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RESEARCH ARTICLE

Analgesic and anti-inflammatory activities of *Polygonum stagninum*

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Abstract

The *n*-hexane, ethyl acetate (EtOAc), and methanol extracts of the aerial parts of *Polygonum stagninum* Buch.-Ham. ex Meissn. (Polygonaceae), a Bangladeshi medicinal plant, were assessed for analgesic and anti-inflammatory properties in experimental mice and/or rat models. In the acetic-acid-induced writhing test in mice, all extracts displayed a dose dependent analgesic effect. The most potent analgesic activity was observed with the EtOAc extract at the dose of 400 mg/kg body weight, with an inhibition of writhing response of 50.3% compared to 62.2% for the positive control aminopyrine. Among the extracts, *n*-hexane extract at the doses of 200 and 400 mg/kg body weight showed the highest levels of anti-inflammatory activity after 2 h, with the inhibition of paw edema of 60.1% and 64.1%, respectively, and this effect was much better than that of the conventional anti-inflammatory agent phenylbutazone (maximum inhibition of 38.3% after 4 h).

Keywords: Polygonum stagninum*; Polygonaceae; analgesic; anti-inflammatory*

Introduction

Polygonum stagninum Buch.-Ham. ex Meissn. (Polygonaceae) is a common pond weed known as "ratooti sag" or "bara bishkatali" in Bengali. This plant grows abundantly in seasonally flooded roadside ditches and small ponds in Bangladesh, India, and Thailand, and also in many other countries in South-East Asia (Balza et al., 1989). The genus *Polygonum* is well known for producing pharmacologically active compounds, and also for its use in oriental traditional medicine systems. A number of *Polygonum* species have traditionally been used as analgesic and antipyretic agents, e.g. *P. aviculare* L., *P. bistorta* L., *P. chinense* L., *P. cuspidatum* Siebold. & Zucc., *P. glabrum* Willd., *P. multiflorum* Thunb., *P. odoratum* Lour., *P. orientale* L., *P. perfoliatum* L., *P. persicaria* L., *P. tinctorium* Aiton., and *P. tinctaricum* L., anti-inflammatory agents, e.g. *P. aviculare* L., *P. barbatum* L., *P. bistorta* L., *P. chinense* L., *P. cuspidatum* Siebold. & Zucc., *P. flaccidum* Auct., *P. hydropiper* L., *P. japonicum* L., *P. lapathifolium* L., *P. multiflorum* Thunb., *P. orientale* L., *P. perfoliatum* L., *P. persicaria* L., *P. punctatum* Elliott, *P. sanguinaria* L., and *P. viviparum* L., and as diuretic agents, e.g. *P. acre* Kunth., *P. amphibium* L., *P. aviculare* L., *P. bistorta* L., *P. cuspidatum* Siebold. & Zucc., *P. hydropiper* L., *P. odoratum* Lour., *P. perfoliatum* L., *P. punctatum* Elliott, and *P. virginianum* L. (Phytochemical and Ethnobotanical Databases, 2008). Previous phytochemical studies on this plant revealed the presence of cinnamic acid derivatives, flavonoids, and proanthocyanidin polymers (Balza et al., 1989; Datta et al., 2002). As part of our ongoing bioactivity and phytochemical studies on *Polygonum* species (Datta et al., 2000, 2001a, 2001b,

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2002, 2004a, 2004b, 2007), we report on the analgesic and anti-inflammatory properties of the extracts of *P. stagninum* in animal models.

Materials and methods

Plant material

The aerial parts of *Polygonum stagninum* were collected from Kajla, Rajshahi, Bangladesh and authenticated by Professor Naderuzzaman (Department of Botany, University of Rajshahi, Bangladesh). A voucher specimen (DACB-29353) representing this collection has been retained in the Bangladesh National Herbarium, Dhaka, Bangladesh.

Extraction

Sun-dried and ground aerial parts (800 g) were cold-extracted successively with *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH), 4 L volume and 5 days' duration for each. The extracts were concentrated by evaporation under reduced pressure at 40°C to yield 19.8, 9.5, and 27.8 g of *n*-hexane, EtOAc, and MeOH extracts, respectively.

Animals

Swiss albino mice (3–4 weeks old, 20–25 g) and Long Evans adult rats (9–10 weeks old, 130–170 g) of either sex were used in this study. These experimental animals were obtained from the Animal House of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions and had access to feed and water *ad libitum*. Experiments on animals were performed strictly in accordance with the guidelines provided by the Institutional Animal Ethics Committee. Each experimental group was composed of five mice/rats. The first group served as control animals, and they were treated with 1% Tween 80 in water. The second group of animals were treated with positive control. The other groups of animals were treated with the extracts at different doses (200 and 400 mg/kg).

Test samples and positive controls

Suspensions of the extracts of *P. stagninum* were prepared in dimethylsulfoxide (DMSO) and diluted with normal saline and triturated thoroughly, or in 1% Tween 80 in distilled water. Aminopyrine (New Dragon Industrial Co., Ltd., China) and phenylbutazone (Shandong H&T Corp., China) were used as positive controls, respectively, for analgesic and anti-inflammatory tests. All test samples and the positive controls were administered orally by a feeding needle.

Assessment of analgesic activity

The analgesic activity of the extracts was assessed by the acetic-acid-induced writhing method (Koster et al., 1959; Whittle, 1964; Williamson et al., 1996; Zakaria et al., 2001; Silva et al., 2003) using Swiss albino mice. Experimental animals were randomly selected and divided into eight groups consisting of five mice in each group. Each group received a particular treatment, i.e. group A: control (0.5 mL DMSO in 2 mL of normal saline); group B: positive control (aminopyrine, 50 mg/kg body weight); groups C–E: extracts at dose of 200 mg/kg body weight; and groups F–H: extracts at dose of 400 mg/kg body weight. Forty minutes after administration of the above treatments, each group was treated with intraperitoneally (i.p.) administered 0.7% acetic acid to induce pain sensation evident from the test animals squirming their bodies at regular time intervals, referred to as "writhing." The number of writhes (i.e. abdominal contractions and stretches) that occurred within the first 10 min following acetic acid administration were counted and recorded. Analgesic agents that reduced this pain sensation could be observed from a reduced number of writhings compared to the control group. The analgesic effect of the test extracts was compared with that of the positive control aminopyrine, a well known painkiller.

Assessment of anti-inflammatory activity

Anti-inflammatory activity of the extracts was evaluated by a carragenan-induced edema model in Long Evans adult rats (Winter et al., 1962). In this study, edema was induced at the sub-plantar region of a rat's hind paw by administration of 1% carrageenan solution. The reduction of paw edema volume with the introduction of test sample would indicate anti-inflammatory activity. The edema volume was measured every hour up to 4 h. The average percent decrease or increase in paw volume with time was calculated and compared against the control group. Experimental animals were randomly selected and divided into eight groups consisting of five mice in each group. Each group received a particular treatment, i.e. group A: control (1% Tween 80 in distilled water); group B: positive control (phenylbutazone, 100 mg/kg body weight); groups C–E: extracts at dose of 200 mg/kg body weight; and groups F–H: extracts at dose of 400 mg/kg body weight. Thirty minutes after administration of the test samples, 0.1 mL of 1% carrageenan in sterile saline was injected into the sub-plantar surface of the right

hind paw of each rat. Paw volumes were measured up to a fixed mark by mercury displacement as viewed by traveling microscope at 1, 2, 3, and 4 h after the administration of the test sample.

Statistical analysis

Experimental values are expressed as mean ± SEM. An independent sample *t*-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results and discussion

Analgesic and anti-inflammatory activities of the *n*-hexane, EtOAc, and MeOH extracts of the aerial parts of *P. stagninum* were determined by a series of well established and validated experimental methods using mice and/or rat models.

The acetic-acid-induced writhing method (Koster et al., 1959; Whittle, 1964) is an analgesic-mediated behavioral observation method that demonstrates a noxious stimulation in mice. In this test (Table 1), a dose dependent analgesic effect was noted with the extracts. The most potent analgesic activity was observed with the EtOAc extract at the dose of 400 mg/kg body weight (inhibition of writhing response 50.3%). This response was quite close to that observed with aminopyrine (50 mg/kg), causing 62.2% pain inhibition. The order of analgesic activity of the extracts was as follows: EtOAc > *n*-hexane > MeOH. As the EtOAc extract was the most potent of the three extracts, it could be assumed that the compounds responsible for the analgesic activity of this plant were of medium polarity, possibly non-conjugated flavonoids previously reported from this plant. The analgesic property observed with the extracts of this plant was in line with the traditional use of a number of *Polygonum* species to treat various pains (Phytochemical and Ethnobotanical Databases, 2008).

Intraperitoneal administration of acetic acid (0.7%), a painful stimulus, results in localized inflammation by releasing free arachidonic acid from tissue phospholipids through the action of phospholipase A_2 and other acyl hydrolases (Koster et al., 1959). The synthesis of eicosanoids from arachidonic acid involves three major pathways. All eicosanoids with ring structures, e.g. prostaglandins, thromboxanes, and prostacyclin, are synthesized via the cyclooxygenase pathway. The leukotrienes, HETE (hydroxy eicosatetraenoic acid) and HPETE (hydroperoxy eicosatetraenoic acid), are hydroxylated derivatives of straight-chain fatty acids and are synthesized via the lipooxygenase pathway. The released prostaglandins, mainly prostacyclin (PGI_2) and prostaglandin E, have been reported to be responsible for pain sensation by exciting the Aδ-fibers. Activity in the Aδ-fibers causes a sensation of sharp, well localized pain. Analgesic activity was determined by measuring the writhing effect that was produced by the administration of acetic acid and inhibition of the writhing effect produced by the test agents. Any agent that lowers the number of writhings demonstrates analgesia by inhibiting prostaglandin synthesis, a peripheral mechanism of pain inhibition. This hypothesis is in consonance with those authors who postulated that acetic-acid-induced writhing test methods are useful techniques for the evaluation of peripherally and centrally acting analgesic drugs (Koster et al., 1959; Williamson et al., 1996; Silva et al., 2003). Therefore, it is reasonable to assume that the analgesic effect of the extracts of *P. stagninum* might have been mediated both peripherally and centrally.

Anti-inflammatory activity of the extracts was determined by the carrageenan-induced edema model in Long Evans adult rats (Winter et al., 1962). Edema is a pathophysiological inflammatory condition characterized by swelling, redness, elevated temperature, and pain. Thus, the reduction of carrageenan-induced edema could be used as a measure of the anti-inflammatory property of the test sample in the rat. Administration of the extracts at different doses produced statistically significant inhibition ($p < 0.05$) of edema within

Table 1. Effect of *Polygonum stagninum* extracts in mice observed in the acetic-acid-induced writhing test.

Group	Treatment	Dose* (mg/kg)	Mean writhings**	% Writhings	% Writhing inhibition
A	Control: DMSO (0.5 mL) in 2 mL of normal saline	—	37.8 ± 0.94	100 ± 3.06	0
B	Aminopyrine	50	14.3 ± 0.74	37.8 ± 3.02	62.2
C	*n*-Hexane extract	200	21.2 ± 0.54	56.1 ± 3.00	43.9
D	EtOAc extract	200	21.0 ± 0.93	55.6 ± 3.04	44.4
E	MeOH extract	200	30.4 ± 1.07	80.4 ± 3.00	19.6
F	*n*-Hexane extract	400	19.3 ± 1.03	51.1 ± 3.04	48.9
G	EtOAc extract	400	18.8 ± 0.90	49.7 ± 3.02	50.3
H	MeOH extract	400	27.9 ± 1.05	73.8 ± 3.02	26.2

*Administered orally (p.o.) 40 min before 0.2 mL of 0.7% acetic acid administration.

**Counted for 10 min, starting 10 min after acetic acid administration.

$p < 0.05$ vs. control, Students *t*-test; values are mean ± SE ($n = 5$).

Table 2. Effect of *Polygonum stagninum* extracts in rats observed in the carrageenan-induced edema test.

			Edema volume (μL) (% inhibition)			
Group	Treatment	Dose (mg/kg, p.o.)	1 h	2 h	3 h	4 h
A	Control (1% aq. Tween 80)	10 mL/kg	36.4 ± 2.15	64.6 ± 2.69	71.4 ± 0.83	63.0 ± 1.26
B	Phenylbutazone	100 (i.p.)	27.2 ± 0.79	42.4 ± 1.86	45.2 ± 1.94	38.6 ± 0.76
			(24.4)	(34.7)	(36.7)	(38.3)
C	*n*-Hexane extract	200	30.8 ± 2.94	25.8 ± 2.67	41.4 ± 1.51	49.8 ± 2.08
			(15.4)	(60.1)	(42.0)	(21.0)
D	EtOAc extract	200	27.4 ± 1.96	45.6 ± 2.55	51.6 ± 1.28	52.6 ± 3.08
			(24.7)	(29.4)	(27.7)	(16.5)
E	MeOH extract	200	27.6 ± 2.83	45.8 ± 3.12	55.2 ± 2.04	40.4 ± 1.31
			(24.2)	(24.5)	(22.7)	(35.9)
F	*n*-Hexane extract	400	29.4 ± 1.28	23.2 ± 1.11	40.6 ± 1.34	50.4 ± 1.84
			(19.2)	(64.1)	(43.1)	(20.0)
G	EtOAc extract	400	26.8 ± 1.39	45.2 ± 1.58	52.4 ± 2.34	55.2 ± 2.25
			(26.4)	(30.0)	(26.6)	(12.4)
H	MeOH extract	400	28.4 ± 0.92	46.2 ± 1.39	57.2 ± 2.36	39.84 ± 1.63
			(22.0)	(28.5)	(20.7)	(36.8)

The initial hind paw volume of the rat was determined volumetrically. Each point represents the mean ± SEM of five rats.
$p < 0.05$, compared with control group (Students *t*-test).
Inhibition (%) = 100(1 – (a/b)) where a = mean paw volume of treated animals after egg albumin injection; b = mean paw volume of control animals after egg albumin injection.

2–4 h of carrageenan administration (Table 2). Among the extracts, the *n*-hexane extract at the doses of 200 and 400 mg/kg body weight showed the highest levels of activity after 2 h, with inhibition of paw edema at 60.1 and 64.1%, respectively, and this effect was much better than that of the conventional anti-inflammatory agent phenylbutazone (maximum inhibition of 38.3% after 4 h). The anti-inflammatory property observed with the extracts of this plant was in line with the traditional use of a number of *Polygonum* species to treat various inflammatory diseases (Phytochemical and Ethnobotanical Databases, 2008).

Administration of extract inhibited edema starting from the first hour and during all phases of inflammation, which was probably due to inhibition of different aspects and chemical mediators of inflammation. The effect of the extracts in the inflammation process induced by carrageenan suggested that the extracts might have affected the time-delay system in a similar fashion to glucocorticoids (Ahamed et al., 2005).

The results demonstrated significant analgesic and anti-inflammatory properties of the extracts of *P. stagninum*, which were similar to, and in some cases better than, those of the positive controls, and indicated that this plant could be a potential source for the discovery and development of newer analgesic and anti-inflammatory "leads" for drug development.

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***Declaration of interest*:** The authors report no conflicts of interest.

References

Ahamed KFHN, Kumar V, Raja S, Mukherjee K, Mukherjee PK (2005): Antinociceptive and anti-inflammatory activity of A*araucaria bidwillii* Hook. Iran J Pharmacol Ther 4: 105–109.

Balza F, Abramowski Z, Towers GHN, Wiriychitra P (1989): Identification of proanthocyanidin polymers as the piscicidal constituents of *Mammea siamensis*, *Polygonum stagninum* and *Diospyros diepenhorstii*. Phytochemistry 28: 1827–1830.

Datta BK, Datta SK, Rashid MA, Nash RJ, Sarker SD (2000): A sesquiterpene acid and flavonoids from Polygonum viscosum. Phytochemistry 54: 201–205.

Datta BK, Rashid MA, Kundu JK, Rouf ASS, Sarker SD, Datta SK (2001a): Isolation and structure elucidation of viscoazucine, a novel sesquiterpene from Polygonum viscosum. Pharmazie 56: 578–579.

Datta BK, Rashid MA, Datta SK, Sarker SD (2001b): Viscozulenic acid: a novel sesquiterpene acid from *Polygonum viscosum*. Pharml Biol 39: 198–201.

Datta BK, Datta SK, Rashid MA, Sarker SD (2002): Flavonoids from *Polygonum stagninum* (Polygonaceae). Biochem Syst Ecol 30: 693–696.

Datta BK, Datta SK, Khan TH, Kundu JK, Rashid MA, Nahar L, Sarker SD (2004a): Anti-cholinergic, cytotoxic and anti-HIV-1 activities of sesquiterpenes and a flavonoid from *Polygonum viscosum*. Pharm Biol 42: 18–23.

Datta BK, Datta SK, Chowdhury MM, Khan TH, Kundu JK, Rashid MA, Nahar L, Sarker SD (2004b): Analgesic, antiinflammatory and CNS depressant activities of sesquiterpenes and a flavonoid glycoside from Polygonum viscosum. Pharmazie 59: 222–225.

Datta BK, Nahar L, Rahman MM, Gray AI, Auzi AA, Sarker SD (2007): Polygosumic acid, a new cadinane sesquiterpene, from *Polygonum viscosum* inhibits the growth of drug-resistant *Escherichia coli* and *Staphylococcus aureus* (MRSA) *in vitro*. J Nat Med 61: 391–396.

Koster R, Anderson M, De-Beer EJ (1959): Acetic acid for analgesic screening. Fed Proc 18: 412–418.

Phytochemical and Ethnobotanical Databases (2008): Dr. Duke's Phytochemical and Ethnobotanical Databases, USA. Available on-line at http://www.ars-grin.gov/duke/.

Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ (2003): Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J Ethnopharmacol 89: 277–283.

Whittle BA (1964): The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br J Pharmacol Chemother 22: 246–253.

Williamson EM, Okpako DT, Evans FJ (1996): Pharmacological methods in phytotherapy research. In: *Selection, Preparation and Pharmacological Evaluation of Plant Materials*, Vol. 1. Chichester, John Wiley, pp. 184–186.

Winter CA, Risley EA, Nuss GW (1962): Carrageenan-induced edema in the hind paw of rat as an assay for anti-inflammatory drugs. Proc Soc Biol Med 11: 544–547.

Zakaria MN, Islam MW, Radhakrishnan R, Chen HB, Kamil M, Al-Gifri AN, Chan-Attas A (2001): Antinociceptive and anti-inflammatory properties of *Caralluma arabi*. J Ethnopharmacol 76: 155–158.