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Anti-inflammatory Activity of Bougainvillea spectabilis Linn

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ABSTRACT

Anti-inflammatory activity of the ethanolic extract of the leaves of Bougainvillea spectabilis Linn. was studied in wistar rats using the carrageenan induced left hind paw edema and cotton pellet induced granuloma model. The ethanolic extract (300 mg/kg, p.o.,) produced the inhibition of carrageenan induced rat paw edema. It also showed reduction on the granuloma weight in the cotton pellet granuloma method. The results indicated that the ethanolic extract produced significant ($P<0.05$) anti-inflammatory activity when compared with the standard and untreated control.

Keywords: Ethanolic extract, Bougainvillea spectabilis, Carrageenan-induced, Cotton-pellet induce, Anti-inflammatory

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INTRODUCTION

Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation [1]. Hence, search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects are justified. There is much hope of finding active anti-rheumatic compounds from indigenous plants as these are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature, with no hazardous reactions.

The enzyme, phospholipase A_2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A_2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cycloxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation [2, 3].

B. spectabilis a plant belonging to the family of Nyctaginaceae, grows as a woody, scandent or straggling shrub, up to 10 m in height climbing by the help of throns, commonly cultivated throughout India as an ornament in gardens, lawns and roadsides [4]. In industrial and busy metropolitan areas, the plants are planted on roadsides as dust filters or biomonitors of air pollution. The plant contains biochemical defense agents, o-dihydrophenols, anthrocyanin, lignin and proline. The traditional medical practitioners of Kolli hills, Tamilnadu, are using this plant to cure inflammation. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory activity in rats.

MATERIALS AND METHODS

Plant material

The leaves of the plant were collected from the foothill of Yercaud, Salem, Tamilnadu, India in the month of June 2010 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Salem. A voucher specimen (BSM-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of leaves of B. spectabilis was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water [5]. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug.

Animals

Wistar rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water ad libitum. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies [6]

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD_{50} was calculated.

Carrageenan induced rat paw edema

Edema was produced by the method described by Winter et al [7]. The rats were divided into three groups of six animals each. First group received 1 ml of normal saline, second group received 10 mg/kg p.o., Indomethacin and third group received ethanolic extract (300 mg/kg, p.o.,) of B. spectabilis. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was that of Singh and Ghosh [8]. The paw volume was measured immediately after injection (0 h) and followed by every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume and the percentage of inhibition was calculated.

Cotton Pellet Granuloma model

In cotton pellet granuloma model [9] the animals were divided into three groups as described in the carrageenan induced paw edema model. The animals were anaesthetized with pentobarbitone (30 mg/kg. s.c.). The back skin was shaved and disinfected with 70 % ethanol.

An incision is made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with indomethacin (10 mg/kg, p.o.,) and ethanolic extract of B. spectabilis for 7 days. Then, the pellets were dissected out and dried until the weight remains constant. The net dry weights, i.e. after subtracting the weight of the cotton pellet were determined.

Statistical analysis

All values were expressed as mean±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple range test and differences below $P<0.05$ are considered as significant.

RESULTS AND DISCUSSION

The average percentage yield of ethanolic extract of B. spectabilis was found to be 5.6 % w/w. The LD_{50} was found to be 2925 mg/kg for ethanolic extract of B. spectabilis.

Table 1: Effect of ethanolic extract of *B. spectabilis* on carrageenan induced rat paw edema

Treatment	Dose (mg/kg, p.o.)	Mean change in paw volume (ml) after 3 h	% Decrease in paw volume
Control (Normal saline)	**1 ml**	**0.37±0.001**	-
Indomethacin	**10**	**0.13±0.001***	**64.9**
Ethanolic extract of *B. spectabilis*	**300**	**0.15±0.001***	**59.5**

**P*<0.05 when compared with control. Values are expressed as mean±SEM (n=6)

The effect of ethanolic extract of B. spectabilis on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the ethanolic extract was found to have significant anti-inflammatory activity in rats. The ethanolic extract of B. spectabilis reduced the edema induced by carrageenan by 59.5 % on oral administration of 300 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 64.9 %. The effect of ethanolic extract of B. spectabilis on cotton pellet induced granuloma in rats is shown in Table 2. In this the mean weights of the cotton pellets were determined. The weight of the granuloma for the control group of animals was found to be 58.2±2.04 mg. Treatment with the ethanolic extract of B. spectabilis (300 mg/kg, p.o.,) decreased the granuloma weight to 27.3±0.82 mg. Treatment with Indomethacin (10 mg/kg, p.o.,) produced a granuloma weight of 20.7±0.65 mg. The ethanolic extract of B. spectabilis and Indomethacin, both inhibited the granuloma tissue formation. The inhibition of the test extract and standard drug was found to be 53.1 and 64. 4 %, respectively.

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Table 2: Effect of ethanolic extract of *B. spectabilis* on cotton pellet induced granuloma in rats

Treatment	Dose (mg/kg, p.o.)	Granuloma wt. (mg)	% inhibition
Control (Normal saline)	**1 ml**	**58.2±2.04**	-
Indomethacin	**10**	**20.7±0.65***	**64.4**
Ethanolic extract of *B. spectabilis*	**300**	**27.3±0.82***	**53.1**

**P*<0.05 when compared with control. Values are expressed as mean±SEM (n=6)

Carrageenan induced inflammation is a biphasic phenomenon [10]. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action. In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroflasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the ethanol extract of B. spectabilis. Thus it can be concluded that leaves of the plant B. spectabilis possess significant anti-inflammatory activity in rats.

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