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***In vivo* Sedative and Cytotoxic Activities of Methanol Extract of Leaves of *Crataeva nurvala* Buch-Ham**

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Abstract: The present study was designed to investigate the sedative and cytotoxic activities of a crude methanol extract of leaves of *Crataeva nurvala* Buch-Ham. Sedative activity was evaluated by using hole cross, open field and Elevated-Plus Maze (EPM) tests at 400 mg kg⁻¹ body weight. The crude extract decreased the locomotor activity of mice in hole cross, open field and EPM tests. The cytotoxic activity of this extract was determined by brine shrimp lethality bioassay where the LC₅₀ value was found to be 55.46 µg mL⁻¹ as compared to that of 0.451 µg mL⁻¹ exhibited by standard vincristine sulphate. The result shows that the crude extract of the leaves of *C. nurvala* have significant (*p<0.05) sedative and cytotoxic activities.

Key words: *Crataeva nurvala*, sedative, cytotoxicity

INTRODUCTION

Crataeva nurvala Buch-Ham (synonym-*Crataeva magna* (Lour) DC; family-Cappariaceae), commonly known as Bama or Varuna (Bhattacharjee, 1998), is widely distributed throughout Bangladesh, India and tropical regions of the world (Kirtikar and Basu, 1984). It is a deciduous tree which grows upto 7-9 m in height. Traditionally it is useful as laxative, analgesic, antipyretic, anti-inflammatory, antihelminthic, antitumor, diuretic, demulcent, stomachic (Drury, 1978), rubefacient and febrifuge (Walia *et al.*, 2007; Sanayaima *et al.*, 2006). The previous phytochemical studies with *C. nurvala* led to the isolation of dodecanoic anhydride, methyl pentacosanoate (Gagandeep and Kalidhar, 2006), phragmalin triacetate (Haque *et al.*, 2008), lupeol, friedelin, betulinic acid, sterols, saponins and diosgenin of which lupeol is reported to treat hypercrystalluria, hyperoxaluria and hypercalciuria (Anand *et al.*, 1994). In the process of our continuous study with medicinal plants of Bangladesh (Kuddus *et al.*, 2012; Al Amin *et al.*, 2012), the present study has been undertaken to evaluate the sedative activity and cytotoxicity of *C. nurvala* as well as to find out the logical evidence for its folk uses.

MATERIALS AND METHODS

Collection of plant materials: Leaves of *C. nurvala* were collected from Chittagong University campus in April 2012 and identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh.

Animals: Swiss albino mice of both sex and weighing 32-37 g were collected from International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were housed in plastic cages having dimension of 28×22×13 cm under standard laboratory conditions (relative humidity 55-65%, room temperature 23.0±2.0°C and 12 h light: dark cycle) and acclimatized for 7 days and fed with standard diet and water. The ethical guidelines for the investigation of experimental animals were followed in all tests (Bowd, 1980) and the protocol was approved by the institutional committee.

Preparation of extract: The collected leaves were thoroughly washed with water, chopped, air dried for a week at 35-40°C and pulverized with an electric grinder. The powder obtained was extracted with methanol at room temperature for 7 days with occasional shaking and

stirring. The filtrate so obtained was concentrated to dryness by evaporation of solvent using a rotary evaporator under reduced temperature and pressure.

Assay for sedative activity

Open field test: This experiment was carried out according to published method (Gupta *et al.*, 1971) where 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 high. The number of squares visited by the mice was counted for 3 min, on 0, 30, 60, 90 and 120 min during the study period.

Hole cross test: This test was done for CNS depressant activity in mice (Takagi *et al.*, 1971). The animals were divided into three groups-negative control, positive control and test animals. The test groups received methanol extract of *C. murvala* leaves at 400 mg kg⁻¹ b. wt orally whereas the control group received vehicle (1% Tween 80 in water). A steel partition was made 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 total number of passages of a mouse through the hole from one chamber to another was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral administration with test substances. In this test, diazepam (1 mg kg⁻¹ b.wt.) was used as the positive control.

Elevated plus maze (EPM) test: This experiment was performed by the method, the experimental details of which could be found elsewhere (Lister, 1987). It utilizes an equipment consisted of two open arms (5×10 cm) and two closed arms (5×10×15 cm) radiating from a platform (5×5 cm) to give the apparatus a plus sign in appearance. The apparatus was situated 40 cm above the floor in which 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. The maze floor and walls were made with dark opaque wood. Sixteen minutes after administration of the test agents, each animal was placed at the center of the maze facing one of the enclosed arms. During the five min test period, the number of open arms entries was recorded. The entry into an arm was defined as the point when the animal places all four paws onto the arm. This procedure was conducted in a sound free room and observations made from an adjacent corner.

Cytotoxic activity: The cytotoxicity assay was performed on brine shrimp nauplii by standard method (Meyer *et al.*, 1982) using vincristine sulphate as standard. The test sample was prepared by dissolving in

dimethyl sulfoxide solution, DMSO (not more than 50 µL in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 25, 50, 100, 200, 400 and 800 µg mL⁻¹. A vial containing 50 µL DMSO diluted to 5 mL⁻¹ with sea water was used as negative control. Then matured shrimps were applied to each of all experimental and control vials. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From the data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\text{Mortality (\%)} = N_i/N_0 \times 100$$

where, N_i is Number of killed nauplii after 24 h of incubation, N_0 is Number of total nauplii transferred i.e., 20. The median lethal concentration, LC_{50} was then determined using Probit analysis.

Statistical analysis: All data were expressed as mean±STD and were analyzed by one way ANOVA followed by using Dunnett's test. The difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Sedative activity: Open field test: In this test, the number of squares traveled by the mice was suppressed significantly in the test group throughout the study period. The CNS depressant activity obtained for the extract was statistically significant (Table 1). About 30 and 90 min after the administration of the extract, the number of movements were 58.33 and 11.0 per min as compared to that of 45.99 and 9.33 displayed by standard diazepam.

Hole cross test: The total number of hole crossed from one chamber to another by the mice was counted for control, standard and test groups (Table 2). In the hole cross test, the extract showed a decrease in locomotor activity in the test animals during observation period as evident by the reduction in number of hole crossed by the treated mice (number of movements after 120 min is 3.66 per minute) as compared to the control group. The result was comparable to the reference drug diazepam and was statistically significant ($p < 0.05$).

Elevated plus maze (EPM) test: Result of EPM test is presented in Table 3. The extract at the dose of 400 mg kg⁻¹ body weight, significantly decreased the percentage of entries of mice into the open arms as well as the percentage of time spent in the open arms of the EPM.

Table 1: CNS depressant activity of crude methanol extract of *C. nurvala* as determined by open field test in mice

Group	Treatment	Dose	Route	No. of movements				
				0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in water	10 mL kg ⁻¹ b.wt.	p.o	55.66±6.55	50.33±4.45	39.00±5.22	37.33±4.21	39.99±3.61
Standard	Diazepam	1 mg kg ⁻¹ b.wt.	i.p.	70.33±2.55	45.99±3.11	26.00±5.12*	9.33±6.12*	11.33±2.66*
Test	CM extract	400 mg kg ⁻¹ b.wt.	p.o	80.33±5.71	58.33±8.12*	19.33±7.12	11.00±9.72*	5.33±8.77*

Values are expressed as mean±SD (n = 5), CM: Crude methanolic extract of leaf of *C. nurvala*. One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Table 2: CNS depressant activity of crude methanol extract of *C. nurvala* by hole cross test in mice

Group	Treatment	Dose	Route	Number of movements				
				0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in water	10 mL kg ⁻¹ b.wt.	p.o	16.33±2.33	15.99±2.19	11.66±3.31	7.99±0.52	5.33±0.612
Standard	Diazepam	1 mg kg ⁻¹ b.wt.	i.p.	18.99±2.74	9.66±3.21	7.00±2.94*	4.66±1.64*	3.00±1.21*
Test	CM extract	400 mg kg ⁻¹ b.wt.	p.o	12.33±4.41	7.33±4.23	6.66±0.87*	5.66±0.88*	3.66±1.32*

Values are expressed as mean±SD (n = 5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Table 3: CNS depressant activity of methanolic extract of leaves of *C. nurvala* by elevated plus maze test in mice

Group	Treatment	Dose	Route	Entry into open arm (%)	Time spent in open arm (%)
Control	1% Tween 80 in water	10 mL kg ⁻¹ b.wt.	p.o	54.41±3.24	40.45±4.23
Standard	Diazepam	1 mg kg ⁻¹ b.wt.	i.p.	39.11± 4.78*	36.60±3.42*
Test	CM extract	400 mg kg ⁻¹ b.wt.	p.o	38.55±8.41*	35.50±1.64*

Values are expressed as mean±SD (n = 5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Now a day scientists are trying to produce low cost suitable medicines for the treatment of neurological disorders like seizure, anxiety, panic, agitation and insomnia. Gama- aminobutyric acid (GABA) is the major neurotransmitter in the brain which ends up producing an inhibitory effect on neurotransmitter release by binding with GABA receptor resulting hyperpolarization of the neuron and diminishing the chance of occurring a successful action potential. The pharmacological action of sedative drugs increases the effect of the GABA neurotransmitter by binding to the benzodiazepine site on the GABAA (via the constituent chlorine atom) leading to the depression of central nervous system (Riss *et al.*, 2008). The study has examined some neuropharmacological activities of methanolic extract of *C. nurvala*. The plant extract was found to possess central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in hole cross, open field and EPM tests. So, the methanolic extract of *C. nurvala* leaves may produce sedative action by hyperpolarization of the CNS via GABA receptor in the brain.

Cytotoxic activity: The brine shrimp lethality bioassay is rapid (24 h), simple (e.g., no aseptic techniques are required), easily mastered, inexpensive and requires small amounts of test material (2-20 mg or less). The bioassay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity. Since its introduction, this *in vivo* lethality test has been

successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays once the active compounds have been isolated.

The LC₅₀ value of the extract was 55.46 µg mL⁻¹ and that for standard vincristine sulphate was 0.451 µg mL⁻¹ which showed. No mortality was found in the control group, using DMSO and sea water. The result of brine shrimp lethality bioassay was also promising. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp nauplii was found to be increased with the increase in sample concentration.

CONCLUSION

In conclusion, we can say that *Crataeva nurvala* Buch-Ham contains chemical constituents having sedative and cytotoxic activity. This could provide a rationale for traditional uses of the plant as antitumor agent and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

REFERENCES

- Al Amin, M., I.A. Chowdhury, K.M.M. Mahbub, M. Sattar, M. Shahriar, M.R. Kuddus and M.A. Rashid, 2012. Anti-inflammatory and analgesic activities of *Asteracantha longifolia* Nees. Bangladesh Pharm. J., 15: 171-176.

- Anand, R., G.K. Patnaik, D.K. Kulshershta and B.N. Dhawan, 1994. Proceeding 24th Indian Pharmacological Society Conference. Ahmedabad, Gujarat, India, A10.
- Bhattacharjee, S.K., 1998. Handbook of Medicinal Plants. Pointer Publishers, Jaipur, Pages: 228.
- Bowd, A.D., 1980. Ethics and animal experimentation. Am. Psychol., 35: 224-225.
- Drury, H., 1978. The Useful Plants of India. International Book Distributors, Dehradun, India, Pages: 353.
- Gagandeep, Meera and S.B. Kalidhar, 2006. Chemical constituents of *Crataeva nurvala* (Buch-ham) leaves. Indian J. Pharm. Sci., 68: 804-806.
- Gupta, B.D., P.C. Dandiya and M.L. Gupta, 1971. A psychopharmacological analysis of behavior in rat. Jap. J. Pharmacol., 21: 293-298.
- Haque, M.E., M.N. Islam, D.D. Gupta, M. Hossain, H.U. Shekhar and B.A. Shibib, 2008. Triterpenoids from the stem bark of *Crataeva nurvala*. Dhaka Univ. J. Pharm. Sci., 7: 71-74.
- Kirtikar, K.R. and B.D. Basu, 1984. Indian Medicinal Plants. Vol. 2, Bishan Singh Mahendra Paul Singh, Dehradun, Pages: 830.
- Kuddus, M.R., M.S. Alam, S.R. Chowdhury, F. Rumi, M.A. Sikder and M.A. Rashid, 2012. Evaluation of membrane stabilizing activity, total phenolic content, brine shrimp lethality bioassay, thrombolytic and antimicrobial activities of *Tagetes patula* L. J. Pharmacogn. Phytochem., 1: 57-63.
- Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology, 92: 180-185.
- Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.
- Riss, J., J. Cloyd, J. Gates and S. Collins, 2008. Benzodiazepine in epilepsy: Pharmacology and pharmacokinetics. Acta Neurol. Scand., 118: 69-86.
- Sanayaima, R.K., A. Kaur, A. Agrawal and S.B. Babbar, 2006. Cryopreservation of *in vitro*-grown shoot tips of *Crataeva nurvala*, Buch. Ham, an important medicinal tree. Cryoletters, 27: 375-386.
- Takagi, K., M. Watanabe and H. Saito, 1971. Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethane. Its acylates on the central nervous system. Jap. J. Pharmacol., 21: 797-810.
- Walia, N., A. Kour and S.B. Babbar, 2007. An efficient, *in vitro* cyclic production of shoots from adult trees of *Crataeva nurvala* Buch. Ham. Plant Cell Rep., 26: 277-284.