolytic test	--
4.	Test for triterpenoids/steroids	Salkowski's test	+
		Liebermann Burchard test	+
5.	Test for flavonoids	Shinoda test	--
6.	Test for tannins	Ferric chloride test	--
		Gelatin test	--

+ Present, -- Absent

Table 2: Activity score in Actophotometer method and mean fall off time using Rota-rod method:

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

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean ± S.E.M. (ANOVA test followed by Dunnett's t-test). Significance variation against control at, * p<0.001. Percent reduction in parenthesis calculated with reference to basal score.

Table 3: Mean score using open field performance method:

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

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean ± S.E.M. (ANOVA test followed by Dunnett's t-test). Significance variation against control at, *p<0.02, **p<0.001.

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.¹⁸

GABAergic activity in the brain can be increased by three ways, by GABA agonists; barbiturates and benzodiazepines directly increase inhibitory chloride conductance and/or upregulate the effect of synaptically released GABA on the GABA_A receptor respectively.¹⁹ Diverse drugs which are used in various psychological 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 receptors²⁰, 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 barbiturates²¹; as consequence of Ca⁺⁺ channel blockade, Ca⁺⁺ entry into presynaptic nerve terminals is blocked, leading to inhibition of release of excitatory neurotransmitters such as glutamate. This results in net reduction of excitatory synaptic transmission.

In present 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. An-

other important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS; decreased activity results from depression of the central nervous system.²² The extracts significantly decreased the locomotor activity as observed in the results of the Actophotometer test. (Table-2)

Moreover, anxiolysis was studied by measuring external signs like ambulation, rearing, preening and defecation in open field test. OFT 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.²³ 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 induced suppression of behavior.^{23,24}

As mentioned in results, EEAA possesses various phytochemical substances such as triterpenoid saponins (A & B) possessing oleanolic acid as aglycone, alkaloid achyranthine, water soluble base betaine and steroids. CNS depressant and anxiolytic activity of EEAA reflected in results of this study is 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,²⁵ saponins²⁶ and flavonoids^{25,26}

from their phytochemical analysis. 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^{27,28} 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.^{25,26}

Conclusion

From the results obtained, 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 our earlier studies may complement each other and thus, may be used in variety of painful and excitatory conditions.

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