ORIGINAL ARTICLE

ANTI-INFLAMMATORY AND ANALGESIC ALKALOID FROM *SIDA CORDIFOLIA* LINN.

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ABSTRACT

The analgesic and anti-inflammatory activities of a new alkaloid, 1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin -3-ylamine (compound 1) isolated from *Sida cordifolia* Linn. were investigated in animal models. In the acetic acid induced writhing model, the compound 1 showed 25.4 ($P<0.05$) and 52.43% ($P<0.01$) inhibition of writhing response at doses of 25 and 50 mg/kg body weight respectively. The alkaloid also produced significant increase in the tail flick latency in radiant heat tail-flick method. In Carrageenan induced rat paw edema the compound 1 produced 16.93 and 24.43 % inhibition of paw edema at the doses of 25 and 50 mg/kg body weight respectively at the third hour of study.

Keywords: *Sida cordifolia*, analgesic, anti-inflammatory activity.

INTRODUCTION

Sida cordifolia Linn. (Family: Malvaceae.) commonly known as brela (Bengali) is a herb which grows 3-5 feet in height and is widely distributed in various parts of Bangladesh. The plant is extensively used as a common herbal drug in the Indian subcontinent (Kirtikar and Basu, 1980). The water extract of the leaves was reported to possess analgesic and anti-inflammatory activities in animal models (Franzotti et al, 2000). The roots, leaves, stems and seeds of the plant are used in the traditional medicine against chronic dysentery, asthma and gonorrhea in the subcontinent (Chopra et al, 1958; Yusuf and Kabir, 1999). The water extract of the whole plant is specially used in the treatment of rheumatism (Yusuf and Kabir, 1999). Previous phytochemical investigations on the roots of this plant have shown the presence of ephedrine, vasicinol, vasicinone and N- methyl tryptophan (Asha and Bannerjee, 1985; Gunatilaka et al, 1980; Ghosh and Dutt, 1930). In continuation of our studies on medicinal plants available in Bangladesh for their chemical constituents and biological activities we isolated 1,2,3,9-Tetrahydro-pyrrolo [2,1-b] quinazolin -3-ylamine (Structure shown in figure 1) from the aerial parts of *S. cordifolia* Linn. In the present paper we report the analgesic and anti-inflammatory effects of the alkaloid on animal models.

MATERIALS AND METHODS

Plant materials

The aerial parts of *S. cordifolia* were collected from the hilly region of the district of Chittagong situated in the south-eastern region of Bangladesh and identified by the experts of National Herbarium of Bangladesh where a voucher specimen (accession No. 31238) was deposited.

Extraction and isolation of the compound

The air-dried aerial parts of the plant were pulverized to powder (5.5 kg) and were successively extracted with chloroform (3 ×72h), methanol (3 ×72h) and 80% ethanol (3 ×72h). The methanol extract (30 g) was acidified (pH 2) with 2M hydrochloric acid and the final volume was adjusted to 400 ml. The aqueous acidic solution was then extracted with ethyl acetate (3 × 300 ml) to remove neutral components. After removal of neutral components the aqueous layer was then made alkaline (pH 9) with 30% ammonium hydroxide solution and repeatedly extracted with ethyl acetate (3 X 300 ml). The combined extract was washed with water, dried, and evaporated under reduced pressure to yield the crude alkaloid (4.2 g) as a solid brown mass H. The compound 1 (112 mg) was separated from mass H by using preparative TLC technique where (1:1:1) methanol, ethyl acetate and chloroform was used as a developing solvent system. The compound was characterized and identified by analyzing

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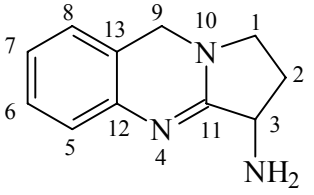
its spectral data. UV λ_{max} (MeOH) nm: 293, 232. IR ν_{max} (KBr) cm^{-1}: 3450 (N-H), 3071 (C-H, aromatic) 1635 (C=N). ^{1}H-NMR (CD_3OD) δ: 1.98 (2H, m, H-C2), 2.3 (2H, m H-C1), 3.30-3.40 (2H, m, NH_2), 4.61 (1H, t, H-C3), 4.58 (2H, dd, H-C9), 6.97 (3H, m, H-C5,6,7) and 7.13 (1H, t, J = 14.8, H-C8). ^{13}C-NMR (CD_3OD) δ: 47.74 (C1), 30.49 (C2), 72.40 (C3), 120.68 (C5), 125.70 (C6), 124.05 (C7), 129.35 (C8), 49.63 (C9), 164.46 (C11), 142.99 (C12) and 120.68 (C13). Mass fragments m/e (%): 187 (100) [M^+], 171 (15), 159 (16), 131 (18), 104 (10) etc.



1,2,3,9-Tetrahydro-pyrrolo [2,1-b] quinazolin -3-ylamine

Fig. 1: Structure of compound 1.

Drugs and chemicals

The following chemicals and drugs were used: aminopyrine (Sigma-USA), acetic acid (Merck, Germany), morphine (Jayson Pharmaceuticals Ltd., Bangladesh), Carrageenan (Sigma-USA) and phenylbutazone (Sigma- USA).

Animals

Swiss albino mice (20-25 g) and Long Evans rats (140-160 g) of either sex were obtained from the animal house of International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were given standard feed developed by ICDDR, B and water *ad libitum* and kept in the laboratory environment (12 h dark/12 h light cycle) for seven days for acclimatization. Animals were fasted for overnight and weighed before the experiment.

Acetic acid induced writhing test

The peripheral analgesic activity was determined by the acetic acid induced writhing inhibition method (Whittle, 1964). The pre-screened Swiss albino mice employed for this experiment were divided into groups shown in table 1. The test compound 1 was given p.o .in suspension of 2% Tween 80 at doses of 25 and 50 mg/kg *body weight* and the control group received the vehicle only. The inhibition of writhing in mice by compound 1 was compared against inhibition of writhing by a standard analgesic agent, aminopyrine given p.o. at a dose of 50 mg/kg body weight. Acetic acid (0.7%) at a dose of 0.1 ml/10g was administered intraperitoneally to induce pain. The number of writhing was calculated for 10 minutes after the acetic acid injection. The percentage of pain protection was calculated.

Radiant heat tail-flick method

The analgesic activity was determined by radiant heat tail-flick model in mice (D'Amour and Smith, 1941). The test compound was given p.o. at doses of 25 and 50 mg/kg *body weight* and the control group received the vehicle only. Morphine (2 mg/kg) was given subcutaneously as the standard analgesic agent. Tail-flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was 10 sec to avoid any tissue injury during the process. Tail-flick latency was measured at 30, 60 and 120 minutes after the drug administration.

Carrageenan induced rat paw edema

The anti-inflammatory activity of compound 1 was measured by Carrageenan-induced rat paw edema model (Winter et al, 1962). The animals were divided into groups as shown in table 3. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of Carrageenan in normal saline, in the right hind paw of the rats, 1 h after oral administration of the test compounds. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 1, 2, 3, 4 and 24 h after the Carrageenan injection. The test compound was given orally at 25 and 50 mg/kg. Phenylbutazone suspension at a dose of 80 mg/kg, p.o. was used as the standard anti-inflammatory drug and the control group was given vehicle only.

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. P value <0.05 was considered significant.

RESULTS

The analgesic activity of the compound 1 was shown in tables 1-2. The compound 1 at doses of 25 and 50 mg/kg *body weight* showed significant reduction in the number of writhing with 25.4 ($P< 0.05$) and 52.43% ($P< 0.01$) respectively. The results were statistically significant and comparable to that of standard drug aminopyrine (67.57% inhibition) at a dose of 50 mg/kg body weight. In radiant heat tail-flick model, the compound also produced significant increase in the tail flick latency.

In Carrageenan induced rat paw edema test for acute inflammation, the compound 1 exhibited significant inhibition of paw volume by 16.93 and 24.43 % at doses of 25 and 50 mg/kg body weight respectively, which was comparable to that of standard drug phenylbutazone (31.94% inhibition) given at a dose of 80 mg/kg body weight at 3rd hour of Carrageenan administration (table 3).

DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate the potential analgesic activity of drugs. It has been suggested that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons (Collier et al. 1968). It is sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and to narcotics and other centrally acting drugs (Collier et al. 1968; Santos et al, 1998; Reichert et al, 2001). Recently, it was found that the nociceptive activity of acetic acid may be due to the release of cytokines, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells (Ronaldo *et al.,* 2000). Thus, the present study presented here might indicate that the antinociceptive action of compound 1 in the acetic acid-induced writhing test could be due to inhibition of the release of TNF-α, interleukin-1β and interleukin-8 by resident peritoneal cells. However, this possibility remains to be tested in future studies. In the tail-flick method the alkaloid increased the stress tolerance capacity of the animals and indicated the possible involvement of a higher center (Whittle, 1964). The Carrageenan-induced paw edema in rats is believed to be biphasic (Vinegar, 1969). The first phase is due to the release of histamine or serotonin and the second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome (Crunkhorn and Meacock,

Table 1: Effects of compound 1 on acetic acid induced writhing response in mice.

Treatment	Dose (mg/ kg)	Writhings	% Inhibition
Control (vehicle, 10ml/kg)	-	30.8 ± 2.18	-
Compound 1	25	23.0 ± 2.03*	25.40
Compound 1	50	14.7 ± 1.26**	52.43
Aminopyrine	50	10.0± 0.58**	67.57

40 min after drug treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 10 min after the injection, the number of writhing was counted for 10 min.
Values are Mean ± SEM (n = 6); ***P*<0.01, **P*<0.05 compared to control.

Table 2: Effects of compound 1 on radiant heat tail-flick response in mice.

Treatment	Dose (mg/ kg)	Pre-treatment (sec)	Reaction time (sec)		
			30 min	60 min	120 min
Control	-	4.07 ± 0.21	3.52 ± 0.16	2.75 ± 0.14	2.37 ± 0.18
Compound 1	25	3.80 ± 0.18	5.13 ± 0.20**	5.67 ± 0.36**	5.28 ± 0.14**
Compound 1	50	3.82 ± 0.14	5.40 ± 0.30**	5.80± 0.26**	5.72 ± 0.35**
Morphine	2	3.28 ± 0.08*	7.50 ± 0.24**	7.65 ± 0.55**	7.42 ± 045**

Values are Mean ± SEM (n = 6); ***P*<0.01, **P*<0.05 compared to control.
[c]Morphine was administered subcutaneously.

Table 3: Effects of compound 1 on carrageenan induced rat paw edema.

Group	Dose (mg/kg)	Carrageenan induced rat paw edema Mean ± SEM (% inhibition of paw volume)				
		1 h	2 h	3 h	4 h	24 h
Control	-	103. 7±2.23	108.5±3.73	95.5±4.52	92.8±2.36	70.5±3.34
Compound 1	25	95.17±3.07 (8.1)	95.00±4.59 (12.44)	79.3±4.59* (16.93)	79.2±3.59 (14.72)	67.2±5.70 (4.72)
Compound 1	50	89.7±5.17* (13.5)	87.0±2.48** (19.82)	72.2±2.50** (24.43)	73.2±2.52* (21.18)	64.8±1.64 (8.03)
Phenylbutazone	80	72.4±1.32** (30.14)	71.0±4.04** (34.56)	65.0±2.84** (31.94)	66.0±3.80** (28.9)	64.0±2.44 (9.22)

Values are Mean ± SEM (n = 6); Paw volume is expressed in change of height (in mm) of Hg bath (in parentheses, % inhibition of edema). ***P*<0.01, **P*<0.05 compared to control.

1971; Di Rosa and Willoughby, 1971). Therefore, it can be inferred that the inhibitory effect of compound 1 on Carrageenan-induced inflammation could be due to the inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

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