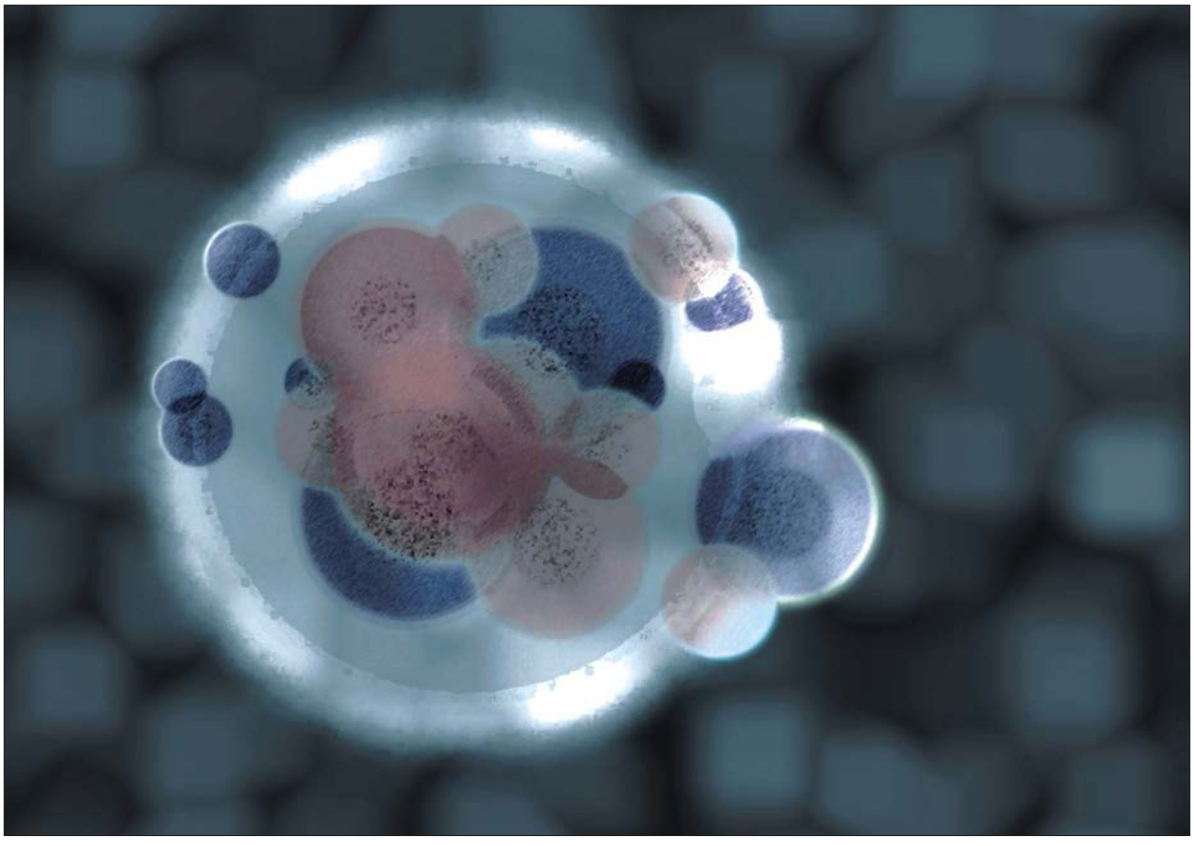

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Analgesic and Anti-inflammatory Principle from *Sida cordifolia* Linn

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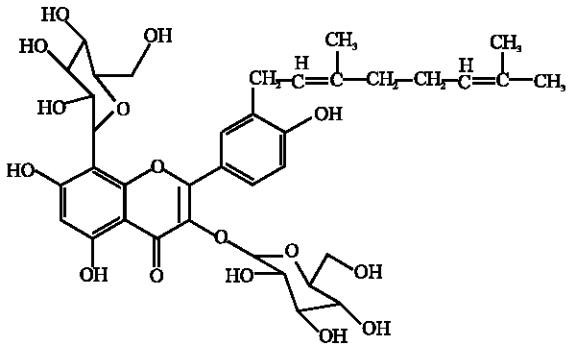
Abstract: A new flavonol glycoside, 3'-(3",7"-dimethyl- 2",6"-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucoside[1], isolated from *Sida cordifolia* Linn. was investigated for analgesic and anti-inflammatory activities in animal models. In the acetic acid induced writhing model, the drug at a dose of 25 and 50 mg kg^{-1} body weight showed statistically significant ($p<0.0001$) inhibition of writhing response of 25.12 and 52.30%, respectively. The drug also produced significant increase in the tail flick latency in a dose depended manner ($r = 0.92$) which were also statistically significant ($p<0.0001$). In carrageenan induced rat paw edema the compound produced 16.15 and 28.52% inhibition of paw edema at doses of 25 and 50 mg kg^{-1} body weight at the 3rd h of study.

Key words: *Sida cordifolia Linn.*, analgesic activity, anti-inflammatory activity, acetic acid induced writhing, radient heat tail flick, carrageenan induced paw edema

INTRODUCTION

Sida cordifolia Linn. is an herb belonging to the family Malvaceae. It grows to a height of 3-5 feets and is extensively used as a common herbal drug in the Indian subcontinent[1,2]. The roots, leaves, stems and seeds of *S. cordifolia* Linn. are used in the traditional medicine against chronic dysentery, asthma and gonorrhea in the subcontinent[3,4]. The water extract of the whole plant is specially used in treatment of rheumatism.[4] Earlier phytochemical studies on the roots have shown the presence of ephedrine, vasicinol, vasicinone and N-methyl tryptophan[5-7]. In continuation of our studies on medicinal plants available in Bangladesh for their chemical constituents and biological activities we isolated 3'-(3",7"-dimethyl- 2",6"-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucoside (Structure1) from the aerial parts of *S. cordifolia* Linn. In the present study we report the effect of this compound on analgesic and anti-inflammatory activities in animal models.

Structure 1:



3'-(3",7"-dimethyl- 2",6"-octadiene)-8-C-ß-D-glucosyl-kaempferol 3-O- ß -D-glucoside

MATERIALS AND METHODS

Extraction and isolation of the compound: The aerial parts of *S. cordifolia* Linn. were collected from the hilly region of the district of Chittagong situated in the south-eastern region of Bangladesh and identified by the National Herbarium of Bangladesh. The air-dried aerial parts were grounded to powder. The powder (5.5 kg) of *S. cordifolia* Linn. was successively extracted with chloroform (3x72 h), methanol (3x72 h) and 80% ethanol (3x72 h). The 80% ethanol extract was concentrated to one third of its volume under pressure at 40°C. The extract was then partitioned with n-hexane, dichloromethane, ethyl acetate

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Table 1: Effects of compound 1[a] on acetic acid induced writhing response in mice

Treatments	Dose (mg kg^{-1}, p.o.)	Writhings[b]	Inhibition(%)
Control	-	32.5±2.48	-
(vehicle, 10 mL kg^{-1})	25	24.33±2.78*	25.12
Compound 1	50	15.50±1.47*	52.30
Aminopyrine	50	10.5±0.76*	67.69
One-way ANOVA	F	22.6	
	df	3, 20	
	p	<0.0001	

[a]1 h r after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1mL/10 g); immediately after the injection, the number writhing was counted for 10 min. [b]Values are Mean±SEM (n = 6); One-way ANOVA; *p<0.01, compared to control.

and n-butanol. The ethyl acetate extract yielded a yellowish mass (5 g) on removal of the solvent. The extract was adsorbed onto silica gel and placed over a column of silica gel and eluted with $CHCl_3$-EtOAc-MeOH (1:1:1). The eluents were monitored by TLC and divided into three fractions. Fraction 1 (test tube 1-15) gave the pure compound 1 (50 mg) which was characterized and identified by analyzing its spectral data.

Experimental 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 kept under fasting for overnight and weighed before the experiment.

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

Acetic acid induced writhing test: The peripheral analgesic activity was determined by the acetic acid induced writhing inhibition method[8]. 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 was compared against inhibition of writhing by a standard analgesic agent, aminopyrine given p.o. at a dose of 50 mg kg^{-1}. Acetic acid (0.7%) at a dose of 0.1 mL/10 g was administered intraperitoneally to create pain sensation. The number of writhes was calculated for 10 min immediately 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[9]. Morphine 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 min after the drug administration.

Anti-inflammatory study: The anti-inflammatory activity of compound 1 was measured by using carrageenan-induced rat paw edema model[10]. 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 drugs. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 1, 2, 3, 4 and 24 h after the carrageenan injection. Phenylbutazone suspension at a dose of 80 mg kg^{-1} p.o. was used as the standard anti-inflammatory drug.

Statistical analysis: The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A value $p<0.05$ was considered significant.

RESULTS

As shown in Table 1, the compound 1 at a dose of 25 and 50 mg kg^{-1} showed dose dependent ($r = 0.88$) reduction in the number of writhing with 25.12 and 52.30% of inhibition respectively. The results were statistically significant ($p< 0.0001$) and similar to that of standard drug aminopyrine (67.69% inhibition) at a dose of 50 mg kg^{-1} body weight.

In radiant heat tail-flick model, the compound showed significant increase in the tail flick latency in a dose depended manner ($r = 0.92$). The result was found to be statistically significant ($p<0.0001$) in comparison to the control (Table 2).

In carrageenan induced rat paw edema test for acute inflammation, the compound 1 exhibited statistically significant ($p<0.0001$) inhibition of paw volume by 16.15 and 28.52% at a dose of 25 and 50 mg kg^{-1} body weight, respectively, which was comparable to that of standard drug phenylbutazone (32.98% inhibition) given p.o. at a dose of 80 mg kg^{-1} body weight at 3rd h of carrageenan administration (Table 3).

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

Treatments	Dose (mg kg^{-1})	Pre-treatment[c] (sec)	Reaction time (sec)[c] 30 min	60 min	120 min
Control	-	3.88±0.21	3.50±0.15	2.73±0.13	2.40±0.20
(vehicle, 10 mL kg^{-1})	25	3.73±0.21^{NS}	5.25±0.22**	5.88±0.31**	5.43±0.33**
Compound 1	50	3.72±0.14^{NS}	5.60±0.32**	5.95±0.20**	5.65±0.34**
Morphine	2[b]	3.23±0.08*	7.45±0.24**	7.68±0.52**	7.53±046**
One-way ANOVA	F	2.73	48.6	39.2	37.3
	df	3, 20	3, 20	3, 20	3, 20
	P	NS	<0.0001	<0.0001	<0.0001

[a]per oral administration of vehicle and drug, radiant heat intensity was 5 amp. [b]morphine was administered sub-cutaneously. [c]Values are Mean ± SEM (n = 6); One-way ANOVA; **p<0.01, *p<0.05 compared to control

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

Group	Dose[a] (mg kg^{-1})	Carrageenan induced rat paw edema[b] (% inhibition of paw volume) 1 h	2 h	3 h	4 h	24 h
Control	-	107.00±3.35	112.0±3.97	97.00±6.22	94.0±2.74	69.0±1.65
	25	94.33±5.02	97.33±3.09*	81.3±4.45*	81.2±4.84	66.0±4.91
		(11.83)	(13.09)	(16.15)	(15.03)	(4.34)
Compound 1	50	89.66±5.17*	87.0±4.27**	72.2±3.22**	73.2±2.52*	64.83±1.64
		(16.2)	(22.32)	(28.52)	(22.12)	(6.04)
PBZ	80	72.42±1.32**	71.0±4.04**	65.0±2.84**	66.0±3.80**	60.66±2.09
		(32.32)	(36.6)	(32.98)	(29.75)	(12.08)
One-way ANOVA	F	6.76	10.7	6.91	3.45	0.49
	df	7, 40	7, 40	7, 40	7, 40	7, 40
	p	<0.0001	<0.0001	<0.0001	<0.01	NS

[a]1h after drug treatment, p.o., carrageenan was administered in rat hind paw [b]Values are Mean±SEM (n = 6); Paw volume is expressed in change of height (in mm) of Hg bath (in parentheses, % inhibition of edema). One-way ANOVA; **p<0.01, *p<0.05 compared to control. PBZ = Phenylbutazone

DISCUSSION

The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells[11], acid sensing ion channels[12] and the prostaglandin pathways[13]. The significant antinociceptive activity of compound 1 might be due to the effect of the drug in the prostaglandin pathways. In the tail-flick method of analgesic activity assay, the drug increased the stress tolerance capacity of the animals and hence also indicated the possible involvement of a higher center[8].

The carrageenan-induced paw edema in rats is believed to be biphasic[14]. 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[15,16]. 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.

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