

Bioactive Alkaloid from *Sida cordifolia* Linn. with Analgesic and Anti-Inflammatory Activities

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ABSTRACT

In the present study, the analgesic and anti-inflammatory activities of a new alkaloid (5'-Hydroxymethyl-1'-(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one) (compound 1), isolated from *Sida cordifolia* Linn. was investigated in animal models. The analgesic activity was investigated in the acetic acid induced writhing and the radiant heat tail flick model in mice and the carrageenan induced rat paw edema model was used for anti-inflammatory study. The compound produced significant ($p < 0.01$) analgesic activity in both models. The compound also exhibited significant ($p < 0.01$) inhibition of rat paw edema induced by carrageenan. These results indicated that compound 1 possessed analgesic and anti-inflammatory activities.

Keywords: *Sida cordifolia*, Analgesic, Anti-inflammatory

Conventional anti-inflammatory drugs such as steroidal anti-inflammatory drugs (SAID) and nonsteroidal anti-inflammatory drugs (NSAID) are used in the treatment of most of the acute and chronic pain and inflammatory disorders including rheumatoid arthritis. However long-term use of these agents may produce serious adverse effects. Thus, it is worth developing new plant-derived anti-inflammatory and analgesic agents with fewer adverse effects.

Sida cordifolia Linn. (Family: Malvaceae) commonly known as berela (Bengali) is herb that is extensively used as a common herbal drug in the Indian subcontinent [1]. The water extract of the leaves was reported to possess analgesic and anti-inflammatory activities in animal models [2]. The water extract of the whole plant is specially used in the treatment of rheumatism [3]. Earlier phytochemical studies on the roots had shown the presence of ephedrine, vasicinol, vasicinone and N-methyl tryptophan [4-6]. In continuation of our studies on medicinal plants available in Bangladesh for their chemical constituents and biological activities we isolated (5'-Hydroxymethyl -1'-(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one) (structure shown in Fig 1) from the aerial parts of *S. cordifolia* Linn. In the present paper we report the analgesic and anti-inflammatory activities of this compound in mice and rats, respectively.

MATERIALS AND METHODS

Plant Materials

The aerial parts of plant were collected from the hilly region of the district of Chittagong, Bangladesh and identified by the expert of National Herbarium of Bangladesh. A voucher specimen (accession No. 31238) was deposited in the Herbarium for future reference.

Extraction and Isolation of Alkaloid

The air-dried plant parts were pulverized to powder. The powder (5.5 kg) was 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 × 300 mL). The combined extracts were washed with water, dried, and evaporated below 40°C temperature and reduced pressure to yield the crude alkaloid (4.2 g) as a solid brown mass H. Alkaloid 1 (120 mg) was separated from mass H by using preparative TLC technique where

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

Treatment	Dose (mg/kg, p.o.)	Writhings	% Inhibition
Control (vehicle, 10 mL/kg)	-	30.8 ± 2.18	-
Compound 1	25	25.5 ± 1.43	17.30
	50	16.3 ± 1.65*	47.03
Aminopyrine	50	10.0 ± 0.58*	67.57

Values are Mean ± SEM (n = 6); *p < 0.01, compared to control.

(1:1:1) methanol, ethyl acetate and chloroform were used as a developing solvent system. The compound 1 was characterized and identified by analyzing its spectral data. UV λ_{\max} (MeOH) nm: 243. IR ν_{\max} (KBr) cm^{-1} : 3400 (O-H), 1701 (C=O saturated). $^1\text{H-NMR}$ (CDCl₃) δ : 0.88 (3H, t, H-C7'), 1.28 (3×2H, s, H-C3',4',6'), 2.07 (2H, m, H-C2), 2.32 (1H, m, H-C5'), 2.47 (2H, m, H-C3), 2.59 (2H, m, H-C2'), 3.63 (2H, d, CH₂OH), 5.03 (2H, dd, J = 14.8, 14.8, H-C9), 5.26 (1H, t, J = 11.6, H-C1), 7.03 (1H, d, J = 7.6, H-C5), 7.10 (1H, t, J = 14.8, H-C7), 7.26 (1H, t, J = 15.2, H-C6), 8.30 (1H, d, J = 8.4, H-C8). $^{13}\text{C-NMR}$ (CDCl₃) δ : 87.25 (C1), 29.71 (C2), 30.06 (C3), 119.68 (C5), 123.79 (C6), 127.66 (C7), 124.28 (C8), 44.25 (C9), 157.66 (C11), 134.78 (C12), 134.34 (C13), 203.91 (C1'), 31.94 (C2'), 24.76 (C3'), 22.70 (C4'), 52.76 (C5'), 68.02 (CH₂OH), 29.37 (C6') and 14.12 (C7'). GC mass m/e (%): 314 (5) [M+], 291 (7), 273 (30), 253 (5), 228 (25), 209 (14), 185 (10), 129 (20), 104 (75), 83 (54), 57 (100) etc.

Animals

Swiss albino mice (20-25 g) and Long Evans rats (120-140 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 overnight and weighed before the experiment.

Drugs and Chemicals Used

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).

Acetic Acid Induced Writhing Response

The peripheral analgesic activity of compound 1 was determined by the acetic acid induced writhing inhibition method [7]. The pre-screened Swiss albino mice employed for this experiment were divided into groups

shown in Table 1. The inhibition of writhing in mice by compound 1 (25 and 50 mg/kg, p.o.) was compared against inhibition of writhing by a standard analgesic agent, aminopyrine given p.o. at a dose of 50 mg/kg body weight. The control group received the vehicles used to prepare the test solutions. 40 min after the administration of test materials each of the mice was injected intraperitoneally with acetic acid (0.7%) at a dose of 0.1 mL/10g to create pain sensation. The number of writhing was calculated for 10 min 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 [8]. The animals were divided into four groups each containing six mice. The test compound 1 was administered orally at doses of 25 and 50 mg/kg. Morphine (2 mg/kg, s.c.) was used 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 30, 60 and 120 minutes after the drug administration.

Anti-inflammatory Study

The anti-inflammatory activity was measured by using carrageenan-induced rat paw edema model [9]. 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 compound (25 and 50 mg/kg, p.o.). Phenylbutazone suspension at a dose of 80 mg/kg, p.o. was used as the standard anti-inflammatory drug.

The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 1, 2, 3, 4 and 24 h after the carra-

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 (vehicle, 10 mL/kg)	-	4.07 ± 0.21	3.52 ± 0.16	2.75 ± 0.14	2.37 ± 0.18
Compound 1	25	3.93 ± 0.20	4.68 ± 0.20*	4.98 ± 0.20**	4.71 ± 0.19**
	50	3.83 ± 0.15	5.05 ± 0.36**	5.40 ± 0.21**	5.06 ± 0.30**
Morphine	2	3.28 ± 0.08*	7.5 ± 0.24**	7.65 ± 0.55**	7.42 ± 0.45**

Values are Mean ± SEM (n = 6); **p < 0.01, *p < 0.05 compared to control.

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

Group	Dose (mg/kg)	Carrageenan induced rat paw edema				
		Mean \pm SEM (% inhibition of paw volume)				
		1 h	2 h	3 h	4 h	24 h
Control (vehicle, 10 mL/kg)	-	103.7 \pm 2.23	108.5 \pm 3.73	95.5 \pm 4.52	92.8 \pm 2.36	70.5 \pm 3.34
Compound 1	25	95.3 \pm 2.72 (8.03)	99.0 \pm 2.89 (8.75)	81.7 \pm 3.28 (14.49)	79.3 \pm 3.77 (14.54)	67.8 \pm 4.71 (3.78)
	50	88.0 \pm 3.45* (15.11)	84.2 \pm 4.73* (22.43)	71.0 \pm 2.48* (25.65)	72.0 \pm 4.16* (22.44)	65.2 \pm 2.64 (7.57)
Phenylbutazone	80	72.4 \pm 1.32* (30.14)	71.0 \pm 4.04* (34.56)	65.0 \pm 2.84* (31.94)	66.0 \pm 3.80* (28.90)	64.0 \pm 2.44 (9.22)

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

geenan injection. Results were expressed as percentage of inhibition of edema, calculated by the formula $(1 - V_t/V_c) \times 100$ where V_t and V_c are the mean paw volume in the treated and controlled groups, respectively.

Statistical analysis

The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values with p <0.05 were considered significant.

RESULTS

The alkaloid (25 mg/kg and 50 mg/kg) produced a significant reduction in the number of writhing induced by acetic acid with 17.30 and 47.03% (p < 0.01) respectively. The standard drug aminopyrine produced 67.57% inhibition of writhing response at a dose of 50 mg/kg body weight (Table 1).

The effect of the test compound 1 on radiant heat tail-flick model was shown in (Table 2). The result indicated that compound 1 caused significant increase in the tail flick latency.

In carrageenan induced rat paw edema test for acute inflammation, the compound 1 exhibited statistically significant (p <0.01) inhibition of paw volume by 14.49 and 25.65% at doses of 25 and 50 mg/kg body weight respectively at 3rd hour of carrageenan administration. The standard drug phenylbutazone produced 31.94% inhibition given p.o. at a dose of 80 mg/kg body weight (Table 3).

DISCUSSION

The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs. The response is thought to be mediated by peritoneal

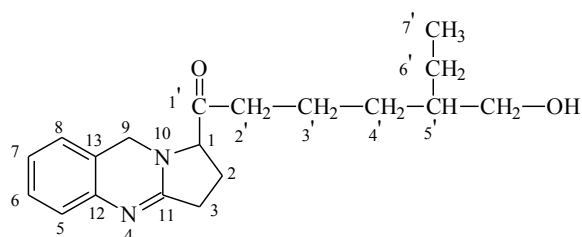


Fig 1. Structure of compound 1.

mast cells [10], acid sensing ion channels [11] and the prostaglandin pathways [12]. The significant antinociceptive activity of compound 1 might be due to the effect of the compound in the prostaglandin pathways. In the tail-flick method of analgesic activity assay, the compound increased the stress tolerance capacity of the animals and hence also indicated the possible involvement of a higher center [7].

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis [13, 14]. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve [15]. The first phase, which occurs between 0 to 2.5 h after injection of the phlogistic agent, has been attributed to the release of histamine or serotonin [16]. The edema volume reaches its maximum approximately 3 h post treatment and then begins to decline. The second phase of inflammatory reaction which is measured at 3 h is caused by the release of bradykinin, protease, prostaglandin and lysosome [16, 17]. Therefore, it can be inferred that the inhibitory effect of compound 1 on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Thus, the results of the present study demonstrated that the alkaloid (5'-Hydroxymethyl-1'-(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one) has significant analgesic and anti-inflammatory activities. However a more extensive study is necessary to determine exact mechanism(s) of action.

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