

*Full Length Research Paper*

# Central nervous system depressant and analgesic activity of *Aphanamixis polystachya* (Wall.) parker leaf extract in mice

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In the present study, we have investigated the possible CNS (Central Nervous System) depressant and analgesic action of the methanol extract of *Aphanamixis polystachya* leaf. Its CNS depressant activity was evaluated by using thiopental sodium- induced sleeping time, hole cross and open field tests. The analgesic activity was also investigated for its central and peripheral pharmacological actions using hot plate and tail immersion test and acetic acid -induced writhing test in mice respectively. The extract decreased the motor activity and exploratory behavior of mice in hole cross and open field test ( $p < 0.001$ ). Moreover, the extract significantly maximized the duration of sleeping time when administered with thiopental sodium ( $p < 0.001$ ). The extract, at the dose of 250 and 500 mg/kg, produced a significant ( $p < 0.05$ ,  $p < 0.001$ ) increase in pain threshold both in hotplate and tail immersion methods in a dose dependent manner. The results were comparable to the reference standard Nalbuphine. In acetic acid-induced writhing test, the extract (500 mg/kg) produced a maximum of 75.9% inhibition ( $p < 0.001$ ) of writhing reaction compared to the reference drug Diclofenac-Na (10 mg/kg) (78.1%). These results suggest that the extract possesses strong CNS depressant and analgesic activity in mice.

**Key words:** Aphanamixis polystachya, CNS depressant activity, analgesic activity.  
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## INTRODUCTION

*Aphanamixis polystachya* (Family: Meliaceae) locally known as pitraj in Bangladesh is a large tree with bunches of rounded locular fruits and glossy deep brown seeds, grows wild and planted in forests and roadsides all over the country (Ghani, 2003). The plant is extensively used in traditional system of medicine for various ailments in different Asian countries like spleen and liver complications, tumors, rheumatism (Bangladesh), spleenomegaly, liver complaints, tumors, ulcers, diabetes, jaundice, haemorrhoids, burning sensations, arthritis, ulcers, ophthalmia (India), nervousness, pyrexia (Laos) (Asian medicinal plants database). The plant is reported to possess antitumor (Rabi and Gupta, 1995), hepatoprotective (Gole and Dasgupta, 2002), insecticidal (Talukder and Howse, 1993), antibacterial, antifungal and immunosuppressive (Ghani, 2003) activities. Fruit shell contains triterpene (Chatterjee et al., 1970), leaves contain diterpene alcohol and beta-sitosterol, seeds yield polystachin,

an alkaloid, a glycoside and a saponin (Ghani, 2003). A chromone and 3 flavonoid glycosides have been reported from roots (Jain and Srivastava, 1985). Recently, a new lignan, polystachyol, 2 lignan glycosides, lyonoside and nudiposide and a sterol, ergosta-4, 6, 8 (14), 22-tetraen-3-one, with stigmasterol and oleic and linoleic acids, have been isolated from a methanol extract of the dried bark of *A. polystachya* (Sadhu et al., 2006). In contrast to the traditional claims, very few biological works of medicinal interest have so far been carried out on this plant. In order to evaluate the pharmacological basis for the use of the plant in folk medicine for the treatment of rheumatism and CNS disorders, the present study was designed to investigate the analgesic and CNS depressant activity of the crude methanol extract of the leaves of *A. polystachya*.

## MATERIALS AND METHODS

### Plant material

The leaves of *A. polystachya* were collected from Jamalpur in July 2007 and were identified by the experts of national herbarium,

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Mirpur, Dhaka, Bangladesh (Accession No. 577) and a voucher specimen was kept for future reference. The leaves were thoroughly washed with water and dried in a hot air oven at 50°C for 2 days and at 40°C for the next 3 days.

### Extraction

The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 60°C. The solvent was completely removed and obtained dried crude extract which was used for investigation.

### Animal

For the experiment male Swiss albino mice of 3 - 4 weeks of age, weighing between 20 - 25 gm, were collected from the animal research branch of the international center for diarrheal disease and research, Bangladesh (ICDDRDB). Animals were maintained under standard environmental conditions (temperature:  $23.0 \pm 2.0^\circ$ ), relative humidity: 55 - 65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

### CNS depressant activity

#### 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 centre of the cage. The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the extract.

#### 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 5 mice each. The test group received *A. polystachya* 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 coloured 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 120 min after oral administration of the test drugs.

#### Thiopental sodium-induced sleeping time

All mice were injected intraperitoneally a sub-hypnotic dose of thiopental sodium (10 mg/kg) 20 min after a similar injection of diazepam or per oral administration of the extract. Sleeping time was determined as the interval between the loss and the recovery of the righting reflex (Ferrini et al., 1974). All groups of mice (n = 5) were injected with thiopental sodium (10 mg/kg i.p) 15 min after administration of either diazepam (1 mg/kg) or *A. polystachya* extract (250 and 500 mg/kg) and the time interval between losing and regaining of righting reflex was measured as sleeping time.

### Analgesic activity

#### Hot plate method

The animals were divided into 4 groups with 5 mice in each group.

Group I animals received vehicle (1% Tween 80 in water, 10 ml/kg body weight), animals of group II received nalbuphine at 10 mg/kg body weight while animals of group III and group IV were treated with 250 and 500 mg/kg body weight (p. o.) of the crude extract. The animals were placed on Eddy's hot plate kept at a temperature of  $55 \pm 0.5^\circ\text{C}$ . A cut off period of 15 s, was observed to avoid damage to the paw (Franzotti et al., 2000). Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples (Toma et al., 2003).

### Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma et al., 2003). The animals were treated as discussed above. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next 3 readings. A latency period of 15 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

### Acetic acid-induced writhing test

The analgesic activity of the samples was also studied using acetic acid- induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed et al., 2004).

### Statistical analysis

Statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparisons for analgesic screening tests and Tuckey's multiple comparisons for neuropharmacological tests. The results obtained were compared with the vehicle control group. P values < 0.05, 0.001 were considered to be statistically significant.

## RESULTS

### CNS depressant activity

#### Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 to 120 min (Table 1). Hole cross test of *A. polystachya* treated groups showed significant decrease of movement from its initial value at 0 to 120 min. The result was statistically significant ( $p < 0.001$ ).

#### Open field test

Open field test of *A. polystachya* treated groups showed significant ( $p < 0.001$ ) dose dependent decrease of movement from its initial value at 0 to 120 min (Table 2).

**Table 1.** Effects of the methanol extract of *A. polystachya* leaf in mice on hole cross test.

Groups	Dose (mg/kg)	Mean movements on open field before and after drug administration			
		0 Min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)				
Diazepam	1 (i.p.)	18.4 ± 2.50	17.4 ± 0.92	16.6 ± 0.92	14.8 ± 0.37
<i>A. polystachya</i> extract	250 (p.o.)	19.2 ± 2.46	10.1 ± 1.76**	3.4 ± 1.12**	1.3 ± 0.93**
	500 (p.o.)	18.6 ± 2.31	13.8 ± 2.58**	8.6 ± 1.40**	5.6 ± 1.24**
		17.8 ± 2.52	10.2 ± 1.83**	5.7 ± 1.12**	2.7 ± 1.09**

Values are mean ± SEM, (n = 5); \*\* p < 0.001, Tuckey test as compared to control.

**Table 2.** Effects of the methanol extract of *A. polystachya* leaf on open field test in mice.

Groups	Dose (mg/kg)	Mean hole cross (no.) before and after drug administration			
		0 min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	115.2 ± 5.75	105.2 ± 3.32	93.8 ± 4.65	89.3 ± 2.93
Diazepam	1 (i.p.)	86.8 ± 4.47	22.5 ± 3.21**	11.6 ± 2.06**	6.8 ± 1.73**
<i>A. polystachya</i> extract	250 (p.o.)	92.4 ± 4.22	52.2 ± 3.86**	34.4 ± 2.93**	20.8 ± 2.17**
	500 (p.o.)	86.8 ± 4.47	32.8 ± 3.04**	16.2 ± 2.06**	10.1 ± 1.73**

Values are mean ± SEM, (n = 5); \*\* p < 0.001, Tuckey test as compared to control.

**Table 3.** Effects of the methanol extract of *A. polystachya* leaf on thiopental sodium-induced sedative test in mice.

Groups	Dose (mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Group-I	10	13.2 ± 2.312	67.6 ± 4.325
Group-II	10 + 5	9.4 ± 1.312*	102.8 ± 5.648**
Group-III	10 + 250	12.5 ± 2.513	79.3 ± 5.214**
Group-IV	10 + 500	10.2 ± 1.268*	95.8 ± 7.362**

Values are mean ± SEM, (n = 5); \* p < 0.05, \*\* p < 0.001, Dunnet test as compared to control. Group I animals received thiopental sodium (i.p.); Group II animals received thiopental sodium (i.p.) plus diazepam (i.p.) and group III and IV were treated with thiopental sodium (i.p.) plus (p.o.) crude extract of *A. polystachya* respectively.

### Thiopental sodium induced sleeping time

Result of thiopental sodium induced sleeping time test is presented in Table 3. Both doses of the extract potential-  
ted the duration of thiopental sodium induced sleeping time (p < 0.001).

### Analgesic activity

#### Tail immersion test

The tail withdrawal reflex time following administration of the extract was found to increase dose dependently. The result was statistically significant (p < 0.05-0.001) and was comparable to the reference drug nalbuphine (Table 4).

### Hot plate method

Result of hotplate test is presented in Table 5. Both doses

of the extract produced a dose dependent increase in latency time when compared with the vehicle (p < 0.05 - 0.001).

### Acetic acid-induced writhing test

Table 6 shows the effects of the extract of on acetic acid-induced writhing in mice. Oral administration of the extract significantly (p < 0.001) inhibited writhing response induced by acetic acid in a dose dependent manner.

## DISCUSSION

In the present study, the effect of methanol extract of *A. polystachya* on CNS has been evaluated. The result indicated that the extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity is a measure of the level of excitability of the CNS (Mansur et al., 1980) and

**Table 4.** Effects of the methanol extract of *A. polystachya* leaf on tail withdrawal reflex of mice induced by tail immersion method.

Groups	Dose (mg/kg)	Mean reaction time (s) before and after drug administration			
		0 min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	1.98 ± 0.174	2.50 ± 0.187	2.44 ± 0.219	2.32 ± 0.116
Nalbuphine	10 (i.p.)	1.96 ± 0.217	4.28 ± 0.317*	10.10 ± 1.030**	11.53 ± 1.59**
<i>A. polystachya</i> extract	250 (p.o.)	1.63 ± 0.282	3.54 ± 0.645*	5.82 ± 0.670*	4.84 ± 0.458*
	500 (p.o.)	1.93 ± 0.304	4.17 ± 0.519*	6.71 ± 0.529**	5.62 ± 0.862*

Values are mean ± SEM, (n = 5); \* p < 0.05, \*\* p < 0.001, Dunnet test as compared to control.

**Table 5.** Effects of the methanol extract of *A. polystachya* leaf on latency to hot plate test in mice.

Groups	Dose (mg/kg)	Mean latency (s) before and after drug administration			
		0 min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	2.26±0.219	2.45±0.225	2.16±0.197	2.58±0.261
Nalbuphine	10 (i.p.)	2.34±0.088	5.62±0.624**	7.97±0.649**	11.67±1.007**
<i>A. polystachya</i> extract	250 (p.o.)	2.21±0.073	3.27±0.264*	4.57±0.747*	4.30±0.383*
	500 (p.o.)	2.07±0.217	3.57±0.850*	5.66±0.546**	5.45±0.469*

Values are mean ± SEM, (n = 5); \* p < 0.05, \*\* p < 0.001, Dunnet test as compared to control.

**Table 6.** Effects of the methanol extract of *A. polystachya* leaf on acetic acid-induced writhing in mice.

Groups	Dose (mg/kg)	No. of writhing	% protection
Control	1% Tween in water (p.o.)	50.2 ± 3.421	78.1
Diclofenac-Na	10 (i.p.)	11.0 ± 0.940**	50.2
<i>A. polystachya</i> extract	250 (p.o.)	25.0 ± 0.994**	75.9
	500 (p.o.)	12.1 ± 0.906**	

Values are mean ± SEM, (n = 5); \*\* p < 0.001, Dunnet test as compared to control.

any decrease of this activity may be closely related to sedation resulting from depression of the central nervous system (Ozturk et al., 1996).

Both the doses of the crude extract was produced a significant increase in the hypnotic effect induced by the thiopental sodium, in a dose dependent manner, thus suggesting a profound sedative activity. The method employed for this assay is considered as a very sensitive way to detect agents with CNS depressant activity (Kumar et al., 2008). The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts. Literature review of the plant reveals that *A. polystachya* contains flavonoids, saponins, steroids, tannins and triterpenoids (Chatterjee et al., 1970; Ghani, 2003; Jain and Srivastava, 1985). Many flavonoids and neuroactive steroids were found to be ligands for the gamma aminobutyric acid type A (GABAA) receptors in the central nervous system (CNS); which led to the hypothesis that

they act as benzodiazepine-like molecules (Fernández et al., 2006). This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion (Marder and Paladini, 2002; Johnston, 2005).

The methanol extract was also evaluated in the tail immersion, hot plate and acetic acid-induced writhing test for its analgesic activity. The hotplate method and tail immersion test are considered to be selective to examine compounds acting through opioid receptor, the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism (Elisabetsky et al., 1995; Pal et al., 1999). Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids (Ahmed et al., 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ronaldo et al., 2000), acid sensing ion channels (Voilley, 2004)

and the prostaglandin pathways (Hossain et al., 2006).

Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain (Elisabetsky et al., 1995; Pal et al., 199). The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic. Such a mode of action is proposed for opioid analgesic such as morphine. It is also reported that the inhibition of pain could arise not only from the presence of opioids and/or opiodiomimetics but could also arise from the presence of phenolic constituents (De Campos et al., 1997) and also steroidal constituents (Miguel et al., 1996). So, it may be due to the similar type of constituents present in the extract. There are also reports on the role of flavonoid in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al., 2001; Rao et al., 1998). Tannins are also claimed to possess analgesic activity (Vanu et al., 2006).

Since the pharmacological profiles of the present investigation of the methanol extract of *A. polystachya* was similar to that of diazepam it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, the use of *A. polystachya* in folkloric medicine may be due to its CNS action and ability to relieve pain validated by our findings. However, further investigation is underway to determine the exact phytoconstituents that are responsible for the biological activities of the methanol extract of *A. polystachya*.

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