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Analgesic, antiinflammatory and CNS depressant activities of sesquiterpenes and a flavonoid glycoside from *Polygonum viscosum*

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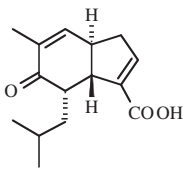
Analgesic, antiinflammatory and CNS depressant activities of four sesquiterpenes, viscosumic acid, viscozulenic acid, viscoazucine and viscoazulone, and a flavonoid glycoside, quercetin-3-*O*-(6″-feruloyl)-β-D-galactopyranoside isolated form the aerial parts of *Polygonum viscosum* (Polygonaceae) have been assessed. All test compounds exhibited CNS depressant activity in open field test, all but viscoazulone showed analgesic activity in Eddy's hot plate test, all sesquiterpenes inhibited acetic acid induced abdominal writhing in mice, and all but viscoazucine and the flavonoid glycoside exhibited mild to moderate antiinflammatory effect on carrageenan induced rat paw edema.

1. Introduction

Polygonum viscosum Buch-Ham. Ex D. Don (Polygonaceae), common name-"Bishkatali", is an annual herb native to Nepal and widely distributed in Bangladesh, northeast India, China and Japan. The genus *Polygonum* is well known for producing a number of pharmacologically active compounds, and also for its use in oriental traditional medicine (Phytochemical Database 2003). Antiinflammatory activity of the aqueous ethanolic extract of *Polygonum bistorta* (Duwiejua et al. 1994, 1999), and antipyretic and antiinflammatory activities of the aqueous and the hydroalcoholic extracts of *Polygonum punctatum* (Simoes et al. 1989) have recently been reported. Compounds having antiinflammatory and antiallergic activities, and tumour cell growth inhibitory activity have previously been isolated, respectively, from *Polygonum chinensis* (Tsai et al. 1998) and *Polygonum hypoleucum* (Kuo et al. 1997). To date, *Polygonum viscosum* has only been reported to have antibacterial property (Hoque et al. 1989). As part of our on-going phytochemical (Datta et al. 2000a–c, 2001a, b, 2002) and pharmacological studies on this species, we now report on the analgesic, antiinflammatory and CNS depressant activities of four sesquiterpenes, viscosumic acid (**1**), viscozulenic acid (**2**), viscoazucine (**3**) and viscoazulone (**4**), and a flavonoid glycoside, quercetin-3-*O*-(6″-feruloyl)-β-D-galactopyranoside (**5**) isolated form the aerial parts of this plant.

2. Investigations, results and discussion

Analgesic activity of the test compounds (**1**–**5**) was studied as a measure of pain perception time in response to thermal stimuli (Woolfe and MacDonald 1944; Eddy and Leimbach 1953; Wood 1985). All test compounds (**1**–**5**), except viscoazulone (**4**), showed statistically significant ($p < 0.01$) analgesic activity in comparison to that of the positive control morphine (Table 1). Among these compounds, viscozulenic acid (**2**) was found to have moderate analgesic activity, while viscosumic acid (**1**), viscoazucine (**3**) and quercetin 3-*O*-(6″-feruloyl)-β-D-galactopyranoside (**5**) showed mild analgesic activity, all having their peak effect at 120 min after administration. Among the structurally related sesquiterpenes **2**–**4**, viscozulenic acid (**2**) has an extra hydroxyl group at C-6 and also a free –COOH



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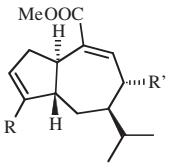
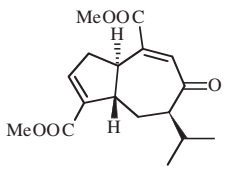


2 R = COOH R = OH

3 R = COOMe R = H



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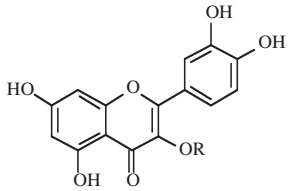

5 R =(6''-feruloyl)-β-D-galactopyranosyl

Table 1: Effect of compounds 1–5 on pain perception time

Compds	Dose (mg/kg)	Pain perception time (in s) Mean ± S.E.M. (p values)				
		0 min	30 min	60 min	120 min	240 min
Control	50	13.05 ± 2.21	11.18 ± 0.82	7.46 ± 0.68	9.64 ± 1.47	10.27 ± 0.96
1	50	8.63 ± 2.05 (0.072)	11.68 ± 1.89 (0.749)	15.95 ± 4.36 (0.05)*	17.15 ± 5.41 (0.361)	11.76 ± 2.7 (0.078)
2	50	10.0 ± 2.05 (0.126)	18.6 ± 0.73 (0.042)*	28.5 ± 3.49 (0.001)***	30.4 ± 5.09 (0.01)**	26.0 ± 3.33 (0.004)**
3	50	8.1 ± 0.92 (0.078)	9.6 ± 1.05 (0.303)	13.05 ± 2.11 (0.049)*	14.78 ± 2.44 (0.285)	10.49 ± 0.93 (0.738)
4	50	8.99 ± 1.55 (0.149)	8.7 ± 1.04 (0.006)**	10.03 ± 1.05 (0.112)	11.38 ± 2.15 (0.541)	10.25 ± 1.7 (0.991)
5	50	10.07 ± 2.097 (0.385)	17.2 ± 2.77 (0.734)	19.49 ± 3.74 (0.002)**	19.5 ± 3.79 (0.006)**	13.79 ± 3.73 (0.001)***
Morphine	05	24.3 ± 4.18 (0.038)*	494.3 ± 22.8 (0.000)***	411.2 ± 27.8 (0.000)***	398.3 ± 9.9 (0.000)***	385.17 ± 9.1 (0.000)***

*p < 0.05, **p < 0.01, ***p < 0.001

Table 2: Effect of compounds 1–5 on acetic acid-induced writhing in mice

Compounds	Doses (mg/kg)	Number of writhing observed Mean ± S.E.M (p value)	% Protection
Control	50	44.25 ± 9.105	–
1	50	25.73 ± 5.83 (0.000)***	41.85
2	50	29.06 ± 3.56 (0.000)***	34.33
3	50	32.01 ± 5.78 (0.001)***	27.66
4	50	28.14 ± 3. (0.002)**	36.41
5	50	33.94 ± 6.92 (0.693)	23.30
ASA	5.0	15.63 ± 4.719 (0.001)***	64.68

* p < 0.05; ** p < 0.01; *** p < 0.001
ASA = Acetyl salicylic acid

group at C-1 which makes **2** more polar than other two (**3**, **4**). This extra polarity might be accounted for its slightly better analgesic activity. On the other hand, viscoazulone (**4**) devoids of any polar functional group in its structure, and this might explain its inactivity as an analgesic.

All sesquiterpenes (**1**–**4**) inhibited acetic acid induced abdominal writhing (Koster et al. 1959) significantly by 34.33% (p = 0.001), 36.41% (p = 0.01), 27.66% (p = 0.001) and 41.85% (p = 0.001) respectively, while

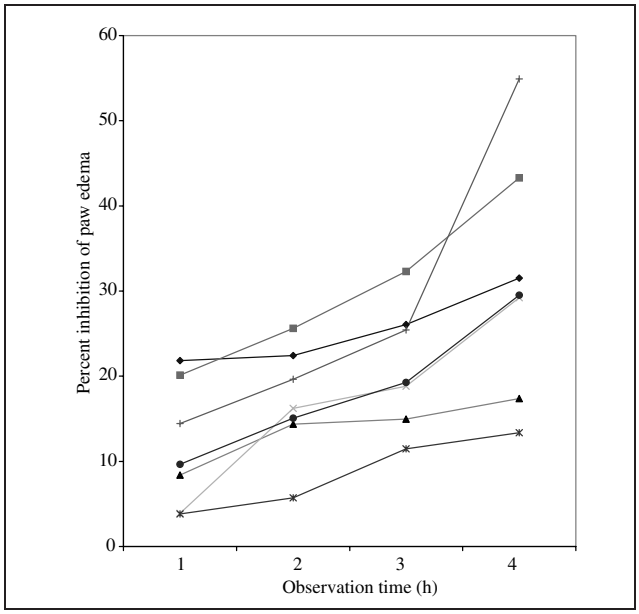

Fig.: Effect of compounds **1**–**5** on carrageenan induced rat paw edema
Compound **1** Compound **2** Compound **3** Compound **4** Compound **5** Aspirin Indomethacin

Table 3: CNS depressant activity of compounds 1–5

Compounds	Dose (mg/kg)	Period of observation (No. of movements) Mean ± S.E.M. (p value)				
		0 min	30 min	60 min	120 min	240 min
Control	–	106.29 ± 4.14	49.71 ± 7.44	48.86 ± 5.72	47.14 ± 3.7	35.71 ± 5.76
1	50	199.92 ± 34.53 (0.946)	119.93 ± 19.63 (0.031)*	99.63 ± 24.64 (0.745)	89.45 ± 6.53 (0.01)**	33.61 ± 8.59 (0.022)*
2	50	152.43 ± 21.47 (0.937)	87.0 ± 12.24 (0.01)**	34.09 ± 10.99 (0.638)	49.96 ± 19.64 (0.049)*	46.65 ± 9.66 (0.042)*
3	50	143.97 ± 39.37 (0.138)	99.12 ± 12.94 (0.042)*	45.91 ± 12.94 (0.005)**	28.94 ± 15.93 (0.037)*	23.92 ± 10.11 (0.039)*
4	50	137.95 ± 15.93 (0.396)	69.52 ± 14.94 (0.002)**	61.91 ± 5.39 (0.497)	30.71 ± 7.94 (0.01)**	27.93 ± 9.74 (0.087)
5	50	189.34 ± 39.53 (0.866)	112.29 ± 27.83 (0.937)	73.64 ± 9.38 (0.036)*	67.64 ± 13.94 (0.749)	35.84 ± 16.21 (0.059)

*p < 0.05, **p < 0.01, ***p < 0.001

the standard drug acetylsalicylic acid caused an inhibition of writhing by 64.68%, which is statistically significant ($p = 0.001$) (Table 2). Inhibition of abdominal writhing by the flavonoid glycoside (**5**) was 23.30% ($p = 0.693$), which is statistically insignificant.

Compounds **1**–**5** were tested for their effect on carrageenan induced rat paw edema (Winter et al. 1962; Godhwani et al. 1987) at a dose of 50 mg/kg body weight taking indomethacin, at a dose of 10 mg/kg body weight and acetylsalicylic acid at a dose of 5 mg/kg body weight as standard drugs. Viscozulenic acid (**2**) exhibited mild to moderate antiinflammatory activity ($p = 0.01$) against carrageenan induced inflammation, when compared with the standard anti-inflammatory agent acetylsalicylic acid (ASA) and indomethacin (IND) (Fig.). It exhibited the highest activity 4 h after i.p. administration (50 mg/kg). Mild antiinflammatory activity was also observed with **1** and **4**. Viscoazucine (**3**) and quercetin-3-*O*-(6″-feruloyl)-β-D-galactopyranoside (**5**) appeared to have no significant effect on carrageenan induced paw inflammation.

The effect of test compounds **1**–**5** on the central nervous system (CNS) was studied by open field test (Gupta et al. 1971) where the effect of the compounds on the movement of experimental animals was recorded. Compounds **3** and **4** exhibited significant CNS depressant activity which was evident from a gradual decrease in movement of the treated mice (Table 3). The decrease in movement with time after administration of the other test compounds (**1**–**2**, **5**) indicated moderate CNS depressant activity of **1** and **5**, and mild activity of **2** at a dose of 50 mg/kg body weight.

The significant analgesic and antiinflammatory activity of most of the test compounds isolated from the aerial parts of *Polygonum viscosum* may explain the Bengali common name of this plant, 'Bishkatali' which means 'pain-killer'.

3. Experimental

3.1. General

The UV (in MeOH) and IR (KBr) spectra were obtained, respectively, on a Beckman DU-640 and Perkin-Elmer 1600 FTIR spectrometer. Optical rotation was measured with a Perkin-Elmer 241 polarimeter. NMR spectra (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR) were obtained on Varian INOVA 500 spectrometer. High-resolution mass spectra were acquired on a JEOL SX102 mass spectrometer. HPLC separation was performed in the Dionex prep-HPLC System coupled with Gynkotek GINA50 autosampler and Dionex UVD340S Photo-Diode-Array detector, and/or Waters prep-HPLC System coupled with a UV-Vis detector. Luna reversed-phase C_{18} preparative column was used. Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) was used for pre-HPLC fractionation.

3.2. Plant material

Whole plant parts of *P. viscosum* Buch.-Ham. Ex D. Don were collected from Panchari, Chittagong, Bangladesh, and authenticated by Professor Abul Hassan (Department of Botany, University of Dhaka, Bangladesh). A voucher specimen (voucher no 764) representing this collection has been retained in the Herbarium of the Department of Botany, University of Dhaka, Bangladesh.

3.3. Extraction and isolation

Ground dried whole plant parts (2.3 kg) were extracted, successively, with *n*-hexane, EtOAc and MeOH. All three extracts were concentrated using a rotary evaporator at a maximum temperature of 45 °C. Four sesquiterpenes, viscosumic acid (**1**), viscozulenic acid (**2**), viscoazucine (**3**) and viscoazulone (**4**), and a flavonoid glycoside, quercetin 3-*O*-(6″-feruloyl)-β-D-galactopyranoside (**5**) were re-isolated by a combination of various chromatographic techniques including preparative RP-HPLC, following the methods previously described in the literature (Datta et al. 2000a, 2001 a, b, 2002).

3.4. Structure determination

Structures of **1**–**5** were determined conclusively by UV, IR, MS, optical rotation and extensive 1D and 2D NMR experiments as described previously (Datta et al. 2000a, 2001 a,b, 2002).

3.5. Analgesic activity testing

3.5.1. Eddy's hot plate method

The analgesic activity of the test compounds was assessed by "Eddy's Hot Plate method (Woolfe and MacDonald) 1944; Eddy and Leimbach 1953; Wood 1985). Morphine sulphate (MPS, Morphine), was used as the positive control at a dose of 5 mg/kg body weight. The test compounds, at a dose of 50 mg/kg body weight, were administered intraperitoneally (i.p.) to five groups of mice, each consisting of 6 animals, while morphine sulphate and normal saline were given intraperitoneally (i.p.) to two other groups of mice. The hot plate was maintained at a constant temperature of 55 °C (± 0.5 °C). Each mouse was placed on the hot surface and the time of response to this thermal stimuli, indicated by the flicking of hind and/or fore paws or by kicking of the legs or by trying to jump-out, was recorded.

3.5.2. Acetic acid induced abdominal writhing assay

The compounds **1**–**5** were tested for their effect on acetic acid (AA) induced writhing reflexes (Koster et al. 1959) in experimental mice taking acetyl salicylic acid (ASA) at a dose of 5 mg/kg body weight, as the positive control. The study design was similar to that of the hot plate method. Muscular contraction was induced by 0.6% solution of acetic acid (0.25 ml/animal). The test compounds, at a dose of 50 mg/kg, were administered (i.p.) 30 min before the administration (i.p.) of acetic acid. The mice were then placed in boxes. Abdominal muscle contractions or writhes were counted at 15 min interval from the time of acetic acid administration to get the average number of writhes.

The percent protection of writhing by both test and standard drug was calculated according to the following equation.

Percent protection = $100\ (X_t/X_c) \times 100$

where X_t = Average number of writhes in treated group, X_c = Average number of writhes in control group

3.6. Antiinflammatory activity testing

The antiinflammatory activity of the test compounds were studied in laboratory animals (Sprague-Dawley rats weighing 120–175 gm) following the method of Winter et al. (1962) with slight modifications (Godhwani et al. 1987) taking indomethacin and acetylsalicylic acid as the positive control. Rats were randomly divided into eight groups, each consisting of 6 rats, of which five groups were treated with five test compounds, one group with indomethacin, one group with acetylsalicylic acid at a dose of 50 mg/kg, 10 mg/kg and 5 mg/kg body weight respectively keeping one group as control supplied only with normal saline. The test compounds, standard drugs and saline were given by oral route of administration. One hour after the administration of the test compounds, 2% carrageenan in physiological saline was injected subcutaneously into the subplantar region of the right hind paw at a dose of 1.0 ml/kg body weight. Immediately after the administration of carrageenan, the volume of the inflammed paw was measured by a digital Plethysmometer-7150 (UGO-Basile, Italy) and the data obtained was recorded as 0 h reading or predrug reading. The paw volume was then measured at +1, +2, +3 and +4 h after the administration of carrageenan and the data obtained was considered as postdrug readings.

The percent of inhibition of oedema volume was obtained by the following equation: % Inhibition = [(Predrug reading – Postdrug reading) × 100]/ Predrug reading

3.7. Open Field Test: CNS depressant activity testing

The effect of compounds **1**–**5** on the central nervous system (CNS) activity was studied by open-field test (Gupta et al. 1971). Five groups of mice, each consisting of 6 animals, were given the test compounds at a concentration of 50 mg/kg body weight intraperitoneally while a sixth group was kept as control. An apparatus having a wall of 40 cm was used for this experiment. The floor of an open field of 0.5 m^2 was divided into a series of squares, each alternatively colored black and white. The number of squares traveled by the animals was recorded for a period of 2 min after the administration of the test compounds.

3.8. Statistical analysis

Data obtained from the experiments are expressed as mean and standard error of the mean. Unpaired t-tests were performed by computer software SPSS (Statistical Package for Social Science) release 9.05 for Windows™, to test the level of significance. Probability (p) value of 0.05 or less ($p < 0.05$) was considered as significant. In these data $pp < 0.05$, $pp < 0.01$, $pp < 0.001$ are represented by a single (*), double (**) and triple (***) asterisk (s).

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