

Original Article**Sedative and anxiolytic effects of different fractions of the *Commelina benghalensis* Linn****S. M. Raquibul Hasan¹, Md. Mokarram Hossain¹, Raushanara Akter², Mariam Jamila³, Md. Ehsanul Hoque Mazumder⁴, Shafiqur Rahman^{5,*}**¹ Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh;² Department of Pharmacy, Stamford University Bangladesh, Dhaka, Bangladesh;³ Department of Pharmacy, Bangladesh University, Dhaka, Bangladesh;⁴ Discipline of Biomedical Science, Faculty of Medicine, Cumberland Campus, University of Sydney, Australia;⁵ Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD, USA.

ABSTRACT: The present study was designed to investigate sedative and anxiolytic properties of the four different fractions (chloroform, pet ether, *n*-butanol and hydromethanol soluble fractions, coded as CFCB, PECB, NBCB and HMCB, respectively) of the aerial parts of *Commelina benghalensis* using rodent behavioral models, such as hole cross, open field and thiopental sodium induced sleeping time tests for sedative property and elevated plus-maze (EPM) test for anxiolytic potential, respectively. All fractions, at the doses of 200 mg/kg, *p.o.* and 400 mg/kg, *p.o.*, displayed dose dependent suppression of motor activity, exploratory behavior (in hole cross and open field tests) and prolongation of thiopental induced sleeping time in mice; maximum effect was shown by chloroform (CFCB) and pet ether (PECB) fractions. In EPM test, chloroform (CFCB) and pet ether (PECB) fractions with similar doses significantly ($p < 0.05$) increased exploration to and time spent by the treated mice in EPM open arms in a way similar to that of diazepam while the effect of NBCB and HMCB fractions on entry to and time spent in open arms was not found to be statistically significant. These findings provide *in vivo* evidence that aerial parts of *C. benghalensis* in general, and chloroform (CFCB) and pet ether (PECB) soluble fraction has significant sedative and anxiolytic effects. Furthermore, these results may justify the scientific basis for the use of this plant in traditional medicine as a modality for anxiety and related disorders.

Keywords: Medicinal plant, *Commelina benghalensis*, sedatives, anxiolytics, elevated plus-maze

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1. Introduction

Anxiety and depressive disorders are the most frequent psychiatric conditions encountered today. Modern life stress, associated trials and tribulations are suspected to be responsible for the upsurge of such psychiatric derangements. It is reported that more than 20% of the adult population suffer from these conditions at some stage during their life (1,2). Since long the benzodiazepines remain to be the most frequently prescribed synthetic drugs of choice for acute anxiety and other allied disorders including depression, epilepsy and insomnia. But chronic use of these drugs have very serious side effects ranging from respiratory, digestive and immune system dysfunctions to deterioration of cognitive function, physical dependence and tolerance (3). In this context, there has been a resurgence of interest in medicine from natural sources (mainly from plant kingdom) with the hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy. Thus the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has advanced significantly in the past decade (4). This is reflected in the large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models. These studies have provided useful information for the development of new pharmacotherapies from medicinal plants for successful use in modern clinical psychiatry.

Commelina benghalensis (family Commelinaceae) is a perennial herb native to tropical Asia and Africa, used in the Indian subcontinent as a folk medicine for the treatment of leprosy, headache, fever, constipation, jaundice and snake bite (5-7). The plant is also used for mouth thrush (8), inflammation of the conjunctiva, psychosis (9), epilepsy, nose blockage in children (10), insanity (11) and exophthalmia. *C. benghalensis* is used medicinally as a diuretic, febrifuge and anti-inflammatory (12-15). It is used as an animal fodder,

eaten by humans as a vegetable in Pakistan, also used there medicinally, but with different purported effects, including as a laxative and to cure inflammations of the skin as well as leprosy (16). The plant is also reported to have antitumor, anticancer and antioxidant activity (5,17,18). Previous phytochemical investigations of the *Commelina* genus were reported on *C. undulata* R.Br., *C. benghalensis* L. and *C. communis* L. from which several types of compounds such as alkaloids, steroids, terpenoids, iridoids, flavonoids, lignans, aliphatic alcohols, polyols, and phenolic acids were obtained (19-27). Moreover, the whole plant of *C. benghalensis* was reported to contain alkaloid, volatile oil, wax (28), vitamin-C and higher levels of both lutein and β -carotene (29,30).

However, only a few biological works of medicinal interest have so far been carried out on this plant to substantiate the traditional claims. Thus, we have evaluated the various fractions aerial parts of *C. benghalensis* for sedative and anxiolytic activities in rodent behavioral models. Additionally, we have determined the pharmacological basis for the use of the plant in traditional medicine for the treatment of major neuropsychiatric disorder involving anxiety.

2. Materials and Methods

2.1. Drugs and chemicals

The following drugs and chemical were used in this study: Diazepam (Square Pharmaceutical Ltd., Bangladesh), thiopental sodium, quercetin, etc. (Sigma Chemicals Co., USA).

2.2. Plant material

The plant was collected from Old Elephant Road, Eskaton Garden, Dhaka in April 2008 when weed beds were in their maximum densities. The whole plant with leaves, stems and roots was collected and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen (Accession No.32784) has been deposited for future reference. The plant was thoroughly washed with water; roots were discarded and the aerial parts were dried in hot air oven at 50°C for 3 days and at 40°C for the next 4 days.

2.3. Extraction and solvent-solvent partitioning

The dried aerial parts were coarsely powdered from which 500 g was extracted with a mixture of methanol: water (7:3, v/v) in a Soxhlet apparatus. The solvent was completely removed and obtained 18 g (yield 3.6%) dried crude extract. Solvent-solvent partitioning was done using the protocol as described by Rahman *et al.* (18). The crude extract was dissolved in 10% aqueous

methanol to make the mother solution which was partitioned off successively by three solvents namely pet ether (3 × 100 mL), chloroform (3 × 100 mL), *n*-butanol (3 × 100 mL). All the three fractions and the residual hydromethanol fraction were subjected to dryness under reduced pressure. The dried extracts thus obtained were used for investigation (18).

2.4. Animal

For the experiment male Swiss albino mice, 3-4 weeks of age, weighing between 20-25 g, were collected from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: $24.0 \pm 1.0^\circ\text{C}$), 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 experimentation.

2.5. Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (31).

2.6. Total flavonoid content determination

Total flavonoid contents of the fractions were estimated using a method previously described by Kumaran and Karunakaran (32) using quercetin as the reference. Each 1 mL of the extracts in methanol (250 $\mu\text{g}/\text{mL}$) was mixed with 1 mL aluminium trichloride in ethanol (20 mg/mL and a drop of acetic acid, and then diluted with ethanol to 25 mL. The absorbance at 415 nm was read after 40 min. Blank samples were prepared from 1 mL of plant extract and a drop of acetic acid, and then diluted to 25 mL with ethanol. These data were used to estimate the flavonoid contents using a standard curve obtained from various concentration of quercetin.

2.7. Thiopental sodium induced sleeping time test

The animals were randomly divided into ten groups consisting of five mice each. The test groups received different fractions of the aerial parts of *C. benghalensis* at the doses of 200 mg/kg and 400 mg/kg body weight (b.w.) while positive control was treated with diazepam (1 mg/kg) and control with vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40 mg/kg) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administration to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex (33).

Table 1. Result of chemical group test of the crude aqueous-methanolic extract of the aerial parts of *C. benghalensis*

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
<i>C. benghalensis</i>	-	++	-	-	-	+++	-

(+): Present, (-): Absent.

2.8. Hole cross test

The method was carried out as described by Takagi *et al.* (34). 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 animals were divided into control, positive control, and test groups containing five mice each. The test groups received four different fractions of the aerial parts of *C. benghalensis* at the doses of 200 mg/kg and 400 mg/kg b.w. orally whereas the control group received vehicle (1% Tween 80 in water). 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 (34).

2.9. Open field test

In open field test, the animals were divided into control, positive control, and test groups containing five mice each. The test groups received different fractions of the aerial parts of *C. benghalensis* at the doses of 200 mg/kg and 400 mg/kg b.w. 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 120 min after oral administration of the test drugs (35).

2.10. Elevated plus-maze (EPM) test

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. Sixty minutes after administration of the test drugs, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded (36). Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound attenuated room; observations made from an adjacent corner.

Table 2. Total flavonoid contents of the different fractions of the aerial parts of *C. benghalensis*

Sample	Total flavonoid (in mg/g, quercetin equivalents)
CFCB	838.72 ± 3.25
PECB	105.82 ± 4.33
NBCB	305.31 ± 2.16
HMCB	51.99 ± 16.23

Values are the mean of duplicate experiments and represented as mean ± SD. CFCB = Chloroform fraction, PECB = Pet ether fraction, NBCB = *n*-butanol fraction and HMCB = Hydromethanol fraction of the aerial parts of *C. benghalensis*.

2.11. Statistical analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. *p* values < 0.05, 0.001 were considered to be statistically significant.

3. Results

3.1. Phytochemical screening

Phytochemical analyses of the crude extract revealed the presence of alkaloid and flavonoid (Table 1).

3.2. Total flavonoid content

The fractions were found to contain high amounts flavonoids (expressed as quercetin equivalents) as measured by total flavonoid content determination assay (Table 2). Flavonoid contents were of the following order: CFCB > NBCB > PECB > HMCB.

3.3. Thiopental sodium induced sleeping time test

In thiopental induced hypnosis test, CFCB and PECB fractions of the aerial parts of *C. benghalensis*, at the doses of 200 and 400 mg/kg, induced the sleep at an earlier stage while NBCB and HMCB fractions were found to have little effect on the onset of thiopental induced sleep. But all fractions dose dependently prolonged the duration of sleeping time in test animals compared to control (Table 3).

3.4. Hole cross test

All fractions, at 200 mg/kg and 400 mg/kg b.w. doses,

produced significant ($p < 0.05$, $p < 0.001$) decrease of locomotion from its initial value during the period of experiment (Figure 1). Maximum suppression of locomotor activity was displayed by chloroform fraction (CFCB), which was comparable to the reference drug diazepam.

3.5. Open field test

The number of squares traveled by the mice was suppressed significantly from the second observation period at both dose levels (200 mg/kg and 400 mg/kg b.w.) of the four fractions of the aerial parts of *C. benghalensis*. The results were dose dependent and

statistically significant (Figure 2). The locomotor activity decreased in the following order: CFCB > PECB > NBCB > HMCB.

3.6. Elevated plus-maze test

CFCB and PECB treatment, at 400 mg/kg b.w., significantly increased the percentage of entries (Figure 3A) of mice into the open arms, and the percentage of time spent (Figure 3B) in the open arms of the elevated plus-maze. However, the effects of treatment of mice with NBCB and HMCB fractions on open arm entries and time spent in open arms were not statistically significant.

4. Discussion

It was observed from the present study that CFCB and PECB fractions of the aerial parts of *C. benghalensis* (200 mg/kg and 400 mg/kg) show strong sedative and antianxiety properties. Both CFCB and PECB fractions dose dependently potentiated the sleep induced by thiopental suggesting that the aerial parts of the plant possesses sleep inducing property. Thiopental, a hypnotic agent, when given at appropriate dose, induces sedation or hypnosis by potentiating GABA mediated postsynaptic inhibition through allosteric modification of GABA receptors. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both (37). Moreover, the study on locomotor activity, as measured by hole cross and open field tests, showed that all

Table 3. Effects of the different fractions of the aerial parts of *C. benghalensis* on thiopental induced sleeping time in mice

Treatment	Dose (mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Control	Vehicle	15.4 ± 4.207	47.2 ± 6.485
Diazepam	1	6.8 ± 2.584	98.6 ± 7.059**
CFCB1	200	9 ± 2.716	84.8 ± 9.588*
CFCB2	400	7.2 ± 2.608	102.4 ± 13.53**
PECB1	200	9.4 ± 2.971	79.6 ± 7.735*
PECB2	400	7.4 ± 3.328	94.4 ± 10.545**
NBCB1	200	15.8 ± 2.608	66.2 ± 7.684
NBCB2	400	12.6 ± 3.328	73.6 ± 7.497*
HMCB1	200	14.6 ± 3.114	63.8 ± 12.295
HMCB2	400	13.6 ± 3.978	69.4 ± 4.102

Values are mean ± SEM, (n = 5); * $p < 0.05$, ** $p < 0.001$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse, p.o.; CFCB = Chloroform fraction, PECB = Pet ether fraction, NBCB = n-butanol fraction and HMCB = Hydromethanol fraction of *C. benghalensis*; 1 = 200 mg/kg b.w., 2 = 400 mg/kg b.w.]

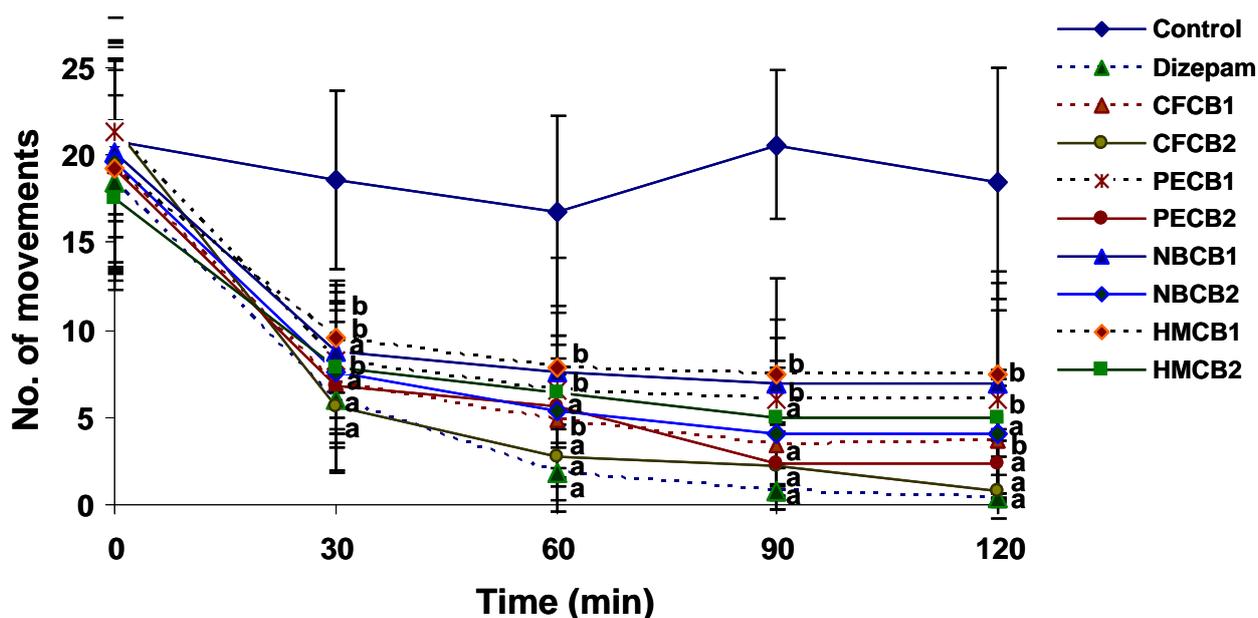


Figure 1. Effects of the different fractions of the aerial parts of *C. benghalensis* on Hole cross test in mice. Values are mean ± SEM, (n = 5); b, $p < 0.05$; a, $p < 0.001$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse, p.o.; CFCB = Chloroform fraction, PECB = Pet ether fraction, NBCB = n-butanol fraction and HMCB = Hydromethanol fraction of *C. benghalensis*; 1 = 200 mg/kg b.w., 2 = 400 mg/kg b.w.]

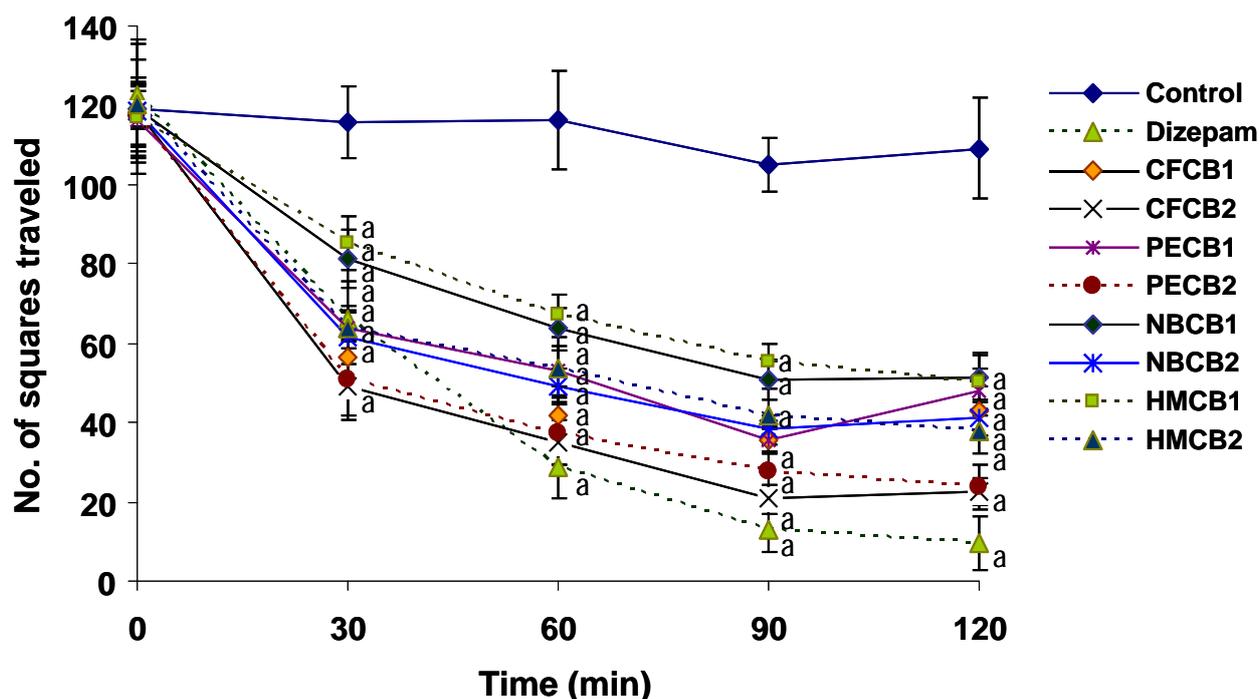


Figure 2. Effects of the different fractions of the aerial parts of *C. benghalensis* on Open field test in mice. Values are mean \pm SEM, ($n = 5$); a, $p < 0.001$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse, *p.o.*; CFCB = Chloroform fraction, PECB = Pet ether fraction, NBCB = *n*-butanol fraction and HMCB = Hydromethanol fraction of *C. benghalensis*; 1 = 200 mg/kg b.w., 2 = 400 mg/kg b.w.].

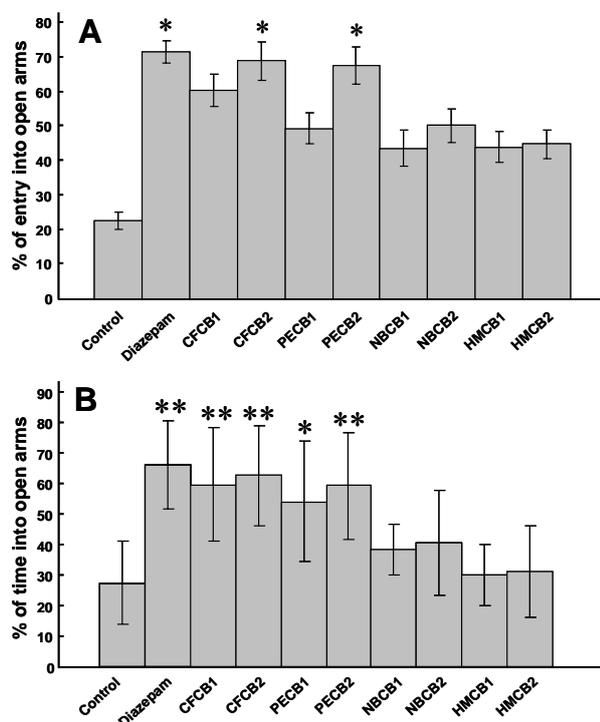


Figure 3. Effects of the different fractions of the aerial parts of *C. benghalensis* on the percentage of entries (A) and the time spent (B) in open arms of the elevated plus-maze during the 5-min test session. Values are mean \pm SEM, ($n = 5$); * $p < 0.05$, ** $p < 0.001$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse, *p.o.*; CFCB = Chloroform fraction, PECB = Pet ether fraction, NBCB = *n*-butanol fraction and HMCB = Hydromethanol fraction of *C. benghalensis*; 1 = 200 mg/kg b.w., 2 = 400 mg/kg b.w.].

fractions of the aerial parts of *C. benghalensis* (at both dose levels) decreased the frequency and the amplitude of movements. Since locomotor activity is a measure of the level of excitability of the CNS (38), this decrease in spontaneous motor activity could be attributed to the sedative effect of the plant extracts (39,40). All fractions significantly decreased the locomotion in mice. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min). Maximum depression of locomotor activity was observed from 3rd (60 min) to 5th (120 min) observation period. The results were also dose dependent and statistically significant (Figures 1 and 2).

However, the evaluation of the putative anxiolytic activity of the four different fractions of the aerial parts of *C. benghalensis* was performed using elevated plus-maze (EPM). The primary measures in the EPM are the percentage of entries into the open arms and of the time spent on the open arms. An anxiolytic effect is suggested when the test drug increases open arms entries without altering the total number of arm entries (41). Although CFCB and PECB treatment, at 200 mg/kg b.w., in mice did not display significant increase in the percentage of entries into open arms, both fractions at 400 mg/kg b.w. showed a significant increase in the percentage of time spent in the open arms of the maze, similar to the effects observed following treatment with the reference anxiolytic drug diazepam, in a dose

dependent manner. These results could indicate an anxiolytic-like activity of the chloroform and pet-ether soluble fractions (CFCB and PECB respectively) of the aerial parts of *C. benghalensis*.

Phytochemical analyses of the crude aqueous methanolic extract of the aerial parts of *C. benghalensis* revealed the presence of alkaloid and flavonoid. Again, total flavonoid content determination assay indicates high amounts of flavonoids in the fractions. There are also reports on the presence of anthocyanins (a kind of flavonoids) in this plant (20,21,31). So the observed bioactivities may be attributed to flavonoid compounds. However, many flavonoids were found to be ligands for the gamma 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. Thus the sedative and anxiolytic effects observed might be due to the interaction of flavonoids with the GABA/benzodiazepine receptor complex in brain (42). This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion (43,44). Electrophysiological experiments with flavone and flavanone derivatives have shown that some of them can modulate GABA-generated chloride currents, either positively or negatively. 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 flavonones have shown that they can modulate gamma aminobutyric acid (GABA)-generated chloride currents, either positively or negatively (45-48). Thus decreased spontaneous motor activity could be attributed to the CNS depressant activity of the aerial parts of *C. benghalensis*.

5. Conclusions

Using behavioral pharmacology models, we have demonstrated that the aerial parts of *C. benghalensis*, in particular chloroform (CFCB) and pet ether (PECB) soluble fractions, possesses strong sedative and anxiolytic potential. Therefore, these fractions could have significant therapeutic utility for the treatment of anxiety and related neuropsychiatric disorders. Furthermore, evidence obtained from the present study may justify the use of this plant in traditional medicine for the treatment of excited mental disorders such as psychosis, insanity, epilepsy, etc.. However, further studies are warranted to understand the underlying mechanism of sedative and anxiolytic activities and to isolate the active phytochemical ingredient(s) responsible for the observed bioactivities in animal models.

Acknowledgements

This research was supported in part by the Ministry of Science, Information and Communication Technology,

Bangladesh. Authors are grateful to Professor Dr. Abdul Ghani, Chairman, Department of Pharmacy, Stamford University Bangladesh.

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(Received August 12, 2009; Revised September 10, 2009; Accepted September 11, 2009)