SHORT COMMUNICATION

Evaluation of effects of *Bauhinia variegata* stem bark extracts against milk-induced eosinophilia in mice

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ABSTRACT

Bauhinia variegata Linn (family: Caesalpiniaceae), popularly known as Rakta Kanchnar, is a medium-sized tree found throughout India. The stem bark of *B. variegata* (BV) is used traditionally in the treatment of asthma, jaundice, tuberculosis, leprosy, and skin diseases. In the present study, we have investigated the role of aqueous (BVA) and ethanol (BVE) extracts of the plant against milk-induced leukocytosis and eosinophilia in albino mice. The results of the study revealed that pretreatment with both the extracts caused significant reduction in the total leukocyte and eosinophil counts in animals in dose-dependent manner. From these results, it can be concluded that the plant BV is having antieosinophilic activity.

Key words: *Bauhinia variegata*, leukocytosis, milk-induced eosinophilia

INTRODUCTION

Bauhinia variegata Linn. (Caesalpiniaceae) is a medium-sized, deciduous tree, found throughout India, ascending to an altitude of 1 300 m in the Himalayas. It is commonly known as Kanchnar in Sanskrit and Mountain Ebony in English.[1] In Sanskrit, the word Kanchnar means "A glowing beautiful lady." The various parts of the plant, *viz*., flower buds, flowers, stem, stem bark, leaves, seeds, and roots, are utilized in various indigenous systems of medicine and are popular among the various ethnic groups in India for curing a variety of ailments. The bark of the plant is medicinally more important and is used by tribals against a variety of ailments. The bark is used in fever, as tonic and astringent, as antileprotic, in skin diseases and wound healing, antigoitrogenic, and as antitumor.[2-5] The bark is also documented as astringent to the bowels, tonic to the liver, and beneficial for the cure of dysmenorrhea, menorrhagia, tuberculosis, asthma, and wounds.[6] In an ethnobotanical survey of the Eastern Ghat region of Andhra Pradesh, the stem bark is documented to be useful for the treatment of asthma.[7] The stem bark of *B. variegata* (BV) is reported to possess hepatoprotective, anthelmintic, and antidiabetic activities.[8-10] In the present investigation, we have evaluated effect of aqueous (BVA) and ethanol (BVE) extracts of BV against milk-induced eosinophilia in albino mice.

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MATERIALS AND METHODS

Collection and Authentication of the Plant Material

The plant material (stem bark) was collected from mature trees around the city of Ahmedabad in October, 2008. The plant material was authenticated at Botanical Survey of India, Koregaon Road, Pune (Voucher specimen No. 165415). A specimen voucher of the plant has been deposited in the department of Pharmacognosy, L. B. Rao Institute of Pharmaceutical Education and Research, Khambhat.

Preparation of Plant Extracts

Dried, coarsely powdered stem bark of BV was defatted using petroleum ether (40-60°C) for 72 hours using Soxhlet apparatus. The marc left was subsequently extracted with ethanol (95%, 60-70°C) for 30 hours and with water (65-70°C). The solvents were removed by distillation under vacuum.

Phytochemical Analysis

Preliminary phytochemical studies of both the extracts were performed for detection of presence of various phytoconstituents, viz., alkaloids, flavonoids, saponins,

glycosides, phenols, steroids, tannins, and terpenoids, according to published standard procedures.[11]

Experimental Animals

The experimental procedure was in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India. The study was approved by the Institutional Animal Ethics Committee, L. B. Rao Institute of Pharmaceutical Education and Research, Khambhat, India, registered under CPCSEA, India (Registration No. 960/a/06/CPCSEA).

Swiss albino mice obtained from Zydus Cadila Limited (Ahmedabad, India) were housed in controlled environment with 12-hour light/dark cycles and free access to food and water. After a seven-day acclimation period, they were randomly assigned to different experimental groups.

Acute Toxicity Study of the Plant Extracts

The acute oral toxicity study was carried out as per the guidelines set by the Organization for Economic Co-operation and Development received from the CPCSEA. BVE and BVA extracts in dose range of 200 to 2 000 mg/kg were administered orally to these animals. They were continuously observed for 2 hours to detect changes in the autonomic and behavioral responses like alertness, spontaneous activity, irritability, urination, etc. Any mortality during experimentation and following 7 days was also recorded. The lethal dose was estimated to be >5 000 mg/kg and on the basis of these results, the doses of 100 and 200 mg/kg were chosen for the experiment.

Milk-induced Leukocytosis and Eosinophilia

The albino mice were randomly divided into six groups (one normal control, second milk intoxicated, and four test groups) containing six animals each. All the mice were anesthetized by using intraperitoneal injection with pentobarbital sodium, and blood was removed from cervical vein. It was then subjected for total cell and differential cell counts. The animals belonging to group I received orally distilled water (10 ml/kg). Animals belonging to group II, III, IV, V, and VI were given boiled and cooled milk (4 ml/kg) subcutaneously. The animals belonging to group III and IV were given 100 and 200 mg/kg of BVA and group V and VI received 100 and 200 mg/kg of BVE, respectively, by oral route 1 hour before milk injection. All mice were anesthetized by intraperitoneal injection with pentobarbital sodium, and blood was removed from cervical vein. Total leukocyte and eosinophil count was done in each group before drug administration and 24 hours after milk injection. The difference in total leukocyte and eosinophil count before and 24 hours after test drug treatment was calculated.[12]

Statistical Analysis

The data are presented as mean ± SEM. The data were analyzed by one-way ANOVA followed by Dunnett's test.

RESULTS

Preliminary Phytochemical Studies

The results of preliminary phytochemical investigation of ethanol extract revealed the presence of an array of active constituents including alkaloids, tannins, flavonoids, steroids, and glycosides, while aqueous extract showed presence of alkaloids, tannins, flavonoids, carbohydrates, and proteins.

Effect of *B. variegata* extracts on milk-induced leukocytosis in mice

Administration of milk (4 ml/kg) by subcutaneous route exhibited significant (***$P < 0.001$) increase in leukocyte count after 24 hours of its administration. In the test group, pretreated with BVA at 200 mg/kg dose and BVE at 100 and 200 mg/kg dose, there was significant (*$P < 0.01$) inhibition was found in leukocytosis [Table 1].

Effect of *B. variegata* extracts on milk-induced eosinophilia in mice

Subcutaneous injection of milk produced significant (***$P < 0.001$) increase in total eosinophil count in milk-intoxicated group. However, the groups treated with BVA and BVE exhibited inhibition in milk-induced eosinophilia. BVA at higher dose of 200 mg/kg and BVE at both the dose level of 100 and 200 mg/kg showed significant reduction in eosinophil count [Table 2].

DISCUSSION

Leukocyte recruited during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein which promote ongoing inflammation. The eosinophil are the most characteristic inflammatory cells in bronchial biopsies taken from asthma patients and may be seen in the submucosal and epithelial layers. An abnormal increase in peripheral eosinophil count to more than 4% of total leukocyte is termed as eosinophilia. In asthmatic patients, there is increase in eosinophil count.

Table 1: Effect of BVA and BVE on milk-induced leukocytosis in mice

Group	Dose	Difference in no. of leukocyte (cu/mm)
Normal control	Vehicle, 10 ml/kg	75±14.28
Milk intoxicated	Milk, 4 ml/kg	4 534±201.11***
BVA	100 mg/kg	3 010±101.12
BVA	200 mg/kg	2 728±210.18*
BVE	100 mg/kg	2 248±102.24**
BVE	200 mg/kg	1 547±182.14**

BVA: Aqueous extract of *B. variegata*; BVE: Ethanol extract of *B. variegate;* Values are expressed in mean ± SEM, comparison was made between normal control and milk-intoxicated group and between milk-intoxicated group and extracts treated group, ***$P < 0.001$, intoxicated group compared with control, *$P < 0.05$, *$P < 0.01$ BVA and BVE compared with intoxicated group

Table 2: Effect of BVA and BVE on milk-induced eosinophilia in mice

Group	Dose	Difference in no. of eosinophil (cu/mm)
Normal control	Vehicle, 10 ml/kg	30.12±2.34
Milk intoxicated	Milk, 4 ml/kg	155.31±7.23***
BVA	100 mg/kg	110.17±8.45
BVA	200 mg/kg	95.22±7.67*
BVE	100 mg/kg	88.17±6.41**
BVE	200 mg/kg	61.32±5.91**

BVA: Aqueous extract of *B. variegata*; BVE: Ethanol extract of *B. variegate*. Values are expressed in mean ± SEM, comparison was made between normal control and milk-intoxicated group and between milk-intoxicated group and extracts treated group, ***$P < 0.001$ intoxicated group compared with control, *$P < 0.05$, *$P < 0.01$. BVA and BVE compared with intoxicated group

The involvement of eosinophil in bronchial mucosa, in which allergic inflammation occurs, is a critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion. In the late phase, especially in the development of allergic asthma, eosinophil plays role as inflammatory cell. Eosinophil secretes mediators such as eosinophil cationic protein, tumor necrosis factor, eosinophil-derived neurotoxin, and prostaglandin, which results in epithelial shedding, bronchoconstriction, and promotion of inflammation in respiratory tract often allergic.[13]

It has been demonstrated that parental administration of milk produces a marked and significant increase in leukocyte and eosinophil count after 24 hours of administration.[14] The milk-induced leukocytosis and eosinophilia in mice model helps to evaluate the stress-induced asthma. The results of present study revealed that aqueous and ethanol extracts of stem bark of BV caused reduction in the count of these inflammatory cells. Among both these extracts, ethanol extract has shown significant activity as compared with aqueous extract in a dose-dependent manner. However, we are screening BVA and BVE for other animal models of asthma to evaluate their efficacy in the management of disease asthma and also working on phytochemical investigation of these extracts to pin point the chemical constituent responsible for the activity.

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