

EFFECT OF *ACHYRANTHES ASPERA* LINN ON MODIFIED FORCED SWIMMING IN RATS

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Summary

Achyranthes aspera Linn is an annual biennial plant known as Prickly chaff flower. The present study is designed to investigate the antidepressant-like effect of the plant by using modified forced swimming test (MFST). The methanolic extract of *A. aspera* (MEAA) is used to investigate the putative psychotherapeutic effects of this plant as antidepressant. The animals were randomly allocated into five groups of six animals each. Group I served as vehicle control, group II, III, IV received *A. aspera* p.o. at the dose rate of 100, 300 and 600 mg/ kg body wt. and group V received the standard drug Desipramine hydrochloride @ 30 mg/kg or Citalopram @ 15 mg/kg body wt. i.p. as standard drug. Following administration, the immobility time in MFST in rats were decreased significantly ($P<0.01$) in *A. aspera* treated group as compared to the control group. There was significant ($P<0.01$) increase in the climbing behavior in the treated groups as well as in the DMI treated group compared to the control group in a dose dependant manner without much effect on the swimming component. The study revealed antidepressant-like activity of *A. aspera*. The pattern of effects observed in the MFST suggests the involvement of the noradrenergic neurotransmitter system. However, further studies are needed to understand the precise mechanism of its action.

Keywords: *Achyranthes aspera*, antidepressant, Citalopram, Desipramine hydrochloride, modified forced swimming test.

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Introduction

At present, depression is one of the most frequent psychiatric conditions commonly encountered, but till date the efficacy of antidepressant drugs are very limited due to their side effects. Hence, need for newer, better tolerated and more efficacious treatment is remaining high. The plant extracts being more efficacious, free from undesirable side effects compared to their pure active principle revalidated the therapeutic benefits of herbs due to totality of constituents rather than the single molecule. In spite of phenomenal development of the synthetic drug industry and antibiotics, medicinal plants still constitute an important part of pharmacopoeias in both the developed and developing countries. These plants are important elements of traditional medicine in virtually all cultures. Therefore, herbal therapies could be considered as alternative/complementary medicines. The search for novel pharmacotherapy from medicinal plants for psychiatric illness has progressed significantly in the past decade [1]. This is reflected in large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models.

The forced swimming test (FST), described originally [2] is widely used as an animal model to assess the antidepressant action [3]. The test is relatively simple to perform and has ability to measure behavioral effects common to antidepressant treatments that have diverse pharmacological and physiological effects. The test can also distinguish drugs that are not antidepressant. Nonetheless, almost all members of an important group of antidepressants-the selective serotonin reuptake inhibitors (SSRIs) - had been found not to be active in classic Porsolt's test in rats [3]. Therefore, certain procedural modifications had to be introduced in order to measure the behavioral effects of those drugs [4, 5]. The MFST, besides allowing to observe the effects of SSRIs in rats was found to be very useful in distinguishing that group of compounds from the compounds acting via the inhibition of noradrenalin reuptake. The latter group, as for example reboxetine or desipramine, increases rather climbing, while SSRIs rather increases swimming behavior [6]. Thus, SSRIs such as Fluoxetine, paroxetine, or sertraline decrease immobility and increase swimming [7, 8], while the selective noradrenergic and dopaminergic reuptake inhibitors, like DMI, maprotiline, and bupropion decrease immobility accompanied by an increase in climbing behavior [9]. The MFST differentiated swimming behavior which was sensitive to SSRIs, 5-HT receptor agonists and climbing behavior which was sensitive to tricyclic antidepressants and drugs with selective effects on catecholamine transmission [5].

Achyranthes aspera L., (Prickly Chaff flower) belonging to the family Amaranthaceae, locally known as Apang, is an annual, biennial, lower portion perennial erect under shrub or rather stiff herb growing upto 0.3 to 1.0 meter in height [10]. It grows throughout the world in tropical and warmer regions. Yunani doctors and local *kabiraj* use the stem, leaves and fruits as a remedy for piles, renal dropsy, pneumonia, cough, kidney stone, skin eruptions, snake bite, gonorrhoea, and dysentery etc. Various extracts of this plant reveal presence of 27-Cyclohexyheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one, a long chain alcohol and 17-pentatriacontanol alkaloid, b-sitos-terol and spinasterol [11]. The plant has antimicrobial [12] antitumor [13] and anti-inflammatory [14] activity. The ethanolic extract of *A. aspera* possesses antiimplantation and abortifacient activity [15].

We have reported excellent antibacterial, wound healing, analgesic, anti-inflammatory and immunosuppressant properties of this plant (Communicated). Antidepressant activity of this plant is not yet reported. We have reported antidepressant like effect of MEAA in all the classic models of depression, where it was found to possess significant antidepressant like activity comparable to the standard tricyclic antidepressant DMI [16]. In order to study the mechanism of action at neurotransmitter level, whether it acts via serotonergic or noradrenergic pathway, the present study was undertaken using MFST in rats and two standard drugs *viz.* Desipramine hydrochloride (DMI), a noradrenalin reuptake inhibitor and citalopram (CIT), a selective serotonin reuptake inhibitor were taken for comparing the mechanism of action at neurotransmitter level.

Materials and Methods

Plant material

The leaves of the plants were collected during the month of Feb - June, 2008 from the medicinal garden of the Department of Pharmacology & Toxicology, College of Veterinary Science, Khanapara, Guwahati and were identified by Taxonomist of NEIST, Jorhat, Assam; a voucher specimen (No AAU/CVSC/PHT/01) was deposited.

Preparation of methanolic extract of A. aspera

Fresh leaves of *A. aspera* were cleaned and washed thoroughly with water and rewashed with distilled water. Washed fresh leaves were dried under shade in clean and dust free environment and grinded and stored in air tight container. About 250 g of powdered leaves were soaked in 1000 ml methanol for 72 hours in beaker. The mixture was stirred every 18 hours using a sterile glass rod. The solvent was filtered every 3rd day using muslin cloth and Whatman filter paper no 1. The filtrate obtained was concentrated in Rotary Evaporator (Equitron, Roteva) at 50^o- 60^oC under reduced pressure leaving a dark brown residue. The *A. aspera* extract thus obtained was transferred to a Petri dish and kept over water bath (50^oC) until the solvent gets completely evaporated. It was stored in air tight container at 4^oC for further use. Recovery was 4.98 % (w/w).

Phytochemical screening

The freshly prepared methanolic extract was subjected to standard screening tests for various constituents [17].

Determination of LD₅₀

The LD₅₀ of *A. aspera* was estimated by the employment of up-and-down stair case method in mice [18]. Doses were adjusted by a constant multiplicative factor viz. 4, for this experiment. The dose for each successive animal was adjusted up and down depending on the previous outcome. The acute toxicity and gross effect of methanolic extract of *A. aspera* was studied in albino mice by using 1/2 LD₅₀ dose. A total of six numbers of male albino mice were selected for the experiment. Animals were observed hourly for six hours and again after 24 hours. The parameters for motor activity and gross effect were determined after administration of *A. aspera* orally at a dose of 2.5g /kg body wt.

Animals

Healthy Sprague Dawley rats of either sex, approximately of same age (6-8 weeks), weighing between 150-250 g were used for the study. They were housed under controlled conditions of temperature (25±3^oC) relative humidity (50±5%), 12:12 hour light-dark cycle and free access to food and water. Animals were housed individually in polypropylene cages containing sterile paddy husk bedding.

The animals were randomly allocated into five groups of six animals each. Group I served as vehicle control, group II, III, IV received *A. aspera* p.o. at the dose rate of 100, 300 and 600 mg/kg body wt. and group V received the standard drug DMI @ 30 mg/kg or Citalopram @ 15 mg/kg body wt. p.o. The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee (770/03/ac/CPCSEA/FVSc, AAU/IAEC/06/21). The animals were fasted for 14 hours before tests to achieve better drug absorption through gastrointestinal tract.

Drugs

Desipramine hydrochloride (DMI) was obtained from Sigma–Aldrich Inc. (St. Louis MO.USA) and Citalopram (CIT) from Lundbeck were used in the study. Both were dissolved in distilled water and administered i.p.

Modified forced swimming test

In order to assess the antidepressant activity of plant extract, the modified Porsolt test [2] was conducted. An important group of anti depressants, the selective serotonin reuptake inhibitors (SSRIs) had been found not to be active in classic Porsolt's test in rats [3]. Hence, certain procedural modifications had to be introduced to measure the behavioral effects of those drugs [4, 5]. The modification that have been introduced, concerned the water depth- in the modified procedure, it was increased from 15-18 cm to 30 cm resulting in worse support of floating animals and not only the immobility time has been measured, but also the mode of active behavior (climbing, swimming, diving or head shaking) [19]. In the first trial, the rats which have not yet treated were forced to swim for 15 min in a glass cylinder (21cm in diameter) filled with water (23-25⁰C) to a 30 cm depth. The next day, animals received different doses of the plant extracts (100 to 600 mg/kg p.o.). One hour after feeding, the animals were again placed inside the cylinders filled in the same way as during the pre-test and observed for 5 min. Upon removal from water the animals were towel dried before placing them to the respective cage.

Behavioral Scoring: A time sampling technique was employed to score three different behaviors [19]. During the test session, the following behaviors in rats were recorded by observers blind to the treatment conditions.

(1) immobility - floating in the water without struggling, and doing only those movements necessary to keep the head above the water; (2) swimming - showing moderate active motions around in the cylinder, more than necessary to merely keep the head above water; and (3) climbing - presenting active vigorous movements with forepaws in and out of the water, usually directed against the walls. Upon removal from the water, rats were towel-dried and finally returned to their home cage.

Statistical analysis

The results of various parameters were subjected to statistical analysis as per standard method [20].

Results

Qualitative phytochemical screening of the MEAA revealed the presence of alkaloid, steroid and triterpenes by various tests.

In acute toxicity study, there was no change of motor activity and gross behavior during 24 hours of observation and the plant extract was found to be safe up to 5g/kg body wt. p.o. The low toxicity of the plant extract suggests that MEAA is relatively safe and devoid of acute toxicity.

In MFST three criteria were measured for antidepressant activity *viz* climbing, immobility and swimming. The MEAA when administered orally at dose of 100, 300 and 600 mg/kg significantly ($P < 0.01$) increased duration of climbing behavior (59.79, 78.10 and 124.18 sec) in a dose dependent manner and is presented in Table 1. For DMI and CIT, climbing behavior was 142.91 and 63.83 sec, respectively as shown in Figure 1.

Administration of the MEAA significantly decreased duration of immobility time (141.65, 93.26 and 69.72) in a dose dependent manner at the same doses and shown in Figure 2. The immobility time was 75.95 and 77.20 sec for DMI and CIT, respectively.

At the same dose level, swimming was recorded as 98.56, 128.64 and 106.10 sec for MEAA and 81.16 and 158.97 sec for DMI and CIT, respectively is depicted in Figure 3.

Table 1. Effect of methanolic extract of *A. aspera* in modified forced swimming test in rats

Sl no	Treatment groups	Dose (mg/kg.p.o.)	Climbing (Sec)	Immobility (Sec)	Swimming (Sec)
Group I	Control	10	62.13 ^a ±1.28	148.23 ^e ±1.13	89.64 ^b ±1.04
Group II	<i>A. aspera</i>	100	59.79 ^a ±1.67	141.65 ^d ±1.46	98.56 ^c ±2.04
Group III	<i>A. aspera</i>	300	78.10 ^b ±1.80	93.26 ^c ±1.30	128.64 ^e ±2.75
Group IV	<i>A. aspera</i>	600	124.18 ^c ±1.30	69.72 ^a ±0.68	106.10 ^d ±0.79
Group V	DMI	30 (i.p.)	142.91 ^d ±1.70	75.95 ^b ±1.34	81.16 ^a ±1.16
Group VI	CIT	15 (i.p.)	63.83 ^a ±1.05	77.20 ^b ±1.64	158.97 ^f ±1.80

(n=6, mean ± SE)

Means bearing same superscript don't differ significantly ($P < 0.01$)

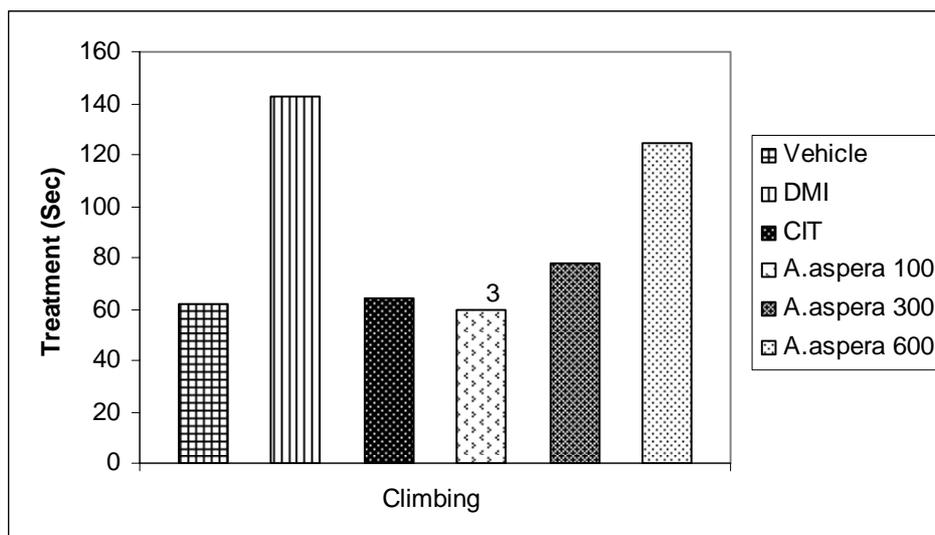


Fig 1. Effect of methanolic extract of *Achyranthes aspera* on climbing behavior in modified forced swimming test in rats

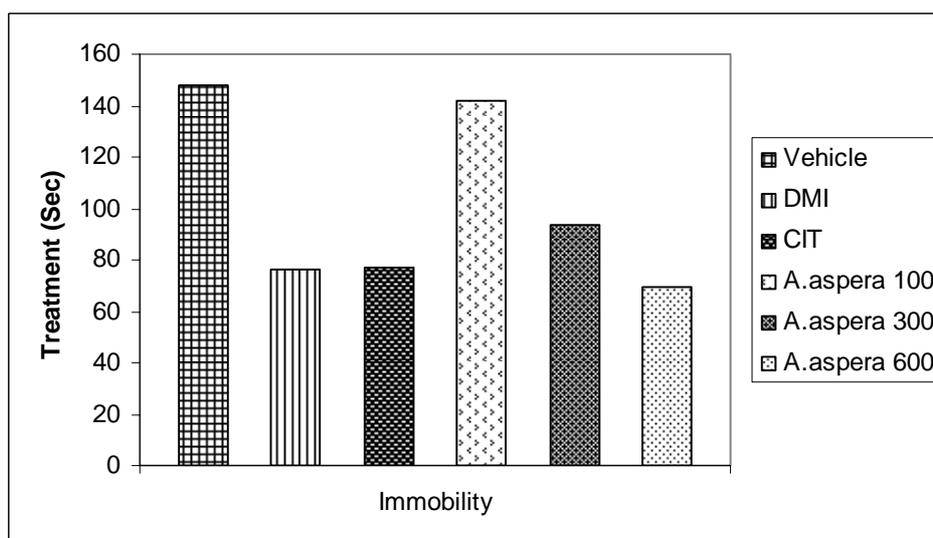


Fig 2. Effect of methanolic extract of *Achyranthes aspera* on immobility time in modified forced swimming test in rats

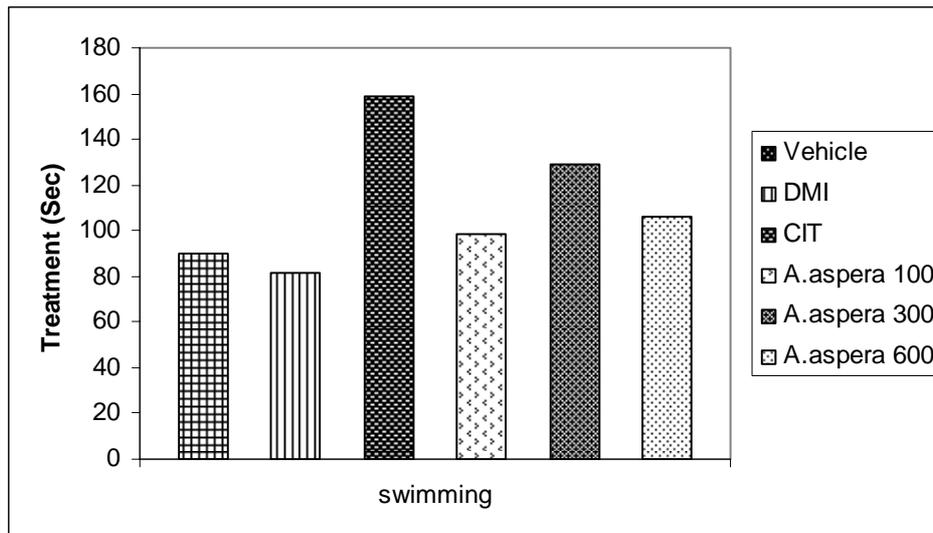


Fig 3. Effect of methanolic extract of *Achyranthes aspera* on swimming behavior in modified forced swimming test in rats

Discussion

The present study characterized the antidepressant-like effect of the MEAA Linn using MFST. The behaviors selected for scoring were climbing, swimming as well as immobility as per modified forced swimming method, which was slightly different from the classical Porsolt's model.

The results demonstrated that administration of MEAA, significantly ($P < 0.01$) decreased the duration of the immobility time in MFST in rat in a dose dependent manner as compared to the control group, indicating the antidepressant activity of *A. aspera*. The extract was able to reduce immobility time and simultaneously enhance active behaviors like climbing. Acute administration of most of the antidepressants decreases immobility [4]. The immobile behavior is thought to reflect either a failure to persist in escape-directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli [21]. A broad spectrum of antidepressants selectively prevents the development of behavioral immobility in the FST [22]. In a study, the hydroethanolic extract of the Brazilian medicinal plant *Trichilia catigua* produces antidepressant-like effects in the forced swimming model in both mice and rats suggesting the antidepressant-like effect of the plant [23]. In our study, reduction of immobility time in case of treatment groups were comparable to that observed after i.p. administration of the reference antidepressant drug DMI without any effect on the swimming component. Similar results were reported by other workers [6] that DMI, the noradrenaline reuptake inhibitor significantly augmented the climbing behavior without any effect on the swimming component. The finding is also in agreement with the report by another author [24] who indicated that drugs affecting noradrenergic system modify rather climbing behavior without any significant change in the swimming in MFST. Another study [25] conducted to evaluate the possible antidepressant action of natural estrogen 17 β estradiol ethynyl estradiol and diethylstilbestrol also confirms the antidepressant-like effect of fluoxetine (FLX) and DMI. The reduction in immobility induced by FLX was accompanied by increased swimming, while in case of DMI an increase in climbing behavior is observed. CIT, which belongs to SSRIs [6], was found to increase swimming behaviour at the cost of climbing and immobility

in the MFST in rats when compared with the effect of DMI at the same dose. Likewise, in our study, CIT, another reference anti depressant drug, swimming behaviour was increased in comparison to the control group. Therefore, the present study revealed that MEAA possess significant antidepressant like activity which was similar to DMI - noradrenaline reuptake inhibitor. Furthermore, it was reported that the presence of monoterpenoid compounds like carvone and thujone in the essential oil of *Aloysia polystachya* are responsible for their anxiolytic and antidepressant like effects [26]. Likewise, the MEAA, which contains triterpenes as one of its constituents, may be responsible for its antidepressant like effect.

The antidepressant-like effect of *Achyranthes aspera* Linn in rats using MFST has not yet been reported so far. Although other kinds of studies are obviously necessary to elucidate the mechanism of action of *A. aspera* in the CNS, the pattern of effects observed in the MFST suggests the involvement of the noradrenergic neurotransmitters system on its antidepressants like effect. However the precise mechanism underlying this change still requires further investigation to confirm and extend these results before the application in human.

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