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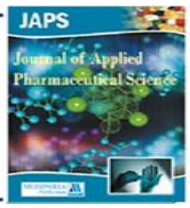

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Central nervous system depressant effects of the ethanolic extract of *Cymbidium aloifolium (L.)*

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ABSTRACT

The ethanolic leaf extract of *Cymbidium aloifolium (L.)* was intended to evaluate the effect on the central nervous system (CNS) using a number of neuropharmacological experimental models in mice. The extract, at the dose of 200 and 400 mg/kg body weight, were shown to have CNS depressant activity by the reduction of locomotor and exploratory activities in the open field and hole cross tests. These results suggest that the extract possess CNS depressant activity. The results of statistical analysis showed that the plant extract had significant, ($p<0.001$) dose dependent, CNS depressant activities when compared to the control.

Keywords: CNS depressant activity, Neuropharmacological, *Cymbidium aloifolium*, locomotor activity.

INTRODUCTION

Cymbidium aloifolium is an orchid found throughout Bangladesh especially in the Chittagong hill tracts (Hossain *et al*., 2009). The plant is reported to have emetic & purgative properties. Leaves have been traditionally used for the treatment of otitis & inflammatory conditions. Salep (flour made from the dried tubers of the orchid) is used as a nutrient & demulcent (Kovacs *et al*., 2007). Dried root powder of *Cymbidium aloifolium* is used to cure paralysis. Tribal people of Chittagong hill tracts use the leaf extract for treating boils and fever. Pasted aerial roots are used for joining fractured bones. It is also used as tonic and in treating weakness of eyes, chronic illness, vertigo, burns and sores. *Cymbidium aloifolium* contains two substituted bibenzyls, dihydrophenanthrene and phenanthraquinone (Cymbinodin-A), which are responsible for biological activity (Hossain *et al*., 2009). Phenanthrenes compounds have been isolated from different plants, mainly in the Orchidaceae family which is used for their cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, antiplatelet aggregation, antiallergic activities & phytotoxicity (Balasubramaniam *et al*., 2000). Few of the biological activities have yet been reported on the leaf part of this plant. Some of the chemical compounds of this plant may possess pharmacological activities. However, no work has been reported on the CNS depressant activities of this plant. That is why the present study has been undertaken to investigate the CNS depressant activities of the crude extract of *Cymbidium aloifolium* in mice.

EXPERIMENT

Plant material

The leaves of *Cymbidium aloifolium* were collected from Baldha Garden,Wari, Dhaka in December 2010 and were identified by the experts of National Herbarium, Mirpur, Dhaka,

Bangladesh (Accession No. **DACB- 35414**) and a voucher specimen was kept for future reference.

Preparation of the Extract

The leaves were thoroughly washed with water. The collected leaves were cut into small pieces & dried in the sun for 15 days. The dried pieces of the plant leaves were ground into fine powder by blender machine. Then the powder was preserved in separate airtight containers for further use. The plant powders (100 gm) were extracted by cold extraction process using ethanol (95% v/v 600 ml) as solvent in a round bottom flask, with occasional shaking & stirring for 7 days. After 7 days the extract was filtered through the cotton & then through the filter paper. Then, the liquid was dried with rotary evaporator to achieve a blackish mass.

Animals

Swiss albino mice (20-30 g) of either sex were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions. The animals were fed with laboratory food and water *ad libitum*. The experiments were done in an isolated and noiseless room.

Drugs and chemicals

Acetic acid & Carrageenan were obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Diclofenac was obtained from Square Pharmaceuticals Ltd., Bangladesh.

Experimental design

The animals were randomly divided into four groups & each group consisting of five mice. The test groups received ELECA (ethanolic leaf extract of *Cymbidium aloifolium)* at the doses of 200 and 400 mg/kg while positive control was treated with diazepam (10 mg/kg) and control with vehicle (1% Tween 80 in water).

PROCEDURE

Open Field Test

The method described by Gupta, Dandiya & Gupta in 1971 was slightly modified & used for screening depressive action of the test drugs on CNS in mice. The animals were divided into control and test groups. The test groups received *Cymbidium aloifolium* ethanolic leaf extracts at the doses of 200 and 400 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 40 cm height wall. The number of squares visited by the animals was counted for 3 mins, at 0, 30, 60 and 90 mins during the study period (Gupta *et al*., 1971).

Hole Cross Test

The method described by Takagi, Watanabe & Saito in 1975 was slightly modified & adopted for determine CNS depressant activity in mice. 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 passages of a mouse through the hole from one chamber to other was counted for a period of 3 mins at 0, 30, 60 and 90 mins after the oral treatment with *Cymbidium aloifolium* leaves ethanolic extracts at the doses of 200 and 400 mg/kg (Takagi *et al*., 1971).

Statistical analysis

The results were expressed as the mean ± SEM (standard error mean). ANOVA (analysis of variance) followed by Dunnett's 't' test was performed as a *post hoc* test to evaluate the statistical significance while taking vehicle treated animals as control; p value of < 0.05 was considered as statistically significant.

RESULTS

Hole cross test

In the open field test, the extract showed a decrease in locomotion in the test animals at both dose levels (200 and 400 mg/kg body weight). The depressant activity was slowly reduced with time. The results were dose dependent & statistically significant (Table 1).

Open field test

In the hole cross test, the extract also showed a decrease in locomotion in the test animals at both dose levels (200 and 400 mg/kg body weight). The results were also dose dependent and statistically significant (Table 2).

Table 1: Effects of the ethanol extract of *C. aloifolium* leaf in mice on hole cross test.

Groups	**Mean movements on open field before and after drug administration**			
	0 min	30 min	60 min	90 min
Control	16.2 ±1.93	13.4 ±1.32	13.2±1.88	11.8 ±1.27
Standard	8 ±2.09**	3.6±1.60**	2.4±0.92**	4.8±2.05**
ELECA (200mg/kg)	11.8±0.96**	8.4±0.50**	8.6±0.67**	9.4±1.36**
ELECA (400mg/kg)	10.2±0.58**	7.8±0.37**	5.2±0.58**	4 ±1.58**

Values are expressed as mean ± SEM, (n = 5); ** $p < 0.001$, Dunnett's 't' test as compared to control. ELECA: ethanolic leaf extract of *Cymbidium aloifolium.*

Table 2: Effects of the ethanol extract of *C. aloifolium* leaf in mice on open field test.

Groups	**Mean hole cross (no.) before and after drug administration**			
	0 min	30 min	60 min	90 min
Control	228.8±3.08	204.4±2.2	190±2.09	181.6±3.15
Standard	123±3.17**	102.4±2.67**	90.8±3.26**	36.2±9.94**
ELECA (200mg/kg)	160±17.19**	145.8±4.65**	114±6.29**	98.4±2.80**
ELECA (400mg/kg)	136.6±14.58**	97.2±3.55**	75.6±6.24**	73.8±10.66**

Values are expressed as mean ± SEM, (n = 5); ** $p < 0.001$, Dunnett's 't' test as compared to control. ELECA: ethanolic leaf extract of *Cymbidium aloifolium.*

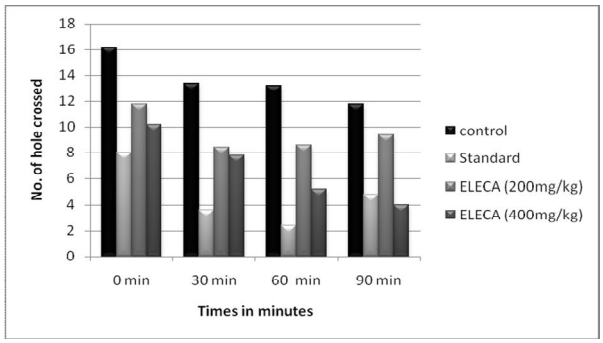

Fig 1: Graphical representations of CNS depressant action by hole cross method.

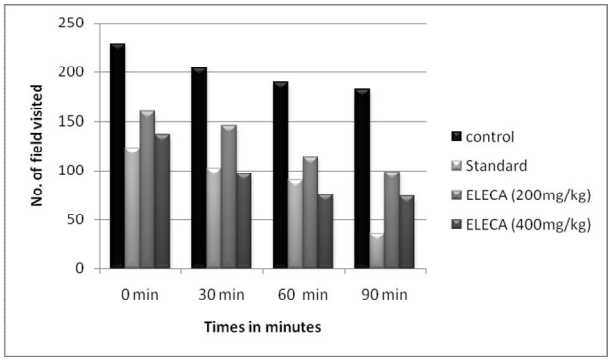

Fig 2: Graphical representations of CNS depressant action by open field method.

Discussion

In the present study, the effect of ethanol extract of *Cymbidium aloifolium* on CNS has been evaluated. The result indicated that the extract significantly decreased locomotor activity which indicates it has CNS depressant activity.

Locomotor activity refers to an increase in alertness and decrease in locomotor activity considered as sedative effect. The major inhibitory neurotransmitter in the central nervous system is Gamma-amino-butyric acid (GABA). Different types of anxiolytic, muscle relaxant, sedative-hypnotic drugs are shown their action through $GABA_A$, that's why the extracts of *Cymbidium aloifolium* may acts by membrane hyperpolarization which potentiating GABA-ergic inhibition in the CNS that leads to either decrease in the firing rate of critical neurons in the brain or direct activation of GABA receptor by the extracts (Khatun *et al*., 2011).

Literature review of the plant reveals that *Cymbidium aloifolium* contains Flavonoids, Reducing Sugar, Cyanogenic glycosides, Terpenoids & Tannin (Maridassa *et al*., 2008). Different types of flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system; which indicate that they act as benzodiazepine-like molecules (Khatun *et al*., 2011).

Earlier pharmacological investigation of the ethanol extract of *Cymbidium aloifolium* was similar to diazepam which led to assume that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, further investigation is needed to determine the exact phytochemicals that are responsible for the biological activities of the ethanol extract of *Cymbidium aloifolium.*

CONCLUSION

In conclusion, it could be suggested that the crude ethanolic extract of *Cymbidium aloifolium* surely possess central nervous system depressant activities. However, further studies are needed to isolate the active principles responsible for the observed activity.

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