Analgesic and Neuropharmacological Investigations of the Aerial Part of *Achyranthes aspera* Linn.

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ABSTRACT

Analgesic and central nervous system depressant activity of the methanol extract of *Achyranthes aspera* Linn. was assessed by using acetic acid induced writhing test, thiopental sodium induced sleeping time determination, hole cross test and open field test in albino mice. The extract (250 and 500mg/kg) showed a dose dependent inhibition of writhing response generation by acetic acid compared to reference drug diclofenac sodium (50mg/kg). Methanol (70%) extract of *Achyranthes aspera* (500 mg/kg) also produced rapid onset and maximized the duration of sleeping time when administered with thiopental sodium. The extract also decreased the motor activity and exploratory behavior of mice in hole cross and open field test. The overall experimental results suggest the analgesic and central nervous system depressant activity of the methanolic extract of *Achyranthes aspera* and justify its use in folkloric remedies.

Key Words: *Achyranthes aspera,* Analgesic activity, Central nervous system depressant activity.

INTRODUCTION

The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success (Akah and Nwambie, 1994). Conventional anti-inflammatory drugs such as steroidal anti-inflammatory drugs (SAID) and nonsteroidal anti-inflammatory drugs (NSAID) are used in the treatment of most of the acute and chronic pain and inflammatory disorders including rheumatoid arthritis. However long-term use of these agents may produce serious adverse effects. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking this into account the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources, the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and antiinflammatory drugs. *Achyranthes aspera* Linn. belongs to the family *Amaranthaceae*, is commonly found as a weed on road side and at waste places throughout Bangladesh and Indian subcontinent (Datta and Mitra, 1953; Ghani, 2003). The plant possesses purgative, diuretic, ecbolic, hypoglycemic, antifungal and antimicrobial activity and used in renal dropsy and general anasarca, piles, colic, pneumonia, cough, kidney stone, boils and snake bites (Ghani, 2003). Leaves are used in leprosy and eczema. Roots are used as astringents to wounds, in abdominal tumor and stomach pain (Ghani, 2003). The benzene extract of the stem bark shows abortifacient activity in the rat (Bhattarai, 1994). Leaf extracts were reported to posses thyroid-stimulating and antiperoxidative properties (Tahiliani and Kar, 2000). The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits (Akhtar and Iqbal, 1991). It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids (Gokhale et al., 2002). The water soluble alkaloid achyranthine isolated from *Achyranthes aspera* possess anti-inflammatory activity (Gokhale et al., 2002). The chloroform extract of the stem led to the isolation of pentatriacontan, 6-pentatriacontanone, hexatriacontane and triacontane (Ali, 1993).

As a part of our ongoing investigations about natural bioactive substances from local medicinal plants of Bangladesh (Alam et al., 2008a, Alam et al., 2008b), in this paper, we reported a study of the analgesic activity and neuropharmacological activities of the plant *Achyranthes aspera.* Biological assays were carried out by acetic acid induced writhing test, thiopental sodium induced sleeping time determination test, hole cross test and open field test.

MATERIALS AND METHODS

Plant

Achyranthes aspera was collected from Gazipur in October 2007, was identified by National Herbarium Bangladesh (Accession no. -DACB 32067).

Preparation of ethanol extracts

Dried ground aeriolar parts (400 gm) were extracted with 95% of methanol in a Soxhlet apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40°C using Buchi rotary evaporator to have gummy concentrate of greenish color extract (yield appx. 5.6%).

Phytochemical screening

The methanol extract of *Achyranthes aspera* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorffs reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulphuric acid. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Animals

Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-30 gm were used for in-vivo pharmacological screening. Mice were collected from the animal research branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were housed in standard environmental conditions and fed with rodent diet and water ad libitum. The departmental animal research ethical committee approved the experimental protocol.

Acetic Acid-Induced Writhing Test.

Antinociceptive response of the extract of *Achyranthes aspera* (200 and 400 mg/kg) was assessed by counting number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) induced by 0.7% acetic acid solution in mice (Koster *et al*, 1959). Number of writhes per animal was counted during 15 min test period, beginning 3 min after the injection of acetic acid. Diclofenac sodium 50 mg/kg b.wt was used as a reference drug.

Thiopental sodium induced sleeping time determination

Pentobarbital induced sleeping time was evaluated according to the method previously described by Turner (1965) and Alam et al., (2007). Thiopental sodium was used instead of pentobarbitone for its short duration of action. Twenty animals were divided into four equal groups containing 5 animals each. The test groups received of *Achyranthes aspera* extracts at the doses of 250 and 500 mg/kg while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, Thiopental sodium (20 mg/kg, i.p., Gonoshasthaya Pharmaceuticals Ltd., Mirzanagar, Dhaka) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental sodium administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of reflex).

Hole cross test

The method was adopted as described by Takagi et al. (1971). A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

Open field test

This experiment was carried out as described by (Gupta et al., 1971).The animals were divided into control and test groups containing five mice each. The test group received *Achyranthes aspera* extract at the doses of 250 and 500 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water).The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120min after oral administration of the test drugs.

RESULTS

Preliminary Phytochemical analysis revealed the presence of flavonoids, tannins, reducing sugars, alkaloids and saponines. Methanolic extracts of *Achyranthes aspera* were found to exhibit good analgesic activity at 500mg/Kg dose level. The methanolic extract produced 34.03% and 81.25% writhing inhibition at oral doses of 250 mg/kg and 500mg/kg body weight of mice whereas the standard diclofenac produced 61.81 % writhing inhibition in mice (Table-1).

Table 1: Effect of the *Achyranthes aspera* methanol extract on Acetic acid induced writhing in mice.

Group	Treatment and Dose	Writhings* (Mean ± SEM)	% of writhing	% of writhing inhibition
Group I	0.7% acetic acid (10mL/Kg)	28.80±0.66	100	0
Group II	Diclofenac sodium (50 mg/Kg)	11.00± 1.92a	38.19	61.81
Group III	Extract of *A. aspera* (250mg/kg**)	19.00± 0.71	65.97	34.03
Group IV	Extract of *A. aspera* (500mg/kg**)	5.40± 0.24a	18.75	81.25

**Administered 45 min before 0.7% acetic acid administration (10 ml/kg, i.p.).
*Counted for 15 min, starting 5 min after acetic acid administration;
[a]$P<0.05$ vs. control, Student's *t*-test; values are mean ±S.E (*N*=5).

Statistical studies revealed that both 250mg/kg and 500mg/kg methanolic extract of *Achyranthes aspera* prolong the duration of the pentobarbital induced sleeping time in mice (Table-2).

Table 2: Effect of the *Achyranthes aspera* methanol extract on Thiopental sodium induced sleeping time determination in mice.

Group	Treatment and Dose	Onset of Sleep (minutes)	Duration of Sleep (minutes)
Group I	Thiopental sodium	7.60± 0.81	82.00±4.64
Group II	Thiopental sodium + Diazepam	6.60± 0.93	178.20±2.27[*a]
Group III	Thiopental sodium + Extract of *A. aspera* (250mg/Kg)	15.20± 0.66[*a]	118.80±8.02[*a]
Group IV	Thiopental sodium + Extract of *A. aspera* (500mg/Kg)	10.20± 0.58	151.80±3.56[*a]

Thiopental sodium was administered via intraperitoneally whereas plant extract was administered through oral rout. Data was collected from the time of drug administration. Values are represented as mean ± Standard error of mean. Differences in means were estimated by means of ANOVA followed by Bonferroni and [a]Dunnett's t post hoc test (N=5) Statistical significance was considered as p< 0.05 in all cases vs. control).

The number of hole crossed from one chamber to another by mice of the control group is increased from 30 minutes to 90 minutes (table-3). Hole cross test of *Achyranthes aspera* 250mg/kg & 500mg/kg dose showed significant decrease of movement from its initial value at 0 to 90 minutes. Open field test of *Achyranthes aspera* 250mg/kg and 500mg/kg dose showed significant decrease of movement from its initial value at zero minute to 120 minutes (Table-4). Initially it was observed that the number of movements of the control group overall increased from 30 minutes to 120 minutes.

Table 3: Effect of *Achyranthes aspera* methanol extracts on Hole cross test in mice

Treatment	Dose (mg/kg, p.o)	Number of Movements			
		0 min	30 min	60 min	90 min
Control		18.40±1.14	12.40±2.07	11.60±2.07	7.80±0.84
Achyranthes aspera *extract*	250	22.60±2.19[*a]	6.00±1.58[*a]	4.20±1.92[*a]	2.40±0.55[*a]
	500	5.60± 1.14[*a]	1.60±0.89[*a]	1.00±0.71[*a]	2.20±1.30[*a]

Plant extract was administered through oral rout. Data was collected after 30 minutes of drug administration. Values are represented as mean ± standard deviation (N=5). General linear model followed by repetitive measures and Bonferroni and [a]Dunnett's t post hoc test. Results were considered significant at $P < 0.05$.

Table 4: Effect of *Achyranthes aspera* methanol extracts on Open field test in mice

Treatment	Dose (mg/kg, p.o)	Number of Movements			
		0 min	30 min	60 min	120 min
Control		115.20±2.17	105.20±2.17	93.80±1.48	89.00±3.08
Achyranthes aspera	250	112.40±9.52*a	60.80±11.56*a	24.40±2.97*a	24.00±7.52*a
Extract	500	100.60±5.59*a	14.60±5.07*a	4.40±1.14*a	0.80±0.84*a

Plant extract was administered through oral rout. Data was collected after 30 minutes of drug administration. Values are represented as mean±Standard deviation (N=5). General linear model followed by repetitive measures and *Bonferroni and [a]Dunnett's t post hoc test. Results were considered significant at $P < 0.05$.

DISCUSSION

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function (Tripathi, 1999). To obtain meaningful results regarding the effect of *Achyranthes aspera* methanolic extracts on the pain management and effect on CNS in mice, a number of methods namely acetic acid induced writhing tests, thiopental sodium-induced hypnosis, open field and hole cross were adopted. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Eddy and Leimback (1953), Koster *et al.* (1959) and Williamson *et al.* (1996) who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. With respect to the writhing test, the research group of Deraedt et al. (1980) described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Hokanson, 1978; Duarte et al., 1988). Thus, the results obtained for the writhing test using acetic acid are similar to those obtained for the edematogenic test using carrageenan. In vivo methods using intact animals are considered to be the best method for investigating the action of drugs on the CNS (Rahman et al., 2006). The most important step in evaluating drug action on the CNS is to observe the behavior of the test animals. Thiopental sodium induced hypnosis test revealed that both extracts, at the doses of 250 and 500 mg/kg body weight, dose dependently induced sleep at a rapid stage as compared to control, and increased the duration of sleep. This is similar with the findings of Fujimori (1995) who proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity (Fujimori, 1995). Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. Anxiety and sedation 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, depression, Parkinson syndrome and Alzheimer's disease (Weinreb et al., 2004). As a rule, there are three ways of increasing GABAergic activity in the brain as follows: GABA agonists, for example, diazepam and phenobarbital, directly increase inhibitory chloride conductances or upregulate the effect of synaptically released GABA on the GABA$_A$ receptor (Petroff, 2002). Diverse drugs which are used in these pathologies might modify the phenomena of the GABA system at the level of the synthesis of GABA mediators, release or re-uptake or metabolism. Thiopental sodium, a barbiturate type of hypnotic agent, when given at appropriate dose, induces sedation or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors (Goodman and Gilman. 2001). Barbiturates potentiates GABA-induced chloride conductance and simultaneously depresses voltage activated Ca++ currents at lower concentration, but at higher concentration, chloride conductance is increased in the absence of GABA (french-Mullen et al., 1993); one consequence of the inhibition of these Ca++ channels could be the blockade of Ca^{++} entry into presynaptic nerve terminals leading to inhibition of the release of excitatory neurotransmitters such as glutamate. This results in net reduction of excitatory synaptic transmission. As shown in the initial part of the present report, the extracts of

Achyranthes aspera possesses various phytochemical substances such as alkaloids, tannins, terpinoids and flavonoids. Many flavonoids were found to be ligands for the γ- aminobutyric acid type A (GABA$_A$) receptors in the central nervous system (CNS); which led to the hypothesis that they act as benzodiazepine-like molecules. This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion (Marder and Paladini, 2002; Johnston, 2005). Electrophysiological experiments with flavone and flavanone derivatives have shown that some of them can modulate GABA-generated chloride currents, either positively or negatively (Goutman et al., 2003; Campbell et al., 2004; Kavvadias et al., 2004; Hall et al., 2005). Due to the increased knowledge of the diversity of GABA$_A$ receptor subtypes, the number of studies with cloned receptors of defined subunit composition has recently risen, and experiments with some natural and synthetic flavones and flavanones have shown that they can modulate γ-aminobutyric acid (GABA)-generated chloride currents, either positively or negatively (Goutman et al., 2003; Campbell et al., 2004; Kavvadias et al., 2004; Hall et al., 2005). Another important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS (Mansur, et al., 1980) and this decrease may be closely related to sedation resulting from depression of the central nervous system (Ozturk et al., 1996). The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident at the 3rd observation (60 min) and continued up to 4th observation period (90 min) (Table-3). Moreover, the validation of anxiety was carried out by measuring external signs, through hole-cross tests. Open field test showed that the depressing action of the extracts was evident from the second observation period in the test animals at the doses of 250 and 500 mg/kg body weight. Maximum depressant effect was observed from 3rd (60 min) to 4th (120 min) observation period. The results were also dose dependent and statistically significant (Table 4). Thus decreased spontaneous motor activity and potentiation of pentobarbitone-induced sleep could be attributed to the CNS depressant activity of the extracts. Finally overall results obtained from this study suggested analgesic and CNS depressant activity of the extracts on experimental animal models. Among the extracts of *Achyranthes aspera* higher dose showed more prominent analgesic and CNS depressant activity compared with other groups of laboratory animal.

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