

# Studies on Antinociceptive, Antiinflammatory and Diuretic Activities of Methanol Extract of the Aerial Parts of *Clerodendron viscosum* Vent.

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The use of plant-based medication is gradually becoming popular through out the world.<sup>1</sup> Plant secondary metabolites play an important role in health care for about 80% of the world's population.<sup>2</sup> Approximately, half of the world's 25 best-selling pharmaceutical agents are derived from natural products.<sup>3</sup> Thus, emphasis is now given on the standardization of herbal medication by screening of biological activities of medicinal plants and isolating active principles from them.

Plants belonging to the genus *Clerodendron* Linn. (alternatively, *Clerodendrum* Linn), Family – Verbanaceae, have been used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy. The plant *Clerodendron viscosum* Vent. (Synonym: *Clerodendrum infortunatum* Gaetrn. Bengali name - Bhand) is an indigenous medicinal plant widely distributed in various parts of India, Ceylon, Malaya and Bangladesh. Traditionally, the plant is used as an

aphrodisiac, antipyretic, and antihelminthic. The plant is useful in relieving thirst and burning sensation, foul odours, and diseases of the blood. Leaves of the plant are prescribed for tumors, certain skin diseases and scorpion stings.<sup>4</sup> Previous phytochemical investigation of the plant revealed the presence of alkylsterols<sup>5</sup> and 2, -(3,4-dehydroxyphenyl)ethanol 1-*O*- $\alpha$ -2 rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-(4-*O*-caffeoyl) glycopyranoside (acteoside)<sup>6</sup> in this plant. The present study has been designed to evaluate antinociceptive, antiinflammatory and diuretic activity of the methanol extract of the aerial parts of *C. viscosum* Vent.

The plant *Clerodendron viscosum* Vent. was collected at flowering stage from Dhaka during November 2000 and was identified (voucher specimen No. DUH-18) by the Department of Botany, University of Dhaka, Bangladesh. After collection, whole plant was sun-dried for eight days and made into a coarse powder by grinding. Pulverized coarse powder of *C. viscosum* Vent. (400 g) was cold extracted using methanol. The methanol extract (MeCV) was filtered off, and evaporated to dryness *in vacuo* at low temperature and reduced pressure by rotary evaporator. The extract (yield,

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0.68%) was then freeze dried and kept in an air-tight container.

*Swiss albino* mice (20-25 g) and *Long Evans* rats (140-160 g) of either sex were obtained from International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). 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. MeCV was dissolved in normal saline by using 0.1% tween-80. Animals were randomly divided into four groups, each consisting of five animals, of which two groups were given the test material MeCV at doses of 150 and 300 mg/kg body weight by gavage. While one group of animals was treated with standard drugs [phenylbutazone (100 mg/kg body weight) for screening of anti-inflammatory activity, aminopyrine (50 mg/kg body weight) for analgesic activity study, and furosemide (3 mg/kg body weight) for assessing diuretic activity], another group of animals served as control receiving saline containing 0.1% tween-80.

The effect of MeCV on acetic acid-induced writhing<sup>7</sup> at doses of 150 and 300 mg/kg body weight was compared to that of aminopyrine at a dose of 50 mg/kg body weight. As shown in Table 1, MeCV exhibited statistically significant ( $p < 0.001$ ) inhibition of acetic acid-induced writhing by 37.95 and 54.91% at doses of 150 and 300 mg/kg body weight, respectively, in a dose-dependent manner (correlation co-efficient,  $r = 0.98$ ). Although MeCV showed significant antinociceptive activity, the effect was not comparable to the inhibition of acetic acid-induced writhing by standard drug aminopyrine (82.14%,  $p < 0.001$ ).

The effect of MeCV on carrageenan-induced rat paw edema<sup>8</sup> was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The study revealed that after 3 h of carrageenan administration, MeCV exhibited statistically significant ( $p < 0.001$ ) inhibition of paw volume by 21.96 and 28.42% at doses of 150 and 300 mg/kg body weight, respectively, which was less than that observed with standard drug phenylbutazone (37.21%,  $p < 0.001$ ) given at a dose of 100 mg/kg body weight (Table 2).

**Table 1. Antinociceptive activity of methanol extract of *C. viscosum* Vent. in mice<sup>a</sup>**

Treatment	Dose (per kg body weight; <i>p. o.</i> )	Writhing <sup>b</sup>	<i>t</i> -value	Percent inhibition of writhing
Control (saline)	10 ml/kg	22.40 ± 0.91	-	-
MeCV	150	13.90 ± 0.35*	08.81	37.95
MeCV	300	10.10 ± 0.44*	12.17	54.91
Aminopyrine	50	04.00 ± 0.35*	18.87	82.14

<sup>a</sup>One hour after treatment, mice were injected intraperitoneally with 0.7% (v/v) acetic acid (0.1 ml /10 g body weight); Ten minutes after treatment, the number of writhings induced by acetic acid was counted for 10 minutes.

<sup>b</sup> Values are mean ± S. E. M. (n = 5); \*  $p < 0.001$ , vs. control; Correlation coefficient ( $r$ ) = 0.98.

**Table 2. Antiinflammatory effect of methanol extract of *C. viscosum* Vent. in carrageenan-induced rat paw inflammation<sup>a</sup>**

Treatment	Dose (mg/kg body weight)	Increase in paw volume (ml x 1000) ± S. E. M. (percent inhibition)				
		1 h	2 h	3 h	4 h	24 h
Saline	10 ml/kg	59.8 ± 1.48	71.0 ± 2.06	77.4 ± 2.27	89.2 ± 1.66	46.8 ± 1.53
MeCV	150	50.6 ± 1.35*** (15.38)	57.2 ± 1.24**** (19.43)	60.4 ± 1.28**** (21.96)	72.2 ± 2.64**** (19.05)	41.2 ± 1.21* (11.96)
MeCV	300	48.8 ± 1.31**** (18.39)	54.4 ± 1.71**** (23.38)	55.4 ± 1.54**** (28.42)	66.8 ± 1.88**** (25.11)	40.2 ± 1.27** (14.10)
PBZ	100	44.8 ± 1.48**** (25.08)	46.6 ± 1.71**** (34.36)	48.6 ± 1.82**** (37.21)	54.4 ± 2.15**** (39.01)	38.2 ± 1.34**** (18.61)

<sup>a</sup> Values are mean ± standard error of mean (S.E.M.), n = 5; \*  $p < 0.1$ , \*\*  $p < 0.02$ , \*\*\*  $p < 0.01$ , \*\*\*\*  $p < 0.001$  vs. control, Student's *t*-test.

In order to study the diuretic activity of *C. viscosum* Vent., effect of MeCV on urination was investigated in *Swiss albino* mice.<sup>9</sup> The urinary output at different hours of study has been presented in Figure 1. Since the standard drug furosemide possesses rapid onset and short duration of action<sup>10</sup> we recorded the urinary output from 1 to 4 h of the administration of MeCV at 1 h interval. According to Gujral *et al.*<sup>9</sup> the diuretic activity of a drug is considered to be good if it is above 1.50, moderate if it is within 1.00 ~1.50, and little if it is between 0.72~1.00. In this respect, MeCV exhibited a score of diuretic activity (Figure 1) below 0.72 at 1, 2, 3 and 4 h of administration, thereby suggesting that the extract lacks diuretic property.

The electrolyte content (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>) of urine collected after 4 h of the administration of Urea (500 mg/kg body weight) and MeCV (300 mg/kg body

weight) was analyzed by using Beckman Synchron EL-ISE Electrolyte System (Germany). The sum of Na<sup>+</sup> and Cl<sup>-</sup> excretion was calculated as a parameter of saluretic activity. The ratio Na<sup>+</sup>/K<sup>+</sup> was calculated for natriuretic activity. The ratio Cl<sup>-</sup>/Na<sup>+</sup>+K<sup>+</sup> was calculated to estimate carbonic anhydrase inhibition.<sup>11</sup>

Analysis of urinary electrolyte excretion (Table 3) after 4 h of intragastric administration of urea and MeCV at doses of 500 and 300 mg/kg body weight, respectively, revealed that MeCV lacks natriuretic or saluretic properties as measured by identical Na<sup>+</sup>/K<sup>+</sup> or the sum of Na<sup>+</sup> and Cl<sup>-</sup>, respectively, in MeCV- and urea-treated groups. Almost identical ion quotient (Cl<sup>-</sup>/Na<sup>+</sup>+K<sup>+</sup>) in MeCV- and urea-treated group indicated that MeCV had no inhibitory property on carbonic anhydrase enzyme.

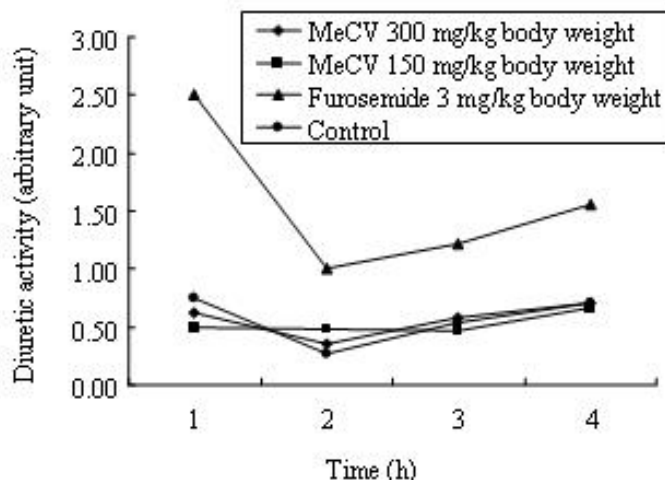


Figure 1. Screening of diuretic activity of MeCV. Mice were given MeCV at doses of 150 and 300 mg/kg body weight by gavage. Total urinary output of 6 mice per group was recorded and data were analyzed for determining diuretic activity.

Table 3. Effect of MeCV on electrolyte excretion\*

Treatment	Na <sup>+</sup> (mmol/l)	K <sup>+</sup> (mmol/l)	Cl <sup>-</sup> (mmol/l)	Na <sup>+</sup> /K <sup>+</sup>	Na <sup>+</sup> +Cl <sup>-</sup> (mmol/l)	Cl <sup>-</sup> /(Na <sup>+</sup> +K <sup>+</sup> )
MeCV	64.45	39.40	97.91	1.636	162.37	0.943
Urea	61.82	39.43	102.45	1.568	164.27	1.011

\*MeCV and urea were administered at doses of 300 and 500 mg/kg body weight, respectively. Each treatment group was consisted of 6 mice.

Although preliminary biological study has revealed that MeCV possesses significant analgesic and anti-inflammatory activities, mechanisms

underlying the observed pharmacological effects are not clear. The assessment of observed pharmacological effects with isolated individual

chemical constituent merit further investigation for better understanding of the molecular mechanisms underlying antinociceptive and anti-inflammatory activities of the methanol extract of aerial parts of *C. viscosum* Vent. However, the present study suggests that the crude extracts of *C. viscosum* Vent., may be used as a herbal remedy for the management of pain and inflammation. Although no sign of toxicity was observed after 24 h of administration of MeCV at a dose of 300 mg/kg body weight, detailed toxicological study should be performed before exploiting this plant extract for therapeutic purpose.

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