

ANTIDEPRESSANT-LIKE EFFECTS OF THE METHANOLIC EXTRACT OF  
*ACHYRANTHES ASPERA* LINN. IN ANIMAL MODELS OF DEPRESSION

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Summary

The antidepressant activity of methanolic extract of the leaves of *Achyranthes aspera* was studied using various animal models of depression viz. forced swimming test in mice, forced swimming test in rats and tail suspension test in mice. The methanolic extract of *Achyranthes aspera* at a dose rate of 100, 300 and 600 mg/kg, distilled water as control and the standard drug Desipramine hydrochloride (DMI) @ 30 mg/kg body wt, p. o. were used to investigate the putative psychotherapeutic effects of this plant as antidepressant. Following its administration, the immobility time in forced swimming test in mice was decreased significantly ( $P < 0.01$ ) in *A. aspera* (@ 300 and 600 mg/kg body wt.) treated group as compared to the control group. But in case of rat, administration of the plant extract, significantly ( $P < 0.01$ ) decreased the duration of the immobility time in forced swimming test in a dose dependant manner from 100 to 600 mg/ kg body weight p.o. In the tail suspension test in mice also there was significant ( $P < 0.01$ ) decrease in the duration of the immobility time in the treated as well as the standard group compared to the control group in a dose dependant manner.

The study thus revealed antidepressant-like activity of leaves of *Achyranthes aspera* as evidenced by different animal models of depression.

**Keywords:** Antidepressant, *Achyranthes aspera*, forced swimming, tail suspension.

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### Introduction

Use of herbal extract in place of crude herbs started with the aim to control quality and precise dosage for better results. 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. *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 [1]. It grows throughout the world in tropical and warmer regions [2, 3]. 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, dysentery etc. [4, 5]. Various extracts of this plant reveals presence of 27-Cyclohexyheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one [6], a long chain alcohol and 17-pentatriacontanol [7], alkaloid [8], b-sitos-terol and spinasterol [9]. The plant has antibacterial [1], antitumor [10], anti-inflammatory [11], abortifacient activity and increases pituitary and uterine wet weights in ovariectomised rats [12] and reproductive toxicity in male rats [13].

Depression is a frequent psychiatric condition commonly encountered but till date the efficacy of antidepressant drugs are very limited. So, there is a need for newer, better tolerated and more efficacious treatment. Therefore herbal therapies should be considered as alternative/complementary medicine. The present study was undertaken to test the antidepressant activity of the methanolic extract of leaves of *Achyranthes aspera* in laboratory animals.

### Materials and methods

#### *Plant material*

The leaves of the plants were collected from the medicinal garden of the Department of Pharmacology, College of Veterinary Science, Khanapara during the month of Feb - June, 2008, identified by Taxonomist of NEIST, Jorhat, Assam and a voucher specimen (AAU/CVSC/PHT/ 01) was deposited.

#### *Preparation of methanol extract*

Fresh leaves of the plant were cleaned from extraneous materials, shade dried, powdered mechanically, weighed and stored in air tight container. About 250 g of powdered material was soaked in 1000 ml methanol for 72 hours in beaker and mixture was stirred every 18 hour using a sterile glass rod. Filtrate was obtained 3 times with the help of Whatman filter paper no 1 and the solvent was removed by rotary evaporator under reduced pressure at  $<45^{\circ}$  C temperature leaving a dark brown residue which was stored in air tight container at  $4^{\circ}$  C until use. Recovery was 6.89 %.

#### *Phytochemical screening*

The methanolic extract of *A. aspera* was tested for the presence of various active principles as per the standard procedure [14].

#### *Determination of LD<sub>50</sub>*

The  $LD_{50}$  of the *Achyranthes aspera* was estimated by the employment of up-and - down stair case method in mice [15]. 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 crude methanolic extract of *Achyranthes aspera* was studied in albino mice by using  $1/2 LD_{50}$  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 *Achyranthes aspera* orally at a dose level of 2.5g /kg b. wt.

**Drugs**

Desipramine hydrochloride (DMI) was obtained from Sigma-Aldrich Inc. (St.Louis MO.USA). DMI was dissolved in distilled water and administered orally at the rate of 30 mg/ kg body weight.

**Animals**

Healthy Sprague Dawley male rats approximately of same age, weighing between 180-200 g and male albino mice weighing 18-22 g were used for the study. The animals were group housed in polypropylene cages under controlled conditions of temperature ( $21\pm 2^{\circ}\text{C}$ ), humidity ( $50\pm 5\%$ ), 12/12 hours of light-dark cycle and free access to standard food pellets and water was provided *ad libitum*. The animals were fasted for 14 hours before the study to achieve better drug absorption through gastrointestinal tract.

The animals were randomly allocated into five groups of six animals each. Group I served as vehicle control, group II, III and IV received *Achyranthes aspera* p.o. at the dose rate of 100, 300 and 600 mg/kg body weight and group V received the standard drug DMI @ 30 mg/ kg body weight p.o. The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee.

**Forced swimming test in mice** [16, 17]

Forced swimming test in mice is a behavioral despair test. The mice were placed individually in glass cylinders (20 cm height  $\times$  10cm diameter) containing 10 cm depth of water at  $25^{\circ}\text{C}$  for 6 minutes. The duration of immobility was recorded during the last 4 minutes of the 6 minutes test period by observers blind to the treatment conditions. Mice were considered to be immobile when floating motionless or making only those movements necessary to keep its head above water. Single administration (*p.o.*) of *A. aspera* extract (100, 300 and 600 mg/kg), DMI (30 mg/kg) or distilled water was given 1 hour prior to the test.

**Forced swimming test in rats** [18, 19].

Forced swimming test in rats was conducted as per standard method. The rats were placed individually in glass cylinders (40 cm height  $\times$  18cm diameter) containing 22 cm depth of water at  $28^{\circ}\text{C}$ . The procedure consisted of a pre-swimming test and swimming test separated by 24 hours. During the pre-swimming, rats were placed in the cylinders for 15 minutes. Rats were removed from the cylinders, dried with a cloth towel and warmed with the electric heater before they were placed back to home cage. The rats were treated with *A. aspera* extract (100, 300 and 600 mg/kg); DMI (30 mg/kg) or distilled water *p.o.* 1 hour prior to test and placed in the cylinder again. Immobility time was recorded during the last 4 minutes of the total 6 minutes test period by observers blind to the treatment conditions. Rats were considered to be immobile when floating motionless or making only those movements necessary to keep its head above the water surface.

**Tail suspension test in mice** [20]

The tail suspension test was performed according to the standard method. The mice were individually suspended 7.5 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. Immobility duration was recorded for the last 4 minutes of the total 6 minutes test period by observers blind to the treatment conditions. Mice were considered to be immobile when they hung passively or were completely motionless. Single oral administration of *A. aspera* extract (100, 300 and 600 mg/kg); DMI (30 mg/kg) or distilled water was given 1 hour prior to the experiment.

**Statistical analysis**

The statistical analysis was carried out as per standard statistical method [21].

## Results

Phytochemical screening of the methanolic extract of the plant revealed the presence of alkaloid by Wagner's and Dragendroff's test, steroid by Salkowski's and Lieberman Burchardt's test and triterpenes by Salkowski's and Lieberman Burchardt's test. The plant extract was found to be safe up to more than 5gm/kg body weight, p.o. There was no acute toxicity at  $\frac{1}{2}$  LD<sub>50</sub> i.e. 2.5 g/kg body p.o.

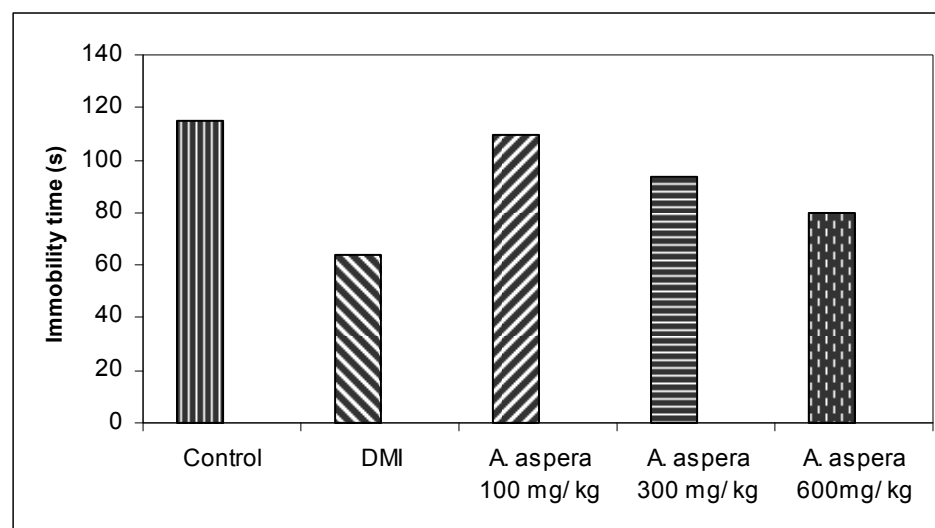
Effects of methanolic extract of *Achyranthes aspera* on the immobility time in forced swimming test in mice at the dose rate of 100, 300 and 600 mg/kg body weight after single oral administration have been presented in Table 1 and Fig 1. The immobility time in forced swimming test in rats at different dose rates after single oral administration are presented in Table 2 and Fig 2. The result of tail suspension test in mice after single oral administration of the plant extract at different doses or vehicle i.e. distilled water as well as the standard drug is depicted in Table 3 and Fig 3.

**Table 1.** Effect of methanolic extract of *Achyranthes aspera* in forced swimming test in mice.

Sl no	Group	Dose (mg/kg p.o.)	Immobility Time (Sec)	Dj
Group I	Control	10	114.83±1.40	
Group II	<i>A. aspera</i>	100	109.53±2.46	1.997 (NS)
Group III	<i>A. aspera</i>	300	93.31±1.48	8.105**
Group IV	<i>A. aspera</i>	600	79.95±1.09	13.268**
Group V	DMI ** P<0.01	30	63.82±1.51	19.207**

The results are expressed as mean ± SEM of n=6 observations \*\*P<0.01 as compared to control. (One way ANOVA followed by Dunnett's test). NS not significant.

$$D_{4, 25; 0.01}=3.00, \quad D_{4, 25; 0.05}=2.27$$



**Fig 1.** Effect of methanolic extract of *Achyranthes aspera* on the immobility time in forced swimming test in mice.

Table 2. Effect of methanolic extract of *Achyranthes aspera* in forced swimming test in rats.

Sl No.	Group	Dose (mg/kg p.o.)	Immobility Time (Sec)	Dj
Group I	Control	10	119.56±2.46	-
Group II	<i>A. aspera</i>	100	105.76±1.89	5.572**
Group III	<i>A. aspera</i>	300	71.44±0.95	19.433**
Group IV	<i>A. aspera</i>	600	51.73±0.92	27.392**
Group V	DMI	30	66.84±0.94	21.292**

The results are expressed as mean ± SEM of n=6 observations \*\*P<0.01 as compared to control. (One way ANOVA followed by Dunnett's test).NS not significant.

\*\* P<0.01  
 D<sub>4, 25; 0.01</sub>=3.00  
 D<sub>4, 25; 0.05</sub>=2.27

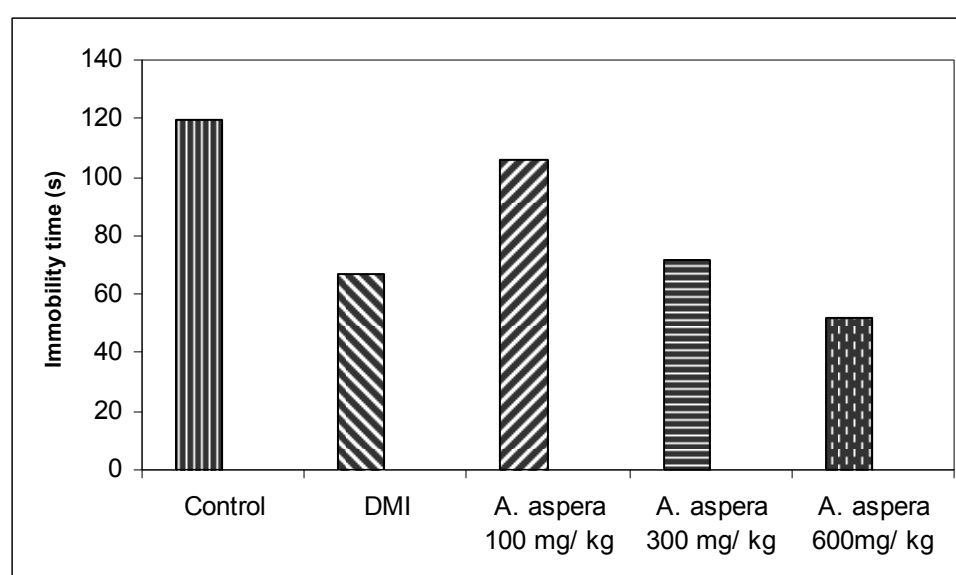
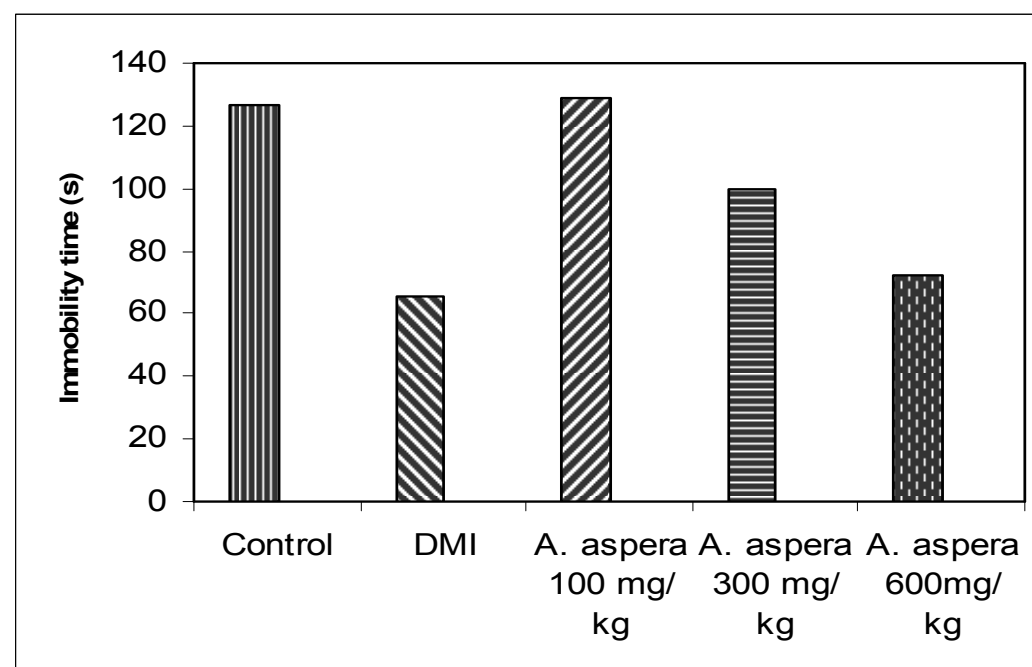


Fig 2. Effect of methanolic extract of *Achyranthes aspera* on the immobility time in forced swimming test in rats.

**Table 3.** Effect of methanolic extract of *Achyranthes aspera* in tail suspension test in rats.

Sl No	Group	Dose	Immobility Time (Sec)
Group I	Control	10	126.67 <sup>a</sup> ±1.28
Group II	<i>A. aspera</i>	100	128.66 <sup>a</sup> ±1.63
Group III	<i>A. aspera</i>	300	99.65 <sup>b</sup> ±1.60
Group IV	<i>A. aspera</i>	600	72.30 <sup>c</sup> ±1.21
Group V	DMI	30	65.51 <sup>d</sup> ±1.90

The results are expressed as mean ± SEM of n=6 observations \*\*P<0.01 as compared to control. (One way ANOVA followed by Dunnett's test). NS not significant. Means bearing same superscript don't differ significantly (P< 0.01)

**Fig 3.** Effect of methanolic extract of *Achyranthes aspera* on the immobility time in tail suspension test in mice.

### Discussion

In the present study, methanolic extract of *Achyranthes aspera* was administered orally at the dose rate of 100, 300 and 600 mg/kg body weight to investigate the putative psychotherapeutic effects of this plant as antidepressant. Following the administration, the immobility time in forced swimming test in mice was decreased significantly ( $P < 0.01$ ) in *A. aspera* (@ 300 and 600 mg/kg body wt.) treated as well as standard group (Fig 1) with simultaneous increase in the swimming time. The test protocol followed in our study by Porsolt *et al.* [16, 17] is a valid test for a broad spectrum of antidepressants, mainly including tricyclics and monoamine oxidase inhibitors, which significantly decrease immobility time in FST [22]. Acute administration of the plant extract, significantly ( $P < 0.01$ ) decreased the duration of the immobility time in forced swimming test in rat in a dose dependant manner as compared to the control group (Fig 2), indicating the antidepressant activity of *A. aspera*. The tail suspension test in mice also significantly ( $P < 0.01$ ) decreased the duration of the immobility time in the test groups compared to the control group which was also dose dependant (Fig 3), corroborating our previous findings of forced swimming test in mice and rats enlightening the antidepressant activity of *A. aspera*.

Initial hypothesis of depression was formulated about 40 years ago, proposing that the main symptoms of depression were due to the functional deficiency of cerebral monoaminergic transmitters such as norepinephrine (NE), 5-HT, and/ or dopamine (DA) located at synapses [23]. In a study the ethanolic extract of the Chinese herbal plant *Xiaobuxin-Tang* when administered at doses of 300 and 600 mg/ kg (p.o.) significantly decreased the duration of immobility time in a dose dependant manner in mice and rat forced swimming tests as well as the extract at a dose of 600 mg/ kg had the same effect in mice tail suspension test [24] suggesting the antidepressant like effect of the plant. Evaluation of the antidepressant effect of alcoholic extract of *Kaempferia parviflora* in aged rat showed decreased immobility time with the increase in swimming time in forced swimming test [25]. Acute administration of most antidepressants decreases immobility [26]. The present result also shows that the methanolic extract of *Achyranthes aspera* when administered orally was effective in reducing the immobility time revealing its significant antidepressant-like effects.

Thus further study in identification and elucidation of the active constituents in this plant may provide useful leads to the development of new and effective plant-based antidepressant drugs.

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### References

1. Aziz A, Rahman M, Mondal AK, et al. 3-Acetoxy-6-benzoyloxyapagamide from *Achyranthes aspera*. *Pharmaceutical J* 2005;4:1816-1820.
2. Hooker JD. The flora of British India, L. Reeve & Co Ltd Kent, 1885:713-730.
3. Prain D. Bengal plants, Botanical survey of India, Calcutta, 1963:646-655.
4. Ghani A. Medicinal plants of Bangladesh, 2nd ed. Asiatic Society of Bangladesh, Dhaka, 2003:71-72.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants, Bishen Singh, Mahendra Pal Singh, New Conaught Place, Dehra Dun, India, 1980:2065-2068.

6. Misra TN, Singh RS, Pandey HS, Prasad C, Singh BP. Two long chain compounds from *Achyranthes aspera*. Phytochemistry 1993; 33:221-223.
7. Misra TN, Singh RS, Pandey HS, Prasad C, Singh BP. Antifungal essential oil and a long chain alcohol from *Achyranthes aspera*. Phytochemistry 1992;31:1811-1812.
8. Kapoor VK, Singh H. Isolation of betaine from *Achyranthes aspera*. Indian J Chem 1966;4:461-463.
9. Ali MK, Rahman MA, Quader MA. Sterols from the leaves of Apang (*Achyranthes aspera*). Dhaka Univ J Sci 2004;52:1-6.
10. Chakraborty A, Brantner A, Mukuinaka T, et al. Cancer chemopreventive activity of *Achyranthes aspera* leaves on Epstein-barr virus activation and two stage mouse skin carcinogenesis. Cancer letters 2002;177:1-5.
11. Vetrichelvan T, Jegadeesan M. Effect of alcohol extract of *Achyranthes aspera* Linn. on acute and sub-acute inflammation. Phytother Res 2003;17:77-79.
12. Workineh S, Eyasu M, Legesse Z, Asfaw D. Effect of *Achyranthes aspera* L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones. African Health sciences 2006;6:108-112.
13. Sandhyakumary K, Bobby RG, Indira M. Impact of feeding ethanolic extracts of *Achyranthes aspera* Linn. on reproductive functions in male rats. Indian J Exp Biol 2002;40:1307-1309.
14. Harborne JB. Phytochemical methods-Guide to modern techniques of plant analysis, 2<sup>nd</sup> ed. Chapman and Hall India, 1991;653.
15. Bruce RD. An up-and-down procedure for acute toxicity testing. Fundam Appl Toxicol 1985;5:151-157.
16. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977;229:327-336.
17. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in rats and mice: strain differences and the effects of imipramine. Eur J Pharmacol 1978;51:291-294.
18. Porsolt RD, Le Pichon M, Jalfre M. Depression: A new animal model sensitive to antidepressant treatment. Nature 1977;266:730-732.
19. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379-391.
20. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 1985;85:367-370.
21. Snedecor GW, Cochran WG. Statistical methods, 8th ed. Oxford and IBH Publishing Co New Delhi, 1994.
22. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity?. Psychopharmacology 1988;94:147-160.
23. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 1965;122:509-522.
24. You- zhi Z, Yun-feng LI, Neng- jiang YU, et al. Antidepressant- like effects of the ethanolic extracts of *Xiaobuxin-Tang*, a traditional Chinese herbal prescription in animal models of depression. Chinese Med J 2007;120(20):1792-1796.
25. Wattanthorn J, Pangpookiew P, Sripanidkulchai K, Muchimapura S, Sripanidkulchai B. Evaluation of the anxiolytic and antidepressant effects of alcoholic extract of *Kaempferia parviflora* in aged rats. Am J Agril Bio Sci 2007;2(2):94-98.
26. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev 2005;29:571-625.