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Evaluation of anti-inflammatory potential of *Bergenia ciliata* Sternb. rhizome extract in rats

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Abstract

The methanol extract of the rhizome of *Bergenia ciliata* Sternb. (Saxifragaceae) has been evaluated for anti-inflammatory potential using two acute rat models (carrageenan- and serotonin (5-HT)-induced rat paw oedema) and a chronic rat model (cotton pouch-induced granuloma). Phenylbutazone (100 mg kg^{-1}), a non-steroidal anti-inflammatory agent, was used as a standard. The methanol extract (100, 200 or 300 mg kg^{-1}) exhibited significant ($P < 0.05$) anti-inflammatory activity in all the animal models. At 300 mg kg^{-1} the methanol extract exhibited maximum inhibition of 32.4 ± 2.89 % in carrageenan-induced rat paw oedema while the standard showed an inhibition of 44.1 ± 2.7 % after 3 h of drug treatment. In the serotonin-induced rat paw oedema model, 300 mg kg^{-1} methanol extract suppressed oedema by 45.33 ± 2.09 %, whereas the standard produced an inhibition of 53.5 ± 4.3 %. In the cotton pouch granuloma model the methanol extract inhibited significantly ($P < 0.001$) the granuloma weight in a dose-dependent manner. In this model, 300 mg kg^{-1} extract produced a maximum inhibition of 31.4 ± 1.09 % in granuloma weight compared with 41.1 ± 1.32 % reduction in granuloma weight for the standard. The methanol extract of *B. ciliata* exhibited significant anti-inflammatory potential at the dose levels examined.

Introduction

Inflammation is commonly divided into three phases, acute inflammation, the immune response and chronic inflammation. Rheumatoid arthritis is an important inflammatory condition in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life (Katzung 1998).

A review of plants exhibiting anti-inflammatory activity showed that different species of 96 genera belonging to five families have such activity (Handa et al 1992). *Bergenia ciliata* Sternb. (Family: Saxifragaceae), commonly known as 'Pakhanbed' (Hindi) is a perennial herb with creeping rhizomes found throughout the temperate Himalayas from Kashmir to Bhutan, between 7000–10 000 ft and the Khasia hills at 4000 ft (Anonymous 1948). As a traditional medicine, the rhizome of this plant can be used fresh or in dried powdered form. If fresh it can be chewed and used for curing diarrhoea and vomiting. It is also useful against fever, cough and pulmonary affections and can be used over boils (Biswas 1955; Rai & Sharma 1994). The rhizomes can be applied to the gums with honey in children when teething to allay irritation (Nadkarni 1976). In Indo China the juice of the plant is used for earache (Kirtikar & Basu 1975).

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In this study we have evaluated the anti-inflammatory potential of the methanol extract of the rhizome of *B. ciliata* Sternb. using several experimental rat models.

Materials and Methods

Plant material

Rhizomes of *Bergenia ciliata* were collected freshly from Baluakhani, North Sikkim, and Kalimpong and Lava forest, Darjeeling district of West Bengal, India, during October and November. Rhizomes were identified by Botanical Survey of India, Sikkim Circle, Gangtok. A voucher specimen (B.C. −1) has been kept in our laboratory for future reference. The rhizomes were shade-dried, pulverized by a mechanical grinder, passed through a 40-mesh sieve, and then stored in a tightly closed container for future use.

Preparation of the extract

The powdered material was extracted with methanol (90% v/v) using a soxhlet apparatus. From the methanol extract, the solvent was removed under vacuum and a semisolid mass was obtained. This dried methanol extract was stored in a desiccator and used for further experiment after suspending in aqueous Tween 80 solution (2%, v/v). The chemical constituents of the extract were identified by preliminary qualitative analysis and confirmed by high-performance thin layer chromatography (HPTLC) for the presence of steroid, flavonoid, tannin and reducing sugar.

Animals used

Albino Wistar rats of either sex (180–200 g; M/S B. N. Ghosh & Co., Calcutta, India) were housed in standard metal cages. They were provided with food and water was freely available. The rats were allowed a one-week acclimatization period before the experimental sessions.

Carrageenan-induced rat paw oedema

The rats were divided into five groups, each group consisting of six animals. Oedema was induced by subplantar injection of 0.1 mL 1% freshly prepared suspension of carrageenan (Sigma Chemical Co., MO) into the right hind paw of each rat. The paw volume was measured before (0 h) and at 2 h after the injection of carrageenan using a plethysmometer (Winter et al 1962). The methanol extract of *B. ciliata* rhizome (100, 200 or 300 mg kg^{-1}) was administered orally to three groups of rats. The fourth and fifth groups of rats received 2% aqueous Tween 80 solution 10 mL kg^{-1} orally (control) or phenylbutazone 100 mg kg^{-1} (standard), respectively, for assessing comparative pharmacological significance. Drug pretreatment was given 1 h before the injection of carrageenan.

Serotonin-induced rat paw oedema

The paw oedema was induced in the right hind paw by subplantar injection of 0.05 mL 1% freshly prepared serotonin solution (Maity et al 1998). Paw volumes were measured 30 min before and after serotonin injection. The rats were treated with methanol extract, control or standard and the paw volumes were measured as described above.

The percentage inhibition of oedema was calculated for both models as described by Kavimani et al (1996).

Cotton pouch-induced granuloma

The rats were divided into five groups with six animals in each group. After shaving off the fur the animals were anaesthetized. Through a single needle incision, sterile preweighed cotton pellets (10 mg) were implanted in the axilla region of each rat as described by D'Arcy et al (1960) with slight modification. Methanol extract (100, 200 or 300 mg kg^{-1}), phenylbutazone 100 mg kg^{-1} (standard) or 2% aqueous Tween 80 solution 10 mL kg^{-1} (control) were administered orally to the respective group of animals for seven consecutive days from the day of cotton-pellet implantation. On the eighth day the animals were anaesthetized again, the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the pellets was taken as measure of granuloma formation (Winter & Porter 1957).

Statistical analysis

The results were expressed as mean ± s.e.m. The significance was evaluated by Student's *t*-test compared with control. $P < 0.05$ implied significance (Woodson 1987).

Results

The methanol extract (100, 200 or 300 mg kg^{-1}) exhibited significant ($P < 0.05$) anti-inflammatory activity in all the animal models. At 300 mg kg^{-1} the methanol extract exhibited maximum inhibition of 32.4 ± 2.89% in carra-

Table 1 Effect of methanol extract of *Bergenia ciliata* Sternb. on carrageenan-induced rat paw oedema.

Treatment	Dose	% Increase in paw volume after 3 h	% Decrease in paw volume
2% v/v Aqueous Tween 80 solution (control)	10 mL kg^{-1}	27.9 ± 1.51	
Phenylbutazone (standard)	100 mg kg^{-1}	15.6 ± 0.75[a]	44.1 ± 2.7
Methanol extract of *B. ciliata* Sternb.	100 mg kg^{-1}	21.7 ± 0.61[c]	22.2 ± 2.2
	200 mg kg^{-1}	20.5 ± 0.87[b]	26.4 ± 3.13
	300 mg kg^{-1}	18.9 ± 0.81[b]	32.4 ± 2.89

Values are mean ± s.e.m., n = 6. [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.02$ compared with control.

Table 2 Effect of methanol extract of *Bergenia ciliata* Sternb. on serotonin-induced rat paw oedema.

Treatment	Dose	% Increase in paw volume after 3 h	% Decrease in paw volume
2% v/v Aqueous Tween 80 solution (control)	10 mL kg^{-1}	35.8 ± 1.95	
Phenylbutazone (standard)	100 mg kg^{-1}	16.6 ± 1.54[a]	53.5 ± 4.3
Methanol extract of *B. ciliata* Sternb.	100 mg kg^{-1}	29.8 ± 0.95[c]	16.8 ± 2.67
	200 mg kg^{-1}	27.6 ± 0.40[b]	22.8 ± 1.13
	300 mg kg^{-1}	19.6 ± 0.75[a]	45.33 ± 2.09

Values are mean ± s.e.m., n = 6. [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$ compared with control.

Table 3 Anti-inflammatory effect of methanol extract of *Bergenia ciliata* Sternb. on cotton-pouch granuloma in the rat.

Treatment	Dose	Weight of granuloma pouch (mg)	% Inhibition
2% v/v Aqueous Tween 80 solution (control)	10 mL kg^{-1}	25.4 ± 0.68	
Phenylbutazone (standard)	100 mg kg^{-1}	14.9 ± 0.34[a]	41.1 ± 1.32
Methanol extract of *B. ciliata* Sternb.	100 mg kg^{-1}	19.9 ± 0.09[a]	21.5 ± 0.33
	200 mg kg^{-1}	19.2 ± 0.15[a]	24.5 ± 0.58
	300 mg kg^{-1}	17.4 ± 0.27[a]	31.4 ± 1.09

Values are mean ± s.e.m., n = 6. [a]$P < 0.001$ compared with control.

geenan-induced rat paw oedema while the standard phenylbutazone showed inhibition of 44.1 ± 2.7 % after 3 h of drug treatment (Table 1). In the serotonin-induced rat paw oedema model (Table 2), the oedema suppression of 300 mg kg^{-1} methanol extract was 45.33 ± 2.09 % whereas the standard produced 53.5 ± 4.3 % of inhibition.

In the model of chronic inflammation (cotton pouch granuloma), the methanol extract inhibited significantly ($P < 0.001$) the granuloma weight in a dose-dependent manner (Table 3). In this model, 300 mg kg^{-1} extract produced a maximum inhibition of 31.4 ± 1.09 % in granuloma weight compared with 41.1 ± 1.32 % for the standard.

Discussion

We have established the anti-inflammatory activity of the methanol extract of *B. ciliata* rhizomes. Carrageenan-induced oedema is commonly used as an experimental model for evaluating the anti-inflammatory potential of natural products (Winter et al 1962; Della Loggia et al 1986; Alcaraz & Jimenez 1988; Mukherjee et al 1997) and is believed to be biphasic. The initial

phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carrageenan; a more pronounced second phase is attributed to the release of bradykinin, protease, prostaglandin and lysosome (Castro et al 1968). The later phase is reported to be sensitive to most of the clinically-effective anti-inflammatory agents (Smucker et al 1967). The inflammation induced by serotonin and its inhibition by the methanol extract suggested that it acted by affecting a time-delayed system similar to the glucocorticoids.

The extract exhibited significant anti-inflammatory activity ($P < 0.001$) in the cotton-pouch granuloma test. This reflected its efficacy to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Arrigoni-Martellie 1977).

The methanol extract of *B. ciliata* exhibited significant anti-inflammatory potential at the dose levels examined. The mechanism of action of the extract requires further investigation.

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