EVALUATION OF ANALGESIC, ANTICONVULSANT AND LOCOMOTOR ACTIVITIES OF ALCOHOLIC EXTRACT OF ACHYRANTHES BIDENTATA BLUME IN MICE

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ABSTRACT*: The alcoholic extract of Achyranthes bidentata (AAB) has been studied for analgesic, anticonvulsant and CNS depressant activities in animal models. Analgesic activity was studied using acetic acid-induced writing test for assessing peripheral analgesic effect and tail immersion test for central analgesic effect. Anticonvulsant activity was performed by maximal electroshock induced convulsions; while the locomotor activity was evaluated using actophotometer. AAB (250-500 mg/kg) significantly reduced the number of writhes induced by acetic acid and elevated pain threshold in hot water test. The extract (500mg/kg) exhibited anticonvulsant activity significantly (P<0.001) against tonic seizures induced by MES. The results of locomotor activity showed the significant (P<0.01) CNS depressant effect at the three doses (250,375 and mg/kg) employed. The results suggest that AAB exhibited analgesic, anticonvulsant and CNS depressant activity in a dose dependent pattern.*

Key words: *Achyranthes bidentata: Writhing; Tail-flick; Tonic extensor; Locomotor.*

INTRODUCTION

Achyranthes bidentata (Amaranthaceae) is an erect, annual herb or a shrub distributed in hilly (400ft. sea level) districts of India, china, Java and Japan[1,2.] The Plant is used in indigenous system of medicine as emenagogue, antifertility, laxative, ecbolic, abortifacient, antihelmintic, antiviral, anticoagulant and antitumour. Also useful to treat cough renal dropsy fistula skin rash, nasal infection, fever asthma, amenorrhoea, piles and snake bits [3-9]. Phytochemical studies revealed that is contains rutin, saponins, achyranthine, caffeic acid, oleanolic acid, inokosterone, ecdysterone, rubrosterone, physcion and amino acids [10-14]. Antinociceptive effect of *Lingha chendooram* and anticovulsant activity of cardiospermum halicacabum were reported from this laboratory [15-16.] In folklore practice, ***A. Bidentata*** has been reported to be useful in arthritis, abdominal cramp, chest pain and as antispasmodic [17-19]. To substantiate this claim the present study was undertaken to evaluate the analgesic, anticonvulsant and locomotor activity of this potential alcoholic extract in various dose levels.

EXPERIMENTAL

Plant Material

Whole parts of ***A. bidentata*** was collected from the hilly regions of Acharapakkam, Kanchipuram District of Tamil Nadu, India. The botanical identity was confirmed by a qualified botanist in the Department of Siddha medicine, Faculty of Sciences, Tamil University, Thanjavur. A voucher specimen (HAD-003) has been kept in our laboratory for future references.

Preparation of Plant extract:

The plant material was reduced to small pieces, dried under shade, powdered in a pulveriser and passed through a 80 mesh sieve. The powdered plant was packed into a Soxhlet apparatus (350g) and extracted with benzene or dewaxing as well as to remove chlorophyll. Then the powder was subjected to hot continuous percolation using alcohol (50% V/V) for 32h. After completion of extraction, filtered and the solvents were removed by distillation under reduced pressure. The extract was dried in a vacuum desiccator (yield 16.01% W/W). The alcoholic extract was dissolved in normal saline and employed for analgesic, anticonvulsant and CNS depressant activity. Part of the extract was subjected to preliminary phytochemical screening [20,21.]

Animals

Swiss adult albino mice of body weights ranging from 25-30 g supplied by The King Institute of Preventive Medicine, Guindy, Chennai were used for the determination of analgesic, anticonvulsant and locomotor activity. They were housed in standard microlon boxes and were given standard laboratory diet (Amrut lab animal feed, Sangli -416 436) and water *ad libitum*.

ANALGESIC ACTIVITY

a) Acetic acid-induced writhing test (Chemical stimulus)

Male albino mice were divided into six groups of 8 mice each. Groupwise, the animals received various doses of AAB i.p. (125,250,375 and 500 mg/kg)[22.] Control group received normal saline and the reference group received 400 mg/kg aspirin [23.] Drug pre-treatment was given one hour before i.p. injection of 0.06% V/V acetic acid (10ml/kg). The severity of pain response (writhing) was assessed by counting number of wriths (construction of abdomen, turning of trunk and extension of hind legs) in mice. Number of writh per animal was counted during a 15 min series beginning 5 min after the injection of acetic acid.

Analgesic activity was calculated as % maximum possible effect (MPE) using the following relation

$$\% \text{ MPE} = \frac{100\text{x}(\text{Mean of wriths in control group} - \text{Mean of wriths in treated groups})}{\text{Mean of wriths in control group}}$$

b) Tail immersion method (Thermal stimulus)

All the mice were screened by exposure to the thermal stimulation. Those showing positive response were divided into groups of six animals each. Normal saline (control), 125,250,375 and 500 mg/kg AAB, and 1 mg/kg fortral (pentazocine) were administered i.p. T the tail (up to 5cm) was then tipped in a water bath at 55 ±0.7^{o}C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cut-off time being 60 seconds. The reading was taken immediately after administration of the test drugs, and 60 min later [24.]

Effect of AAB in electrical seizures

Application of electrical – shock (50m A for 0.2 sec) through corneal electrodes produced convulsions and those showing positive response were divided into six group of 8 animals each. AAB (125,250,375 and 500 mg/kg i.p) was administered in four different groups, control and reference groups were received normal saline and phenytoin sodium (25mg/kg) respectively.

Drug pre-treatment was given 30 min prior to the electroshock and animals were observed for the duration of tonic flexion, tonic extensor, clonus, stupor and death/recovery [25].

LOCOMOTOR ACTIVITY

Albino mice of either sex were divided into five groups of 6 animals each. All the mice were placed individually in a activity cage (INCO) for 10 min. The basal activity score of the animals were noted. 125,250,375 and 500 mg/kg AB was administered i.p to groups of animals; while the animals in the reference group received 3 mg/kg chlorpromazine [26]. After 30 min re-tested each mouse for activity scores for 10 min. Difference between the score before and after drug administration were noted and calculated the percentage decrease in motor activity.

STATISTICAL ANALYSIS

Values are expressed as mean ± SEM and the significance of data obtained was evaluated statistically using the Student's t-test.

RESULTS:

The preliminary phytochemical analysis showed the presence of alkaloids, amino acids, flavonoids and terpenoids in AAB. Alkaloids, amino acids, flavonoids and terpenoids have earlier been elucidated for their structures [10-14]. A dose –dependent and significant analgesic activity was exhibited in the acetic acid-induced writhing assay by AAB. AAB (125 to 500 mg/kg) produced a significant ($P<0.001$) reduction in writhing at the four doses employed; AAB (125 mg/kg), however had very little antinociceptive effect (Table 1) The ED 50 of AAB was works out to 224 mg/kg $_{50}$B.W. (Fig 1) in peripheral analgesic model. Further analgesic studies revealed that AAB produced an elevation of pain threshold to pain produced by thermal stimulation in a dose dependent manner (Fig2) Antinociceptive effect of AAB (500mg/kg) was nearly comparable with the effect produced by fortral (1mg/kg).

Phenytoin and AAB (500mg/kg) showed a significant ($p<0.001$) protection against duration of extensor phase (Fig 3) while compared to control animals. AAB (125 and 250 mg/kg) effect being very less while compared to reference group. But, AAB at 375 mg/kg) showed a significant reduction of extensor phase in its duration as compared to control. The results clearly indicated that the AAB reduced the extensor phase in a dose dependent pattern. AAB (125 to 500 mg/kg) showed significant CNS depressant action in a dose dependent manner (Table 2). AAB decreases the activity scores with respective control group (Prior to drug treatment) and it is due to a pronounced depressant action. Reduction of awareness and depressant action may be due to the action of AAB on CNS.

DISCUSSION

Prostaglandin (PGs) and Leucotriens (LTs) are the most universally distributed eicosanoids in every cell and tissue. PGs and LTs are synthesized locally by release of arachidonic acid form membrane lipids by phospholipase A2 in response to chemical and mechanical and lipoxygenase are required for the conversion of arachidonic acid to PGs and LTs. PGs elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other provoking stimuli. LTs also produces hyperalgesia. Inhibition of cyclooxygenase, the enzyme responsible for the biosynthesis of PGs and certain related autocoid is

generally thought be a major facet of the mechanism of action of aspirin [27,28.] Hence, the mechanism of analgesic action of ***A. bidentata*** may be due to its inhibitory effect on the synthesis of PGs and LTs.

The maximal electroshock-induced convulsions in animals represents grandmal type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generlised tonic-clonic seizure. The most outstanding action of phenytoin showed abolition of tonic extensor phase of MES seizure. Gamma amino butyric acid is produced from glutamic acid by decarboxylation in brain. It acts as a normal regulator of neuronal activity as an inhibitor of neural transmission. The glutamic acid present in the AAB may increases the brain GABA level and thereby it act as anticonvulsive. Analgesic, anticonvulsant and CNS depressant activity of alcoholic extract of plants have earlier been reported due to the presence of alkaloids, flavonoids, rotenoids and triterpenses [29,30.] Since it is well known that alkaloids, flavonoids and triterpenes have shown to possess analgesic, anticonvulsant and CNS depressant effects, it may be concluded that the activity of AAB now reported is due to the presence of achyranthine, glutamic acid, oleanolic acid and rutin in *Achyranthes bidentata.*

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TABLE -1 EFFECT OF AAB ON ACETIC ACID-INDUCED WRITHINGS

Treatment	**Dose (mg/kg)i.p**	**Mean No .of Wriths ± SEM (15 Min)**	**Percent Inhibition of Wriths**
Saline	5 ml	43.2 ± 1.9	----
Aspirin	400	10.7 ± 0.9	75.23***
AAB	125	29.1 ± 1.3	32.64
AAB	250	22.6 ± 1.2	47.69***
AAB	375	14.7 ± 0.7	65.97***
AAB	500	11.8 ± 0.9	72.69***

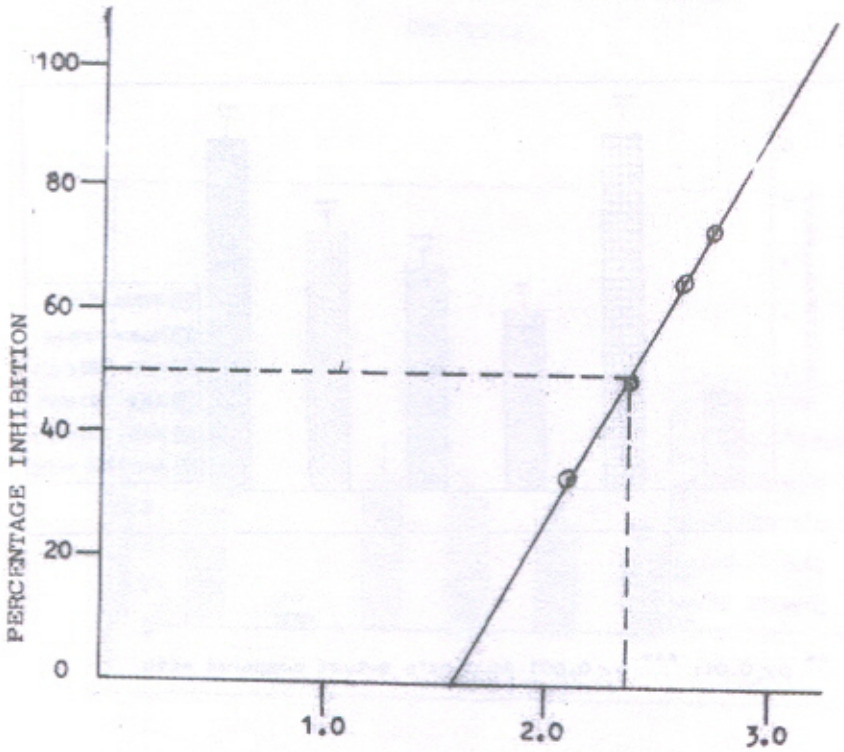
***P<0.001 vs Control
Number of animals used + 8 in each group

TABLE -2. EFFECT OF AAB ON LOCOMOTOR ACTIVITY

Treatment	Dose (mg/kg)	Locomotor activity (Scores) in 10 min		% Decreases in activity
		Before Treatment	After Treatment	
Chlorpromazine	3	127.8 ± 18.4	60.2 ± 8.7	52.89**
AAB	125	143.9 ± 21.3	71.2 ± 10.5	50.52*
AAB	250	155.4 ± 20.2	75.1 ± 9.5	51.67**
AAB	375	166.1 ± 22.3	76.02 ± 9.4	54.23**
AAB	500	161.7 ± 21.8	67.6 ± 8.9	58.19**

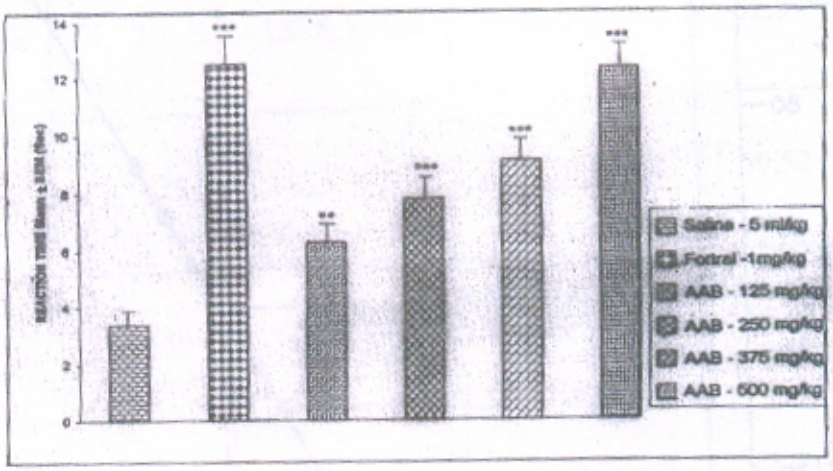
*p<0.05; ** p<0.01 vs Control
Number of animals used =6 in each group

Figure 1. EFFECT OF ACHYRANTHES BIDENTATA ON ACETIC ACID-INDUCED WRITHINGS



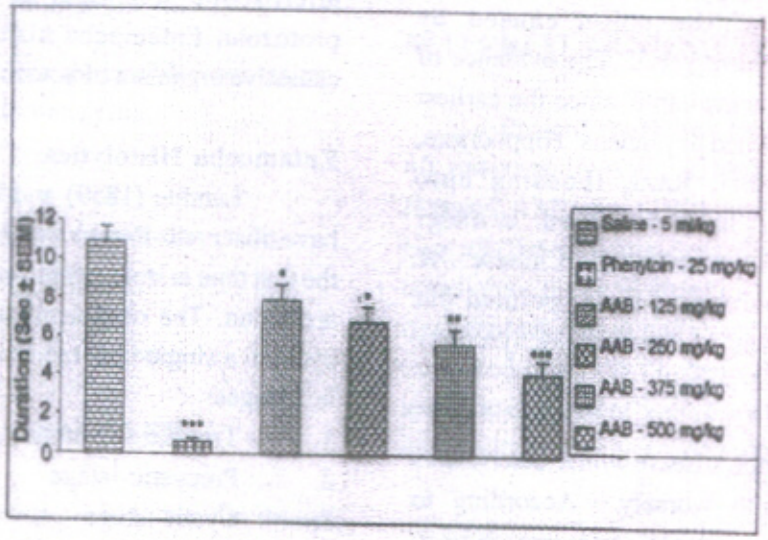
ED_{50} LOG DOSE = 2.35
DOSE = 224 mg/kg

Figure 2. DOSE RELATED CHANGES PRODUCED BY AAB ON PAIN THRESHOLD IN THERMAL-INDUCED PAIN



** $p < 0.01$; *** $p < 0.001$ Student's t-test compared with control (n=6)

Figure 3. DOSE RELATED CHANGES PRODUCED BY AAB ON THE DURATION OF TONIC EXTENSOR PHASE IN MES-INDUCED CONVULSIONS



* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Student's t-test compared with control (n=8)