In Vivo Study for Anti-inflammatory Activity of *Bauhinia variegata* L. Leaves

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Abstract: *Bauhinia variegata* L. (Rakta kanchan) has been traditionally used in India for treatment of a variety of inflammatory conditions, skin diseases, stomatitis, leprosy and for wound healing. Using mostly *in vitro* models, a number of published reports confirmed the anti-inflammatory potential of the leaves, bark and roots of this tree. The isolation of a bioactive triterpene saponin from the leaves and a flavonol glycoside from the roots has also been reported. In the present study, we have evaluated the anti-inflammatory activity of the leaf extract of *B. variegata*, using three *in vivo* animal models: the carrageenan induced rat paw edema, cotton pellets induced granuloma formation, and adjuvant induced arthritis in rat. Both the ethanol extract and the petroleum ether fraction obtained from this extract demonstrated activity in all the three bioassays. The activity was found to be more pronounced in the petroleum ether fraction. These bioactivities compared favorably with diclofenac sodium, which was used as positive control, and confirms the traditional usefulness of this plant for the treatment of both acute and chronic inflammatory conditions.

Keywords: Adjuvant-induced arthritis, Anti-inflammatory activity, *Bauhinia variegata*, Fabaceae, Cotton pellet granuloma, Ethanol extract, Petroleum ether fraction.

INTRODUCTION

India is one of the largest producers of medicinal herbs in the world [1]. The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development [2]. *Bauhinia variegata* L. (Fabaceae), commonly known as 'Rakta kanchan' is distributed in sub-Himalayan and outer Himalaya of the Punjab and Sikkim. It is also found in Burma and China. Traditionally, various parts of this plant are used as anthelmintic, astringent, anti-leprotic, liver tonic, antibacterial and in the treatment of dysmenorrhoea. According to different indigenous medicinal systems of India, the plant is also useful for treatment of skin diseases, wounds, edema, dysentery, ulcers, eye disease, piles, hemorrhoids and snake bite [3-7]. The various biological activities such as antimicrobial, anti-inflammatory, antitumor, cytotoxic and hepatoprotective activities of this plant have also been reported [8-12]. Earlier studies on leaves of *B. variegata* reported the isolation of insulin like protein, an anti-inflammatory triterpenoid saponin and flavonoids. In the present study, we further corroborated the anti-inflammatory activity of the ethanolic extract and petroleum ether-soluble fraction of this extract using *in vivo* models for acute and chronic inflammation.

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MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used were obtained in high purity either from S.D. fine chemicals Pvt. Ltd; Bombay, India or E. Merck (India) Ltd., Mumbai. Carrageenan (Hi–Media Research Laboratories Pvt. Ltd., Mumbai), Tween80 (S.D. fine Chemicals Pvt. Ltd., Mumbai), Complete Freund's Adjuvant (FCA) (Sigma Aldrich St. Louis, USA) and Diclofenac sodium (Diclo) was a gift from M/S Jagsonpal, New Delhi.

Plant Material

The leaves of *B. variegata* were collected from Paneer, India during August 2008. A voucher specimen (voucher no. 324 b) was deposited in NGSM Institute of Pharmaceutical Sciences, Derelakatte, Mangalore, India.

Extraction

The shade dried powdered leaves (5 kg) were exhaustively extracted with 95% ethanol, and the solvent removed by evaporation on a water bath to give 400 g of extract (BVE). A non-polar fraction was obtained by partitioning the ethanol extract (400 g) between petroleum ether (60-80^{o}C) and water to give 40 g petroleum ether –soluble (BVPT).

Animals and Study Groups

Studies were carried out using Albino Wistar rats weighing 180–200 g of either sex. Animals were obtained from

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K.S. Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore, and housed in polyacrylic cages (6 animals per cage), for one week at standard laboratory conditions [(25ºC ±2ºC), relative humidity 60 ± 5% and 12 h light and dark cycle]. The diet was standard pellets (Hindustan Lever Limited, Mumbai, India) and water was given *ad libitum*. One group served as negative control receiving orally 3 mL of 1% Tween 80 each. Another group, the positive control, was given 13.5 mg/kg Diclofenac sodium orally [13]. The study groups (n=6) received orally 100, 200, 400 mg/kg each of freshly prepared suspension (in 1% Tween 80) of either BVE or BVPT.

The study protocols were approved by Institutional Animal Ethical Committee (KSHEMA /AEC/077/2008).

Acute Toxicity Study

Acute toxicity study was conducted to determine the median lethal dose (LD_{50}) of the two extracts, BVE and BVPT, in adult female albino Wistar rats following OECD guideline no. 425 [14]. Animals were administered the extract preparations orally, and observed at half hour intervals for 4 h, then after 24 h. Both extracts were found to be safe up to an oral dose of 2000 mg/kg.

In Vivo Anti-inflammatory Studies

Carrageenan Induced Rat Paw Edema

The carrageenan induced rat paw edema was carried out as described by Winter *et al*. [15] to evaluate acute anti-inflammatory activity of BVE and BVPT. Paw edema was induced by injecting 0.1 mL of 1% (w/v) carrageenan suspension in 0.9% (w/v) sterile saline into the plantar tissue of the left hind paw of all animals, one hour following oral administration of either control vehicle, Diclofenac sodium or plant extracts. The right paw served as reference to measure the degree of inflammation in the left one. Increase in paw volume was measured at four hourly intervals, following carrageenan injection, using a plethysmograph [16]. The percentage inhibition of inflammation, calculated as inhibition of edema volume, was calculated [17] as follows:

Percentage Inhibition of Inflammation= $(1-V_t / V_c)$ X100

V_t is the average paw edema volume of each extract treated group, as well as Diclofenac sodium group;

V_c is the paw volume of the negative control group that only received the vehicle.

Cotton Pellets Induced Granuloma in Rats

The granuloma in albino Wistar rats was induced by implanting cotton pellets [18]. All animals were anaesthetized with ether after shaving the fur, and 10 mg of sterile cotton pellets were inserted, one in each axilla. The extracts, control vehicle and Diclofenac sodium were administered orally every day for 7 days. On the eighth day, the animals were anaesthetized and the cotton pellets surgically removed and cleaned from extraneous tissues. The moist pellets were weighed, dried at 60° C for 24 h and then re-weighed. Increment in dry weight of pellets was taken as measure of granuloma formation.

Adjuvant Induced Arthritis in Rats

Arthritis was induced by the injection of 0.1 mL of FCA, (containing 1 mg/mL of heat killed *Mycobacterium tuberculosis* in paraffin oil and mannide monooleate) into the subplantar region of right hind paw of rat. Two hours prior to the injection, each group of animals received the assigned treatment, which was then continued daily for 14 days. Paw volume was measured on days 0, 3, 7, 11, and 14 [19].

Statistical Analysis

Values were expressed as mean ± S.E.M. Statistical significance of weight or volume change was determined by ANOVA, followed by Dunnet's *t*-test; values with $P<0.05$

Table 1. Anti-inflammatory Activity of BVE and BVPT in Carrageenan Induced Rat Paw Edema Model

Group	Treatment	Dose (mg/kg)	Increase in Paw Volume (mL)			
			1 h	2 h	3 h	4 h
I	Control	-	0.47±0.09	0.71±0.13	0.88±0.17	0.89±0.11
II	Diclo	13.5	0.23±0.03 (51.1)	0.21±0.12* (70.4)	0.18±0.12* (79.6)	0.16±0.07** (82.0)
III	BE	100	0.44±0.17 (6.4)	0.49±0.1 (31.0)	0.40±0.21 (54.5)	0.37±0.22 (58.4)
IV	BE	200	0.36±0.23 (23.4)	0.42±0.14 (40.8)	0.35±0.18 (60.2)	0.32±0.2 (64.0)
V	BE	400	0.30±0.15 (36.2)	0.27±0.06* (62.0)	0.25±0.2* (71.6)	0.22±0.1* (75.3)
VI	BVPT	100	0.38±0.22 (19.2)	0.34±0.11 (52.1)	0.28±0.11 (68.2)	0.26±0.15* (70.8)
VII	BVPT	200	0.32±0.16 (32.0)	0.31±0.09 (56.3)	0.24±0.14* (72.7)	0.21±0.19* (76.4)
VIII	BVPT	400	0.25±0.09 (46.8)	0.22±0.12* (69.0)	0.20±0.13* (77.3)	0.18±0.04** (79.8)

Notes: All the result are expressed in term of Mean ± S.E.M., n=6 animals in each group; number in parenthesis indicates percentage inhibition in increase in paw volume. Statistical significance was determined by ANOVA, followed by Dunnet's *t*-test. * $p<0.05$, ** $p<0.01$, statistically significant.

Table 2. Effects of BVE and BVPT on Cotton Pellets Induced Granuloma Formation in Rats

Groups	Treatment	Dose (mg/kg)	Moist Cotton Pellet		Dried Cotton Pellet	
			Weight (mg)	% inhibition	Weight (mg)	% inhibition
I	Control	-	210.40±11.6	-	52.57±2.4	-
II	Diclo	13.5	93.26±5.8**	55.7	21.02±0.5**	60.0
III	BVE	100	190±9.8	9.7	47.78±1.6	9.1
IV	BVE	200	178.38±8.6	15.2	45.68±2.13	13.1
V	BVE	400	156.72±6.3*	25.5	39.87±1.0*	24.2
VI	BVPT	100	170.48±10.4	19.0	46.43±1.3	11.3
VII	BVPT	200	132.80±8.7*	36.9	31.93±1.4 *	39.3
VIII	BVPT	400	114.63±7.8**	45.5	24.89±0.7**	52.7

Notes: All the result are expressed in term of Mean ± S.E.M. n=6 animals in each group; Statistical significance was determined by ANOVA, followed by Dunnet's *t*-test. * p<0.05, ** p<0.01, statistically significant.

Table 3. Anti-inflammatory Activity of BVE and BVPT in Adjuvant Induced Arthritis in Rats

Group	Treatment	Dose (mg/kg)	Increase in Paw Volume (mL)				
			0 day	3 day	7 day	11 day	14 day
I	Control	-	0.49±0.1	0.64±0.2	0.82±0.09	0.98±0.12	1.12±0.29
II	Diclo	13.5	0.44±0.08 (10.2)	0.52±0.06 (18.8)	0.58±0.21 (29.3)	0.45±0.24 (54.1)	0.33±0.1* (70.5)
III	BVE	100	0.48±0.09 (2.0)	0.62±0.16 (3.1)	0.78±0.13 (4.9)	0.75±0.11 (23.5)	0.69±0.26 (38.4)
IV	BVE	200	0.48±0.18 (2.0)	0.61±0.25 (4.7)	0.69±0.16 (15.9)	0.67±0.3 (31.6)	0.58±0.15 (48.2)
V	BVE	400	0.47±0.16 (4.1)	0.59±0.19 (7.9)	0.66±0.2 (19.6)	0.63±0.21 (35.7)	0.41±0.17* (63.4)
VI	BVPT	100	0.48±0.16 (2.0)	0.60±0.28 (6.3)	0.74±0.25 (9.8)	0.70±0.32 (28.6)	0.62±0.14 (44.6)
VII	BVPT	200	0.47±0.22 (4.1)	0.58±0.11 (9.4)	0.68±0.14 (17.1)	0.59±0.1 (39.8)	0.51±0.26 (54.5)
VIII	BVPT	400	0.46±0.17 (6.1)	0.55±0.13 (14.1)	0.63±0.27 (23.2)	0.53±0.13 (45.9)	0.39±0.05* (65.2)

Notes: All the result are expressed in term of Mean ± S.E.M., n=6 animals in each group; number in parenthesis indicates percentage inhibition in increase in paw volume. Statistical significance was determined by ANOVA, followed by Dunnet's *t*-test. * p<0.05, statistically significant.

and p<0.01 were considered as statistically significant. GraphPad Prism version 4.0, GraphPad Software Inc., was used for statistical analysis.

RESULTS AND DISCUSSION

Carrageenan-induced rat paw edema model has been in use to evaluate anti-inflammatory activity of drugs. The carrageenan induced edema develops by mediators in three phases. The initial phase is caused by histamine release, whereas the second phase is mediated by kinin and bradykinin, and the late phase by prostaglandins [20, 21]. Most anti-inflammatory drugs are effective at the late phase of edema formation [22]. As shown in Table **1**, Diclo (Diclofenac sodium 13.5 mg/kg) showed significant inhibition of rat paw edema at 4 h (82.0%). At 400 mg/kg, the paw edema inhibition following treatment with BVE and BVPT was 75.3% and 80.0% respectively. At this dose, the anti-inflammatory activity of both extracts is comparable to Diclo, and a significant reduction can be observed even 2 h post induction. BVPT appears to be more active (Table **1**).

The anti-inflammatory effect of the extracts was further investigated by the cotton pellets induced granuloma formation in rats, which is a model for chronic inflammation. In this model granuloma formation is due to proliferation of

inflammatory cells like macrophages, fibroblasts and neutophils [23, 24]. Diclofenac sodium and the plant extracts (BVE and BVPT) reduced the wet cotton pellet weight (Table **2**), an indication of reduction in accumulation of exudates at the inflammation site [25]. Administration of Diclo at 13.5 mg/kg resulted in 56.0% weight reduction, whereas, BVPT at 200 and 400 mg/kg reduced the weight by 37.0 and 46.0% respectively. BVE at 400 mg/kg reduced the weight of the wet cotton pellet by 26.0%. As shown in Table **2**, Diclo (13.5 mg/kg) showed significant 60.0% reduction of dried cotton pellet weight. Likewise both BVE (400 mg/kg), and BVPT at 200 mg/kg and 400 mg/kg showed significant reduction in the pellets weight (24.0, 39.0 and 53.0%, respectively), suggesting an anti-proliferative activity [23, 25].

The FCA induced arthritis was assessed by formation of rat paw edema [26, 27]. Diclo at 13.5 mg/kg significantly inhibited the edema (71.0%) on the 14^{th} day of study (Table **3**). The edema inhibition on this day following BVE and BVPT treatment at 400 mg/kg was 63.0 and 65.0% respectively.

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CONFLICT OF INTEREST

None declared.

ABBREVIATIONS

LD_{50} = Median lethal dose

FCA = Complete Freund's adjuvant

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