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Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models

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Abstract

In this study, the anti-inflammatory, analgesic, and antipyretic effects of 50, 100 and 200 mg/kg body weight of methanol extract obtained from *Bauhinia racemosa* stem bark, the so-called MEBR, were investigated. The effects of MEBR on the acute and chronic phases of inflammation were studied in carrageenan, dextran and mediators (histamine and serotonin)-induced paw oedema and cotton pellet-induced granuloma, respectively. Analgesic effect of MEBR was evaluated in acetic acid-induced writhing and hotplate tests. Antipyretic activity of MEBR was evaluated by yeast-induced hyperpyrexia in rats. The anti-oedema effect of MEBR was compared with 10 mg/kg of indomethacin orally. In acute phase of inflammation, a maximum inhibition of 44.9, 43.2, 44.8 and 45.9% ($P < 0.001$) was noted at the dose of 200 mg/kg b.w. after 3 h of treatment with MEBR in carrageenan, dextran, histamine and serotonin-induced paw oedema, respectively. Administration of MEBR (200 mg/kg b.w.) and indomethacin (10 mg/kg b.w.) significantly ($P < 0.05$) decreased the formation of granuloma tissue induced by cotton pellet method at a rate of 50.4 and 56.2%, respectively. The extract also inhibited peritoneal leukocyte migration in mice. The MEBR also produced significant ($P < 0.01$) analgesic activity in both models. Further, the MEBR potentiated the morphine- and aspirin-induced analgesic in mice. Treatment with MEBR showed a significant ($P < 0.01$) dose-dependent reduction in pyrexia in rats. The results suggest that MEBR possess potent anti-inflammatory, analgesic and antipyretic activity.

Keywords: *Bauhinia racemosa*; Anti-inflammatory; Analgesic; Antipyretic

1. Introduction

Bauhinia racemosa L. (Caesalpiniaceae), a small crooked tree with dark scabrous bark, is widely distributed throughout India, Ceylon, China and Timor. The bark and leaves of this plant are reported to be medicinally important in the traditional system of medicine and are used extensively for the treatment of inflammation, headache, fever, tumors, skin infection, disease of the blood, dysentery, and diarrhoea (Kirtikar and Basu, 1975; Wealth of India, 1952). The ethanol extract of leaves of this plant was evaluated for analgesic, anti-inflammatory, antipyretic and antispasmodic activity and was reported to be active (El-Khatiba and Khaleel, 1995). The fresh flower buds of this plant were screened for antiulcer activity (Akhtar and Ahmad, 1995). Dried entire plant showed antimicrobial activity (Ali et al., 1999). The cytotoxic, hypotensive and hypothermic activities of seeds of *Bauhinia racemosa* have also been reported (Dhar et al., 1968).

Several chemical constituents of *Bauhinia racemosa* have been identified mainly as flavonols, coumarins, triterpenoids, stilbenes, steroids and tannins (El-Hossary et al., 2000; Prakash and Khosa, 1976; Anjaneyulu et al., 1984, 1986; Balasooriya et al., 1982).

Previous results from this laboratory have also demonstrated the antioxidant and hepatoprotective effects in rats (Gupta et al., 2004a), and antitumor and antioxidant activity of *Bauhinia racemosa* against Ehrlich ascites carcinoma in Swiss albino mice (Gupta et al., 2004b). The present study was focussed on anti-inflammatory, analgesic and antipyretic

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effects of methanol extract of *Bauhinia racemosa* (MEBR) stem bark in animal models.

2. Materials and methods

2.1. Plant material

The stem bark of the plant *Bauhinia racemosa* L. (Family—Caesalpiniaceae) was collected from Kolli Hills of TamilNadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Kolkata. A voucher specimen (No GMS-1) has been preserved in our laboratory. The stem barks were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the bark was extracted with methanol (yield 9.25%), in a soxhlet apparatus. Phytochemical screening of the extracts revealed the presence of flavonoids, triterpenoids, coumarins, tannins and steroids.

2.2. Chemicals and drugs

Carrageenan (S.D. Fine Chemicals Limited, Bombay), 5-hydroxytryptamine hydrochloride (serotonin), histamine (Sigma, USA) and dextran (Sigma, USA) were used in the study, and indomethacin (Recon Bangalore), aspirin (USV Bombay), paracetamol (IPCA, Bombay) and morphine (M.M. Pharma, New Delhi) were used as the standard drugs.

2.3. Animals

Studies were carried out using male Wistar albino rats weighing 180–200 g and male Swiss albino mice weighing 18–22 g. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University animal ethical committee.

2.4. Toxicity study

The LD_{50} was determined using the graphical method of Litchfield and Wilcoxon (1949), in mice. Briefly, geometric doses of the extract (100–1750 mg/kg) were administered i.p. to 10 groups of mice. Control group received normal saline (5 ml/kg i.p.). Signs of toxicity and mortality within 24–72 h were noted. Confirmatory test was carried out and the LD_{50} was calculated from the graph of percent mortality against probit log dose of the extract.

2.5. Anti-inflammatory activity

2.5.1. Carrageenan-induced rat paw oedema

The rats were divided into five groups ($n = 6$). The different groups were treated with MEBR (50, 100 and 200 mg/kg b.w., p.o.), indomethacin (10 mg/kg, p.o.) and vehicle control (10% propylene) p.o. and the paw volume was measured at 0 h and 3 h after carrageenan injection using plethysmometer (Winter and Porter, 1957). The animals were pretreated with the extract 1 h before the administration of carrageenan. Acute inflammation was produced by the subplantar administration of 0.1 ml of 1% carrageenan in normal saline in the right paw of the rats. The anti-inflammatory effect of MEBR was calculated by the following equation: Anti-inflammatory activity (%) = $(1 - D/C) \times 100$, where D represents the percentage difference in paw volume after MEBR was administered to the rats and C represents the percentage difference of volume in the control groups (Suleyman et al., 1991).

2.5.2. Dextran-induced paw oedema

The animals were treated in a manner similar to that of carrageenan-induced paw oedema models; dextran (0.1 ml, 1% w/v in normal saline) was used in the place of carrageenan (Winter and Porter, 1957).

2.5.3. Histamine- and serotonin-induced inflammation

The anti-inflammatory activity of the MEBR was measured with phlogistic agents (viz. histamine, 5-HT) which act as mediator of inflammation. The paw oedema was induced in rats by subplantar injection of freshly prepared histamine (1 mg/kg b.w.) and serotonin (1 mg/kg b.w.) solutions, respectively, and the paw oedema was measured as mentioned earlier (Winter et al., 1962).

2.5.4. Cotton pellets-induced granuloma

The rats were divided into five groups ($n = 6$). After shaving the fur, the rats were anaesthetized and 10 mg of sterile cotton pellets were inserted, one in each axilla. The MEBR (50, 100 and 200 mg/kg b.w., p.o.) and indomethacin (10 mg/kg b.w., p.o.) and control vehicle were administered orally for seven consecutive days from the day of cotton pellet implantation. The animals were anaesthetized on the eighth day and cotton pellets were removed surgically and made free from extraneous tissues. The moist pellets were weighed and then dried at 60 °C for 24 h, after that dried pellets were weighed again. Increment in the dry weight of the pellets was taken as measure of granuloma formation. The antiproliferative effect of MEBR was compared with the control.

2.6. Mouse carrageenan peritonitis

The mice were divided into five groups ($n = 6$). Inflammation was induced by modification of the technique as