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Analgesic and Neuropharmacological Activities of Methanol Extract From the Leaf of *Nicotiana plumbaginifolia* Viv.

Mehedi Shahriar, Sadia Afreen Chowdhury, Mohammad Zafar Imam and Bidyut Kanti Datta

Laborarory of Pharmacognosy and Phytochemistry, Department of Pharmacy, Stamford University Bangladesh, Dhaka-1217, Bangladesh

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Abstract

The aim of study was to evaluate the analgesic and neuropharmacological activities of the leaf of *Nicotiana plumbaginifolia*. The central analgesic activity of the methanolic extract was evaluated in Swiss Albino mice by using tail immersion, hot plate and acetic acid-induced writhing tests at 100, 200 and 400 mg/kg body weight. These tests showed significant analgesic activity of the extract compared to the standard drug, diclofenac-Na (1 mg/kg b.w.). The neuropharmacological activities were evaluated using hole cross, open field, light/dark box and elevated plus maze stage tests in mice model at 100, 200 and 400 mg/kg body weight. The results of neuropharmacological assays demonstrated potential CNS depressant activity of the extract when compared to the standard drug, diazepam (1 mg/kg b.w.).

Key words: *Nicotiana plumbaginifolia*, Solanaceae, Analgesic, Neuropharmacology.

Introduction

Nicotiana plumbaginifolia Viv. is commonly known as Tex-Mex tobacco, Wild tobacco (Bengali Name: Bon tamak) belongs to the family Solanaceae. It is an annual or perennial weed with hairy stem, which is originated from Mexico and West Indies. It is found road side in damp environment. It attains a height up to 60 cm with spreading radical and slender leaf branches. In India, it has great medicinal properties such as antispasmodic, diuretic, expectorant. It is widely used in the treatment of several human ailments like rheumatism, swelling in order to relieve the pain. Dried leaves are used in the treatment of nausea and travel sickness (Singh *et al.*, 2010).

The plant *N. plumbaginifolia* has traditional and contemporary uses. The latex is used to treat tertiary syphilitic ulceration. The sap from the leaves is used against sores. A decoction of the bark is highly praised for the treatment of tertiary syphilis, while the pounded roots are used against hydrocele and orchids. An infusion of the inner leaves or root is drawn into the nostrils in treating ulceration due to syphilis (Dhar *et al.*, 1968). *N. plumbaginifolia* is used traditionally to treat constipation, haemorrhoidal bleeding, strangulated hernia (smoke by mouth) and malaria or intermittent fever (Anne, 2004). It is also a very common traditional medicinal plant in Bangladesh, Argentina, India, Peru, and USA.

Materials and Methods

Chemicals: Drugs and chemicals used in the study include acetic acid (Merck, Germany), methanol (Merck, Germany), DMSO (Merck, Germany), diclofenac-Na and diazepam (Square Pharmaceuticals Ltd., Bangladesh).

Plant materials and extraction: The leaves of *N. plumbaginifolia* were collected from Bangladesh National Herbarium, Mirpur, Dhaka in August, 2013. The plant was identified by the scientist of Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen has been deposited for future reference. The powdered leaf (500g) was soaked in 2L of methanol for 20 days and then the extract was filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50) °C and reduced pressure to have greenish extract (20 g).

Animals: For the experiment, 2 to 3 weeks old male Swiss Albino mice weighing between 20 to 25 g were collected from the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B). Soft wood powder was used as

Correspondence to: Bidyut Kanti Datta; Tel.: +880-2-835512-14, ext. 256, 277); E-mail: dattabidyut@yahoo.com

bedding of cages. Animals were maintained under standard environmental conditions: temperature (24.0±1.0 °C), relative humidity (55-65%) and 12 h light /12 h dark cycle. Husk and excreta were removed from the cages every day (Hasan *et al.*, 2009).

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, while diclofenac-Na was administered intraperitonially 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 (Hossain *et al*., 2009).

Tail immersion test: The tail-flick test was conducted as per the method modified by D'Amour and Smith (1941). The screening cut-off time was 5 sec, while the test cut-off time was set as 10 sec. The extract was administered orally at three doses (100, 200 and 400 mg/kg body weight) using diclofenac-Na as standard. The post drug reaction times were measured at 0, 30, 60 and 90 minutes later. The tail of the mouse was immersed to a constant level (3 cm) in a water bath maintained at 55 ± 0.5 °C. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 10 seconds was maintained to prevent thermal injury to the animals. A significant increase in reaction time compared to control animals was considered a positive analgesic response (D'Amour and Smith, 1941)**.**

Hot plate test: Hot plate test was used to measure the response latencies based on the procedure described by Eddy and Leimbach (1953). In this experiment, the hot plate was maintained at 50 ± 0.05°C. The reaction time was recorded for animals pretreated with DMSO (10 ml/kg b.w. 30 min before orally) as control, methanol extract leaf (100, 200 and 400 mg/kg body weight 30 min before). Here, diclofenac-Na (1 mg/kg, b.w. intraperitonially, 15 min before) was used as positive control. Animals were placed onto the hot plate chamber and the time of latency was determined. The latent period of response was taken as the index of antinociception and was determined at the pre treatment and after 30 min, 60 min and 90 min after administration of the test drug and standard in the order to minimize the damage on the animal paws. The cut off time was taken as 20 s (Eddy and Liembach, 1953).

Hole cross test: A wood 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 negative control, positive control, and test groups containing five mice each. The test groups received leaf extract of *N. plumbaginifolia* at 100, 200 and 400 mg/ kg body weight orally where as the negative and positive control groups received vehicle (0.1 ml/mice distilled water) and the standard drug diazepam (1 mg/kg b.w.), respectively. 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 and 90 min after oral administration of the test drugs and the standard (Gupta *et. al*., 1971).

Open field test: The animals were divided into negative control, positive control, and test groups containing five mice each. The test groups received leaf extract of *N. plumbaginifolia* at 100, 200 and 400 mg/ kg body weight orally where as the negative control group received distilled water (0.1 ml/mice). Like hole cross test, animals in positive control group received diazepam (1mg/kg b.w.). 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 and 90 min after oral administration of the test drugs and the standard (Gupta *et. al*., 1971).

Light/dark box test on mice: The test was performed as per the method modified by Hascoet and Bourin (1998).

Elevated plus maze test: The test was performed as per the method modified by Komada *et al*., (2008). Though 5 min recording was common, the behavior was recorded for 10 min in our protocol to increase the opportunity to detect the phenotype. The open and closed elevated arms induce an exploration conflict. The measures of the elevated plus maze test are recorded by an observer during the experiment.

Results and Discussion

The leaf of methanol extract of *N. plumbaginifolia* was administered to the mice at 100, 200 and 400 mg/ kg

body weight to determine the effects on acetic acid-induced writhing in mice. The extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner which was comparable to the reference drug, diclofenac-Na (Table 1).

Dose dependent reduction in the number of abdominal constriction was observed in animals treated with different concentration of the methanol extract of *N. plumbaginifolia* at 100, 200 and 400mg/kg body weight and the inhibition of writhing response was observed as 39.38%, 47.32% and 33.22% respectively, as compared to the inhibition of writhing response by diclofenac-Na which was 60.12%.

The tests for analgesic activity were carried out in the laboratory on five groups of mice by tail-flick and hot plate method. Time interval for the test was 30 minutes and the results are given in Tables 2 and 3.

The tail withdrawal reflex time following administration of the leaf extract of *N. plumbaginifolia* was found to increase with increasing dose of the extract.

Table 1. Effect of leaf extract of *N. plumbaginifolia* in acetic acid-induced writhing test on mice.

Test group	Doses (mg/kg b.w.)	Number of writhings	% Inhibition
Control (Purified water)	0.1 ml/mice	61.7 ± 1.47	0.00
Diclofenac-Na	1.0	24.6 ± 0.78	60.12
Group-I	100	37.4 ± 0.60	39.38
Group-II	200	32.5 ± 0.97	47.32
Group-III	400	41.2 ± 0.73	33.22

Values are presented as mean ± SEM, where n= 5.

Table 2. Results of tail-flick test for plant extract of *N. plumbaginifolia*.

Test group	Dose (mg/kg b.w.)	Number of movements			
		0 min	30 min	60 min	90 min
Control (DMSO+ Water)	0.1ml/mice	2.34 ± 0.574	1.7 ± 0.228	1.28 ± 0.073	1.54 ± 0.2
Diclofenac-Na	5.0	1.16 ± 0.112	1.62 ± 0.8	1.22 ± 0.159	1.16 ± 0.0975
Group-l	100	1.8 ± 0.207	1.22 ± 0.1015	1.4 ± 0.202	1.54 ± 0.112
Group-ll	200	1.94 ± 0.1435	2.4 ± 0.4515	2.1 ± 0.2165	1.68 ± 0.188
Group-lll	400	2.44 ± 0.4695	1.8 ± 0.2545	2.06 ± 0.1565	1.76 ± 0.1745

Values are presented as mean ± SEM, where n= 5.

Table 3. Results of hot plate test for plant extract of *N. plumbaginifolia*.

Test group	Dose (mg/kgb.w.)	Number of movements			
		0 min	30 min	60 min	90 min
Control (DMSO+ Water)	0.1ml/mice	11.11 ± 2.165	6.91 ± 0.875	8.49 ± 1.365	10.504 ± 2.00
Diclofenac-Na	5.0	3.496 ± 0.358	5..464 ± 0.710	3.926 ± 0.210	4.354 ± 1.02
Group-l	100	10.734 ± 2.75	8.824 ± 1.689	15.102 ± 3.365	9.852 ± 2.505
Group-ll	200	10.743 ± 2.528	6.404 ± 1.124	8.592 ± 1.498	8.256 ± 1.39
Group-lll	400	5.888 ± 1.760	5.438 ± 1.282	9.036 ± 2.279	7.05 ± 1.299

Values are presented as mean ± SEM, where n= 5.

The data obtained clearly indicated that the leaf extract *of N. plumbaginifolia* showed highly significant dose dependent central analgesic effect in tail-flick (Table 2) and hot plate (Table 3) tests.

The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animals. The activity is a measure of the level of excitability of the CNS and any decrease in CNS activity may be closely related to sedation resulting from depression of the central nervous system. The CNS

potential of the extract was evaluated using hole cross (Table 4), open field (Table 5), light/dark box (Table 6) tests and elevated plus maze stage test (Table 7) to understand the CNS activity in Swiss Albino mice at 100, 200 and 400 mg/kg b.w. The results of these tests revealed potential CNS depressant activity of the extract which was comparable to the standard drug, diazepam (1 mg/kg b.w.).

Both hole cross and open field tests showed that the depressing activity of the methanolic leaf extract was evident from the 2nd observation period in the test animals at the doses of 200 and 400 mg/kg body weight of mice. Maximum depressant effect was observed from 2nd (60 min) to 4th (90 min) observation period at the dose of 400 mg/kg b.w. The results were also dose dependent.

Table 4. Effect of leaf extract of *N. plumbaginifolia* in hole cross test on mice.

Test group	Dose (mg/kg b.w.)	Number of movements			
		0 min	30 min	60 min	90 min
Control	0.1ml/mice	10 ± 1.732	8.4 ± 2.441	4.6 ± 1.913	2.8 ± 0.663
Diazepam	1.0	13.4 ± 2.580	6.2 ± 0.734	3.4 ± 0.678	3.8 ± 1.2
Group-l	100	3.6 ± 1.077	4.4 ± 0.979	2.6 ± 1.02	3.2 ± 1.067
Group-ll	200	8 ± 1.923	6.8 ± 0.663	4 ± 1.264	2 ± 0.547
Group-lll	400	8.8 ± 1.907	7.2 ± 1.280	4.8 ± 1.019	3 ± 0.547

Values are presented as mean ± SEM, where n = 5.* p < 0.05 compared with the control group (Dunnett's test).

Table 5. Effect of leaf extract of *N. plumbaginifolia* in open field test on mice.

Test group	Dose (mg/kg)	Number of movements			
		0 min	30 min	60 min	90 min
Control	0.1ml/mice	54.0 ± 11.679	56.2 ± 16.653	41.4 ± 13.919	28.2 ± 1.2
Diazepam	1.0	113.8 ± 16.611	55.2 ± 33.026	65.0 ± 27.114	26.0 ± 9.327
Group-l	100	25.2 ± 11.888	25.2 ± 7.418	10.2 ± 3.760	8.8 ± 3.878
Group-ll	200	65.4 ± 12.019	30.8 ± 7.651	23.6 ± 3.841	16.4 ± 5.24
Group-lll	400	91.0 ± 10.940	52.8 ± 3.954	18.2 ± 1.984	13.2 ± 2.764

Values are presented as mean ± SEM, where n= 5.* p < 0.05 compared with the control group (Dunnett's test).

Table 6. Effects of leaf extract of *N. plumbaginifolia* in the light/dark test in mice.

Test group	Doses (mg/kg b.w.)	Latency	Transition	Time spent in light compartment (s)	Time spent in dark compartment (s)
Control (Purified water)	0.1ml/mice	35.6 ± 12.70	12.6 ± 1.02	135.6 ± 15.15	184.4 ± 26.18
Diazepam	10	17.8 ± 7.39	19.6 ± 1.49	76.6 ± 5.98	223.4 ± 5.98
Group-I	100	13.8 ± 3.67	4.2 ± 0.58	79.4 ± 20.71	220.6 ± 20.71
Group-II	200	23.6 ± 5.09	15.6 ± 0.74	105.8 ± 1.15	194.2 ± 1.15
Group-III	400	17.4 ± 4.53	9.8 ± 3.13	72.4 ± 19.55	227.6 ± 19.55

Values are presented as mean ± SEM, where n = 5. * p < 0.05 compared with the control group (Dunnett's test).

Table 7. Effects of leaf extract of *N. plumbaginifolia* in elevated plus maze test in mice.

Test group	Doses (mg/kg)	Time spent in open arm (s)	Entries in open arm	Time spent in closed arm (s)	Entries in closed arm
Control (Deionized water)	0.1ml/mice	22.8 ± 9.11	2.4 ± 0.81	286.2 ± 17.62	10.2 ± 2.31
Diazepam	1.0	69.6 ± 6.51	8.2 ± 1.42	262.6 ± 8.12	14.4 ± 2.22
Group-I	100	5.8 ± 3.63	0.6 ± 0.4	343.8 ± 2.39	3.8 ± 0.96
Group-II	200	11.0 ± 6.92	1.0 ± 0.54	318.4 ± 11.24	8.8 ± 2.49
Group-III	400	6.0 ± 3.47	1.0 ± 0.31	335.6 ± 6.80	3.6 ± 2.03

Values are presented as mean ± SEM, where n = 5. * p < 0.05 compared with the control group (Dunnett's test).

Conclusion

Based on the results of the present studies, it can be concluded that the crude leaf extract of *N. plumbaginifolia* possesses neuropharmacological effects and analgesic potential. At higher dose, simple analgesic activity was observed from hot plate, tail-flick and acetic acid-induced writhing tests. Significant activity can also be suggested from the results of neuropharmacological test. Dose dependant activity was observed in all the performed pharmacological investigations. Hence, further studies are suggested to pinpoint the bioactive compounds from the extract of *N. plumbaginifolia* and to better understand the mechanism of such action scientifically.

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