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CNS depressant and sedative effects of *Andrographis paniculata*

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Abstract

Andrographis paniculata aerial parts methanol extract (aerial parts) at 200 and 400 mg/kg (p.o) was administered in the mice to study CNS depressant effect. Different models for CNS depressant activity viz. open field, hole cross and thiopental sodium induced sleeping time tests were used. In hole cross and open field tests, the number of squares traversed decreased significantly. In the thiopental sodium induced sleeping time test, the extract at 400 mg/kg showed a remarkable decrease in onset of action and increased the duration of sleep in comparison with controls. The behavioral studies on mice indicate CNS depressant activity of the methanol extract of *A. paniculata*.

KEY WORDS: *ANDROGRAPHIS PANICULATA,* CNS DEPRESSANT ACTIVITY, HOLE CROSS TEST, OPEN FIELD TEST, SEDATIVE EFFECT

Introduction

At present, anxiety and depressive disorders are the most frequent psychiatric conditions. It is reported that more than 20% of the adult population suffer from these conditions at some stage during their life [1, 2]. Due to the presence of some adverse effects of antidepressant drugs searching for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade [3].

Andrographis paniculata (Family: Acanthaceae) is a herbaceous plant distributed throughout the Bangladesh having a local name Kalomegh. It is widely cultivated in southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created [4]. *A. paniculata* is used for the treatment of pharyngolaryngitis, diarrhea, dysentery and cough with thick sputum, carbuncle, sores, and snake bites [5]. Various preparations and compound formulas of the herb have been used to treat infectious and non-infectious diseases, with significant effective rates reported for conditions such as epidemic encephalitis B, suppurative otitis media, neonatal subcutaneous annular ulcer, vaginitis, cervical rosion, pelvic inflammation, herpes zoster, chicken pox, mumps, neurodermatitis, eczema, and burns [5]. *A. paniculata* has been reported as having antioxidant, anti-inflammatory [6], anti-diabetic [7], antibacterial, antifungal, antiviral, hypocholesterolemic, adaptogenic, hypotensive [8-11], anticancer and immunostimulatory [12] effects. Due to its "blood purifying" activity it is recommended for use in cases of leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fevers [13].

A. paniculata contains diterpenes, lactones, and flavonoids. Four lactones- chuanxinlian A deoxyandrographolide), B (andrographolide), C (neoandrographolide) and D (14-deoxy-11, 12-didehydroandrographolide)- were isolated from the aerial parts in China. Leaves contained two bitter principles- andrographolide and kalmeghin [5]. A diterpene glucoside (deoxyandrographolide-19beta-D-glucoside) has been detected in the leaves and six diterpenoids of the ent-labdane type, two diterpene glucosides and four diterpene dimers (bis-andrographolides A, B, C, and D) have been isolated from aerial parts [14-15]. Two flavonoids identified as 5,7,2,3-tetramethoxyflavanone and 5-hydroxy-7,2,3-trimethoxyflavone were isolated from the whole plant,16 while 12 new flavonoids and 14 diterpenoids have been reported from the aerial parts [16-18].

Despite the widespread traditional use of *Andrographis paniculata* for treating various disorders there are no reports of scientific evaluation of its CNS depressant and sedative activities, thus the experiment was designed to evaluate these activities of methanol extracts of *A. paniculata* aerial parts.

Methods

Plant Materials Collection

A. paniculata aerial parts were collected from Chittagong district, Bangladesh and authenticated by the expert of Bangladesh Forest Research Institute, Chittagong, Bangladesh. A voucher specimen (No. 4026) has also been deposited at the Forest Research Institute, Chittagong, Bangladesh.

Preparation of Extract

After cutting and slicing, the collected plant samples were dried at room temperature (30° ± 2°C) for a week and ground to coarse powder with a mechanical grinder. The dried sample was then soaked in sufficient amount of methanol for ten days at room temperature with occasional shaking and stirring, then, filtered with cotton followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Animals

Male swiss albino mice, 3-4 weeks of age, weighing between 20-25 g, were collected from the Jahangirnogor University, Savar, Bangladesh. The

animals were exposed to alternative 12:12 h light and dark cycle at an ambient temperature of 25 ± 2°C and had free access to pellet diet and purified water. The animals were acclimatized to laboratory condition for one week prior to experimentation.

Chemicals and Drugs

Diazepam injection (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), thiopental sodium (Beacon Pharmaceuticals Ltd., Dhaka, Bangladesh) and normal saline solution (0.9% NaCl) were used. All other reagents were of analytical grade.

CNS Depressant and Sedative Activity

Hole Cross Test

The method was carried out 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 centre of the cage. The animals were divided into control, positive control and test groups containing five mice each. The test groups received *A. paniculata* extract at the dose of 200 mg/kg b.w and 400 mg/kg b.w orally respectively, whereas, the control received vehicle (normal saline water) and the standard drug, diazepam (1 mg/kg b.w.) given to the positive control respectively. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after oral administration of the test drugs and the standard [19].

Open Field Test

The animals were treated as discussed above. The experiment was carried out according to the methods described by Gupta et al., (1971). 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 mice was counted for 3 min on 0, 30, 60 and 120 min during the study period [20].

Thiopental Sodium Induced Sleeping Time Test

The experiment was conducted following the method described by Ferrini et al (1974). The animals were randomly divided into three groups consisting of five mice each. The test group received methanol extract of *A. paniculata* at dose 400 mg/kg (p.o) b.w. while the standard group was treated with diazepam (1 mg/kg, p.o) and control group with vehicle (normal saline water). Twenty minutes later, thiopental sodium (40 mg/kg, i.p) were administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep i.e. time between the loss and recovery of righting reflex [21].

Statistical Analysis

Experiments results were analyzed by one-way ANOVA followed by Dunnett's test using SPSS Data Editor for Windows. Values are represented as mean ± STD (Standard Deviation).

Results

Hole Cross Test

In the hole cross test, compare with the control group, the extract at both doses (200 and 400 mg/kg body weight) showed decrease in locomotion activity in the test animals as evident by the decrease in number of movement from one chamber to another in the cage at all the observation periods except the 4th observation period (60 min). At the 4th observation period, an increase in locomotion activity was observed in the mice by the extract at dose of 200 mg/kg body weight but the result was not statistically significant. Treatment with diazepam, the positive control, showed decrease in locomotion activity at all the observation periods (Table 1).

Open Field Test

The open field model, the number of squares traveled by the mice was suppressed significantly ($P<0.05$) at the doses 200mg /kg and 400 mg/kg

respectively as compared to control animals which was dose-dependent. There was significant decrease in number of squares crossed in the diazepam treated group (Table 2).

Thiopental Induced Sleeping Time

In the thiopental sodium induced sleeping time test, the test group treated with the extract at 400 mg/kg showed a moderate decrease in onset of action and increased the duration of sleep in comparison with controls while positive control group was found to significant (P<0.05) effects on the thiopental induced sleep (Figure 1).

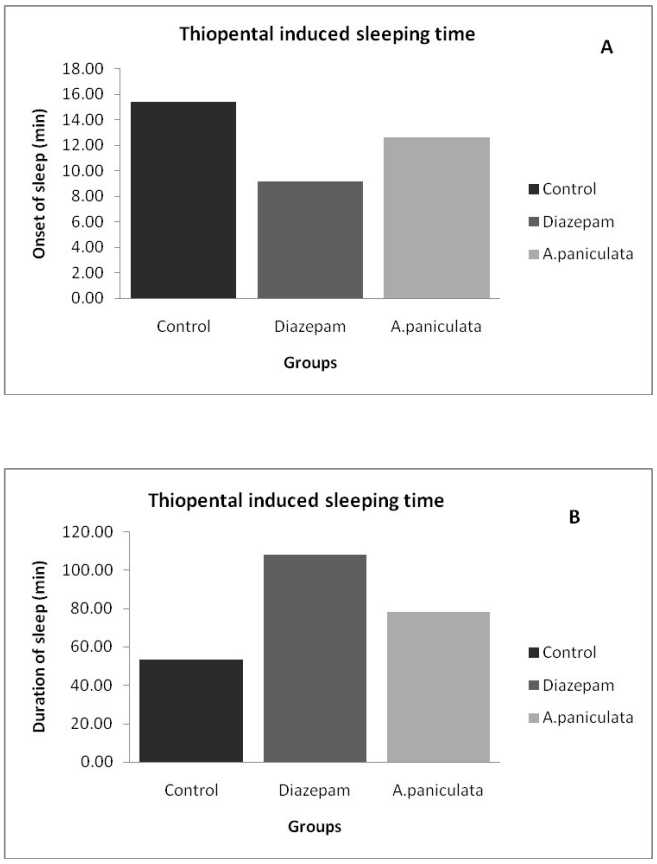

Figure 1: Effects of methanol extract of *A. paniculata* on onset of sleep (A) and duration of sleep (B) after induction of thiopental sodium in mice.

Discussion

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety [22]. Benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders. The serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (BZD's, barbiturates and alcohol), sexual dysfunction, anticholinergic, antihistaminic effects (TCA's) have limited their use in patients. Buspirone, the non-sedative anxiolytic agent is not effective in a high percentage of patients. It is also associated with tachycardia, palpitation, gastric discomfort etc [23,24].

Locomotors activity is considered as an index of alertness and a decrease in that indicates a sedative effect [25]. The plant extract possessed central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in hole cross and open field tests which were dose- dependent. The marked sedative effect of the extract was also found by the reduction in sleeping latency and increase of thiopental sodium induced sleeping time.

Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavanoids, saponins and tannins possess activity against many CNS disorders [26]. Chemical investigations revealed the presence of glycoside and flavanoid. It may possible that the mechanism of CNS depressant action of *A. paniculata* could be due to the binding of any of these phytochemicals to the GABA and/or serotonin receptor complex.

Therefore it was concluded that, *A. paniculata* used in this study might affect certain mediators to cause CNS depression. Further in depth study is needed to understand the mechanism of action at biochemical and physiological level.

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Group	Treatment	Numbers of movements				
		0 min	30 min	60 min	90 min	120 min
Control	Saline	18.10 ± 0.76	16.20 ± 1.26	15.10 ± 0.76	17.00 ± 0.76	14.20 ± 0.50
Standard	Diazepam (1 mg/kg)	16.40 ± 1.00	8.16 ± 1.32*	5.40 ± 1.52*	4.15 ± 1.00	3.80 ± 0.57*
Test 1	Extract (200 mg/kg)	16.11 ± 5.96	12.47 ± 2.31	8.25 ± 4.09*	8.79 ± 2.36	8.80 ± 1.32
Test 2	Extract (400 mg/kg)	17.85 ± 5.96	10.01 ± 2.3*	6.87 ± 4.09*	6.11 ± 2.36	5.10 ± 1.32

Table 1: Effects of methanol extract of *A. paniculata* on number of movements in the hole cross apparatus in mice. All values are expressed as mean ± STD (n=5). *P<0.05, significant compared to control.

Group	Treatment	Numbers of movements				
		0 min	30 min	60 min	90 min	120 min
Control	Saline	72.40 ± 1.52	65.50 ± 2.31	68.35 ± 1.96	60.48 ± 1.54	54.87 ± 1.22
Standard	Diazepam (1 mg/kg)	69.55 ± 1.05*	54.38 ± 1.44	40.15 ± 1.02*	23.10 ± 0.88	16.24 ± 1.48*
Test 1	Extract (200 mg/kg)	67.90 ± 7.10	60.1 ± 11.20*	52.31 ± 9.08	45.23 ± 9.14	34.71 ± 4.57*
Test 2	Extract (400 mg/kg)	70.25 ± 9.22	56.10 ± 8.09*	45.25 ± 11.2*	33.44 ± 7.31	22.15 ± 2.27

Table 2: Effects of methanol extract of *A. paniculata* on number of movements in the open field in mice. All values are expressed as mean ± STD (n=5). *P<0.05, significant compared to control.