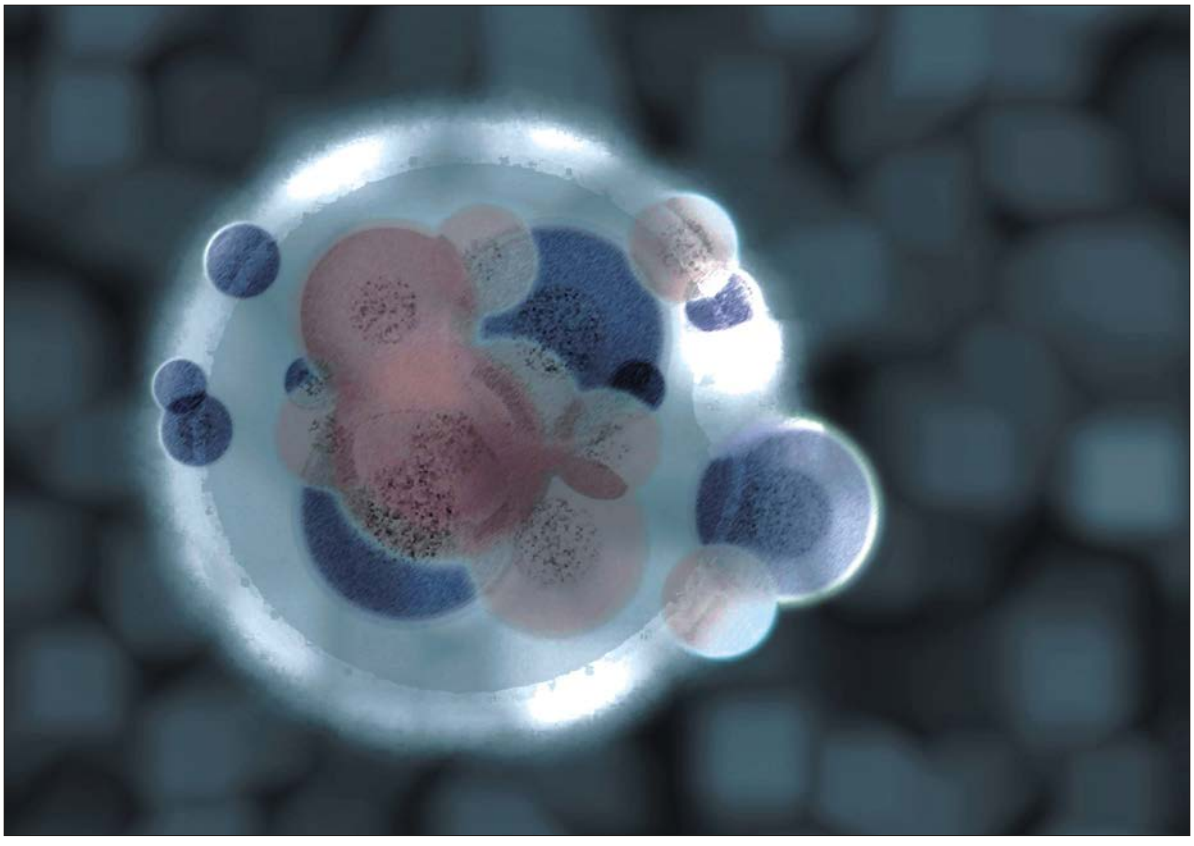

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Analgesic and Anti-inflammatory Activities of *Desmodium triflorum* DC

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Abstract: Successive hexane, ethyl acetate and methanol extracts of *Desmodium triflorum* DC were subjected to evaluate analgesic and anti-inflammatory activities in animal model. In acetic acid-induced writhing test, the ethyl acetate extract at doses of 150 and 300 mg kg^{-1} body weight elicited 47.60 and 57.40% inhibition of writhing reflex, respectively but the methanol extract showed 51.48% inhibition at 300 mg kg^{-1} body weight. Significant elongation of tail flick time was evident both in ethyl acetate and methanol extracts (69.54 and 61.72% elongation, respectively) only at the higher dose. Both the hexane and methanol extracts at a dose of 300 mg kg^{-1} body weight showed statistically significant ($p<0.01$) inhibition of rat paw edema by 35.8 and 38.97%, respectively at the third hour after carrageenan injection.

Key words: *Desmodium triflorum,* Papilionaceae, analgesic activity, anti-inflammatory activity

INTRODUCTION

Desmodium triflorum DC (Bengali-Kulaliya, Malay-Rumput) is a small perennial trailing herb belonging to the family Papilionaceae[1]. The plant is available in all tropical countries. The creeper commonly grows in grassy places, roadsides and lawns in Bangladesh and throughout India. The fresh leaves of the plant are applied to wounds and abscesses that are usually difficult to heal. The paste is sometimes applied to sores and itch. The fresh juice of the plant is often given to the children for coughs. The traditional use of the plant also recommends for use in dysentery and as a laxative. Previous phytochemical investigations showed that the plant contains 2'-O-glucosylvitexin[2]. Though the plant is traditionally used in many parts of Bangladesh, no scientific report is available to validate the folkloric use. As a part of our continuing studies on the medicinal plants of Bangladesh, we investigated the analgesic and anti-inflammatory activities of different extracts of *D. triflorum*.

MATERIALS AND METHODS

Plant materials: *Desmodium triflorum* was collected from Dhaka, Bangladesh. A voucher specimen {accession number: DUH-1400 (15)} was kept in Dhaka University Herbarium, Dhaka, Bangladesh after identification of the plant. Collected plants, after cutting into small pieces, were sun-dried and pulverized into a coarse powder and stored into an air-tight container.

Extraction: The pulverized coarse powder of the plant *D. triflorum* (525 g) was extracted with n-hexane, ethyl acetate and methanol by successive cold extraction. All the extracts obtained were filtered off and evaporated to dryness *in vacuum* at low temperature and reduced pressure by rotary evaporator. The hexane extract was designated as DTH, ethyl acetate extract as DTE and methanol extract as DTM.

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).

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Acetic acid-induced writhing test: The peripheral analgesic activity of different extracts of *D. triflorum* was determined by the acetic acid-induced writhing inhibition method[3]. 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 the plant extracts was compared against to the 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[4]. 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 after 1 h of the drug administration.

Anti-inflammatory activity study: Different extracts of *D. triflorum* was subjected to evaluate their anti-inflammatory activities by using carrageenan-induced rat paw edema model[5]. The animals were divided into groups as shown in Table 2. Acute inflammation was produced by sub-plantar 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 $p<0.05$ was considered as significant.

RESULTS

As shown in Table 1, ethyl acetate extract of *D. triflorum* (150 and 300 mg kg^{-1}, p.o.) showed a significant ($p<0.01$) dose dependent ($r = 0.92$) reduction in the number of writhing with 47.60 and 57.40% of inhibition, respectively. DTM showed 51.48% reduction at a dose of 300 mg kg^{-1}, which was statistically

Table 1: Analgesic activity of different extracts of *Desmodium triflorum* on acetic acid-induced writhing response and radiant heat tail-flick model in mice

		Acetic acid-induced writhing response in mice			Radiant heat tail-flick	
Groups		Dose[a]	Writhings[b]	% Inhibition	Reaction time (sec)[b]	% of elongation
Control		-	36.60±1.76	-	4.05±0.20	-
DTH		150	28.60±1.49**	21.86	4.85±0.23	19.75
		300	26.60±1.64**	27.33	4.97±0.29	22.63
DTE		150	19.20±1.62**	47.60	5.92±0.75*	46.63
		300	15.60±0.91**	57.40	6.87±0.54**	69.54
DTM		150	26.60±1.72**	27.33	5.63±0.28	39.09
		300	17.80±1.73**	51.48	6.55±0.26**	61.72
Aminopyrine		50	11.00±0.73**	69.93	-	-
Morphine		2[c]	-	-	7.97±0.63**	96.70
One-way ANOVA	F		30.60		7.94	
	df		7.40		7.40	
	p		<0.001		<0.05	

[a]1 h after drug treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1 mL/10 g); immediately after the injection, the number of writhing was counted for 10 min, [b]Values are Mean±SEM (n = 6); One-way ANOVA; **p<0.01, *p<0.05 compared to control, [c]Morphine was administered subcutaneously

Table 2: Anti-inflammatory activity of different extracts of *Desmodium triflorum* on carrageenan-induced rat paw edema

		Carrageenan-induced rat paw edema[b] Mean±SEM (% inhibition of paw volume)				
Groups	Dose[a] (mg kg^{-1})	1 h	2 h	3 h	4 h	24 h
Control	-	72.80±1.19	95.20±1.94	113.40±1.76	121.4±2.22	68.20±1.70
DTH	300	51.40±1.71** (29.52)	66.40±1.89** (33.45)	72.80±2.39** (35.73)	82.8±2.21** (31.73)	62.20±2.60 (8.8)
DTE	300	62.80±2.93** (13.73)	80.50±2.84** (25.41)	90.80±2.07** (19.85)	98.33±3.18** (18.95)	64.20±1.49 (5.8)
DTM	300	48.80±2.51** (32.95)	59.60±2.49** (37.30)	69.17±1.70** (38.97)	84.0±2.33** (35.02)	64.00±3.43 (6.1)
PBZ	80	46.60±1.28** (35.92)	54.17±1.54** (43.08)	65.87±1.74** (42.05)	78.85±1.49** (35.02)	55.80±1.76**(18.09)
One way ANOVA	F	29.30	59.80	103.00	60.40	3.97
	df	4.25	4.25	4.25	4.25	4.25
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.05

[a]1 h after dmg 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, compared to control, PBZ = Phenylbutazone

significant and similar to that of standard drug aminopyrine (69.93% inhibition) at a dose of 50 mg kg^{-1} body weight (Table 1).

In radiant heat tail-flick model, percentage of tail flick elongation was 69.54 and 61.72, respectively in ethyl acetate and methanolic fraction of *D. triflorum* only at the higher dose. The result was found to be statistically significant ($p<0.01$) in comparison to the control (Table 1).

In carrageenan-induced rat paw edema test for acute inflammation, DTH and DTM exhibited statistically significant ($p<0.01$) inhibition of paw volume by 35.73 and 38.97%, respectively at a dose of 300 mg kg^{-1} body weight, which was comparable to that of standard drug phenylbutazone (42.15% inhibition, $p<0.001$) given p.o. at a dose of 80 mg kg^{-1} body weight at 3rd h of carrageenan administration (Table 2). The extracts did not demonstrate any significant anti-inflammatory activity at doses of 150 mg kg^{-1} body weight (Data not shown).

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[6], acid sensing ion channels[7] and the prostaglandin pathways[8]. The significant antinociceptive activity of DTE and DTM might be due to the presence of analgesic principles acting with the prostaglandin pathways. In the tail-flick method of analgesic activity assay, both the ethyl acetate and methanol extracts increased the stress tolerance capacity of the animals and hence also indicated the possible involvement of a higher center[3].

The carrageenan-induced paw edema in rats is believed to be biphasic[9]. 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[10,11]. Therefore, it can be inferred that the inhibitory effect of DTH and DTM on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Although different extracts of *D. triflorum* exhibited significant analgesic and anti-inflammatory activities, exact mechanisms underlying the observed pharmacological effects can only be elucidated after isolation of active constituents using a wide range of experimental models.

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