- Short communication

Analgesic Activity of Methanolic Extracts of *Nerium indicum* Mill.

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Nerium indicum Mill. (Bengali name: Korobi or Gundari, Family: Apocynaceae) is a large, branched shrub.[1] It is widely distributed in India, Bangladesh and Nepal and is extensively cultivated throughout the greater part of India as well as in China and Japan.[1] The leaves and the flowers are cardiotonic, diaphoretic, diuretic, emetic, expectorant and sternutatory.[2-4] The whole plant is said to have anticancer properties.[4] Previous phytochemical studies revealed the presence of cardioactive glycosides, formerly designated as neriodorin, neriodorein and kabarin in the roots and bark. The bark also contains scopoletin and scopolin.[5-6]

As a part of our continuing studies on the medicinal plants of Bangladesh, we report here the analgesic activity of *Nerium indicum*.

The plant *Nerium indicum* Mill. (Family-Apocynaceae) was collected from Brac nursery in Pabna, Bangladesh in April, 2004 and was taxonomically identified by the taxonomist of the herbarium of the Department of Botany, University of Dhaka, Bangladesh. The sun dried, coarsely powdered flower (75 g), root (100 g), stem (750 g) and leaves (550 g) were extracted separately with distilled methanol at room temperature. Then the extracts were filtered and the filtrates thus obtained were concentrated with a rotary evaporator and were subsequently defatted to get dried extracts.[7] The yield of flower, root, stem and leaf extracts was 5.4, 3.7, 26.7 and 38.0 g, respectively. The methanolic leaf extract was further fractionated with *n*-hexane, ethyl acetate and methanol to get 7.7, 7.8 and 18.7 g fractions, respectively. The analgesic activity of the extracts was investigated on Swiss Albino mice of either sex, aged 4-5 weeks. The weight of the mice used ranged from 20-24 g. They were purchased from Animal Research Branch of the International Centre for Diarrhoeal Diseases & Research, Bangladesh (ICDDR' B). The animals were supplied with formulated food pellets and water *ad libitum.* To keep the hydration rate constant, the food and water were withdrawn 12 hrs before the experiment. In order to administer the crude extracts at doses of 500, 250 and 125 mg/kg body wt. of mice, 500, 250 and 125 mg of the crude extracts were added separately to the mortar containing approximately 5 ml of normal saline solution with continuous stirring in one direction. Then, one drop of Tween-80 was added to each mortar. After proper mixing, the final volume of the suspension of each extract was made 10 ml by adding normal saline. To stabilize the suspension, it was stirred well by vortex mixture. For the administration of aminopyrine (Sigma, USA) at the dose of 50 mg/kg body weight, 12.5 mg of aminopyrine was taken and 2.5 ml of suspension was made with Tween-80 and normal saline. The *in vivo* analgesic activity of the different crude extracts and

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fractions was studied by the acetic acid induced writhing method.[8] In this method, 0.7% (v/v) acetic acid (0.1 ml/10 g body wt.) is administered intraperitoneally to the experimental animals to produce writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within a given time frame and with respect to the control group. The inhibition of writhing in mice by the plant extract was compared against inhibition of writhing by a standard analgesic, aminopyrine. At zero hour, test samples, control (1% Tween-80 solution in normal saline), aminopyrine were administered orally by means of a long needle with a ball-shaped end. After 40 minutes, acetic acid (0.7% v/v) was administered intraperitoneally to each of the mice of all groups. Five minutes after the administration of acetic acid, number of writhing were counted for each mouse for fifteen minutes. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A P value < 0.05 was considered significant.

In acetic acid induced writhing model, the flower extract of *Nerium indicum* showed 89.14% (p <0.001) and 93.20% (p <0.001) inhibition of writhing response at oral doses of 250 mg/kg and 500 mg/kg body weight of mice, respectively (Table 1). The root extract showed prominent analgesic activity with 59.18% (p < 0.001) and 95.92% (p <0.001) writhing inhibition at oral doses of 125 mg/kg and 250 mg/kg body weight of mice, respectively (Table 1). The results were found to be highly promising (p < 0.001) in comparison to the control and accompanied with dose dependence. The stem extract showed only 6.78% and 27.89% inhibition of writhing response at oral doses of 125 mg/kg and 250 mg/kg body weight of mice, respectively (Table 1). The analgesic activity of stem extract was less significant as compared to that of crude flower and root extracts.

Table 1. Analgesic activity of different extracts of *Nerium indicum* in mice.

Treatment	Dose (mg/kg, p.o.)	Writhings[a]	% Writhing	% Writhing inhibition
Control (1% Tween-80 solution in normal saline)	10ml/kg	12.25 ± 0.60	100	-
Flower extract	250	1.33 ± 0.44 **	10.86	89.14
	500	0.83 ± 0.28 **	6.80	93.20
Stem extract	125	11.42 ± 0.27	93.22	6.78
	250	8.83 ± 0.24 **	72.11	27.89
Root extract	125	5.0 ± 0.18 **	40.82	59.18
	250	0.5 ± 0.22 **	4.08	95.92
Leaf extract	250	0	0	100.00
	500	0	0	100.00
Aminopyrine	50	4.5 ± 0.36 **	36.73	63.27
One-way ANOVA	F	182		
	Df	7, 40		
	P	< 0.0001		

[a] Values are mean ± SEM (n= 6); One-way ANOVA; ** P < 0.001, compared to control.

All the fractions of crude leaf extract of *Nerium indicum* showed 100% inhibition of writhing reflex. This indicates that administration of the fractions of crude leaf extract inhibited the pain sensation produced by acetic acid since the mice did not show any writhing reflex during this investigation.

Acetic acid induced abdominal constriction is a sensitive procedure to establish peripherally acting analgesics. The response is thought to be mediated by the prostaglandin pathways.[9] The promising antinociceptive activity of the methanolic extract of flower and root of *Nerium indicum* might be due to

the presence of analgesic principles which interfere in the biosynthesis of prostaglandins and some other autacoids.

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