

## Antiinflammatory and Antinociceptive Activities of Methanolic Extract of Leaves of *Bischofia javanica* Blume on Experimental Animals

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The methanolic extract of leaves of *Bischofia javanica* was screened (200, 400 and 600 mg/kg p.o) for antiinflammatory activity by acute carrageenan induced paw edema in rats, sub-chronic cotton pellet induced granuloma in rats and chronic study by Freund's adjuvant induced arthritis in rats. In all the three models of antiinflammatory studies, 400 and 600 mg/kg of methanolic extract shows significant ( $p < 0.001$  vs. control) effect. Antinociceptive screening by Writhing test and Tail immersion test supported both the peripheral and central mechanism behind pain relieving effect. This study had rationalised the ethno medicinal use of the plant for cut, burns and injury by tribal people of Sikkim.

**Key Words:** Antiinflammatory, *Bischofia javanica*, Carrageenan, Cotton pellet, Freund's adjuvant, Antinociceptive, Writhing test, Tail flick test.

### INTRODUCTION

Inflammation can be basically defined as a change of the morphological equilibrium in a specific area of the tissue caused by different kinds of agents *i.e.*, physical, chemical or biological<sup>1</sup>. It can be represented by capillary dilatation with fluid accumulation (oedema) and by phagocyte emigration and accumulation (neutrophils, monocytes, macrophages), which also contribute to hyperalgesia generation and loss of tissue function<sup>2</sup>. Inflammation is an important causative agent of human morbidity and mortality, such as systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF)<sup>3</sup>.

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*Bischofia javanica* Blume (Euphorbiaceae), known as kainjal in Nepali, bhillas in Hindi is a evergreen tree widely distributed over the Southeast Asia, Japan and Australia. In India it is distributed over the Sub-Himalayan region, Orissa and south west coast from Konkan to Nilgiris<sup>4</sup>. This plant has been utilized significantly for various ailments like topical treatment for ulcer, sores and boils<sup>5</sup>. It possesses antiulcer, antimicrobial, anthelmintic and antidiarrhetic activities<sup>6</sup>. Tribes of Chhattisgarh and Sikkim use the leaf juice of the plant for the treatment of cancerous wound<sup>7</sup>. *B. javanica* have been reported to contains the phytoconstituents like triterpenoids, glucosides, flavonoids and tannins<sup>8</sup>. Recently betulinic acid and its derivatives from the stem bark of the plant which are having Topoisomerase II inhibitory activities<sup>9</sup>. In this study the crude methanolic extract of leaves of *B. javanica* has been studied for its antiinflammatory, antinociceptive activities.

### EXPERIMENTAL

The leaves of *Bischofia javanica* were collected from Melli region of Sikkim (India) in the month of June 2005. The plant material was identified and authenticated at Botanical survey of India (BSI), Sikkim. A herbarium is also kept in the parent institute for future reference (LS/BJ/03/RPS). The collected leaves of *B. javanica* was shade dried for 15 d and size reduced using laboratory grinder in to coarse powder. It was stored in a well closed container free from environmental climatic changes till usage.

**Preparation of extract:** The powdered leaves were extracted with methanol in soxhlet apparatus. After exhaustive extraction the extract was concentrated by distilling the solvent for further use. The concentrated extract was kept in the desiccator. Yields of the prepared extract was 9.5 % w/w of the dried powder. Phytochemical study of the dried extracts shows the positive test for alkaloid, triterpenoid, steroid, saponins, carbohydrates, proteins and tannins. The dried extract was suspended in 2 % CMC and used for the animal studies.

**Animals used:** Wistar Albino rats (150-200 g) and Swiss Albino mice (20-35 g) of either sex were used in the study. They were maintained at  $25 \pm 2^\circ\text{C}$  in 12 h dark-light cycle. The animals were fed with rat pellet food and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least 1week before the experimental session. All the experimental procedures were done by the guidelines of Institute Animal Ethics Committee (IAEC).

**Acute toxicity study (LD<sub>50</sub>):** Acute toxicity study was performed by giving methanolic extract from low dose level to higher (p.o) to different group of mice 50 % mortality rate was recorded for 24 h 1/10 of the LD<sub>50</sub> was taken as the starting dose for pharmacological screening<sup>10</sup>.

**Antiinflammatory activity:**

**Carrageenan induced paw edema in rats (acute model):** Acute inflammation was produced by injecting 0.1 mL per 100 g rat of 1 % carrageenan in to plantar surface of rat hind paw<sup>11</sup>. The methanolic extract of *B. javanica* was administered at the dose range of 200, 400, 600 mg/kg p.o, the reference drug aspirin at dose of 100 mg/kg p.o and negative control of 2 % CMC 0.1 mL/100 g were administered 0.5 h before carrageenan injection to different groups of animals. The volume was at 0, 1, 2, 3 and 4 h using mercury displacing plethysmometer. The paw volume was measured for the different group of animals were compared, the percentage of inhibition of paw volume was also determined.

$$\% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

where,  $V_c$  = Paw volume of control animals;  $V_t$  = Paw volume of drug treated animals.

**Cotton pellet induced granuloma in rats (sub-chronic model):** Two autoclaved cotton pellets weighing  $10 \pm 1$  mg were implanted in to the both sides of the groin region of each rat<sup>12</sup>. The animals were divided into five groups of six each. The test group were administered with 200, 400 and 600 mg/kg p.o of methanolic extract of *B. javanica* for seven consecutive days. The standard aspirin was also given to the respective group for seven days. 2 % CMC was administered for the control. After 7 d animal were sacrificed by cervical dislocation and the pellets with the granuloma tissues were dried in a oven at 60°C, weighed and compared to control.

**Freund's adjuvant induced arthritis in rats (chronic model):** Male albino rats were divided into five groups. On day one 0.1 mL of Freund's adjuvant was injected in to the plantar pad of each rat. The different group of animals were treated with the respective drug<sup>13</sup> at the specific dose p.o consecutively for 21 d. The paw volume of each group was measured using plethysmometer on day 0 before and on day 21 after drug administration. The percentage of inhibition of edema was calculated.

**Antinociceptive activity**

**Tail-immersion test in mice (opioid screening method):** Swiss albino mice of either sex (20-35 g) were used in this study. Animals were divided in to five groups of six each. The methanolic extract of *B. javanica* was administered at the dose of 200, 400 and 600 mg/kg p.o, pentazocine 5 mg/kg. The animal were held in a suitable restrainer with tail extending out. The tail up to 5 cm was then dipped into a pot of water maintained at  $55 \pm 0.1^\circ\text{C}$ . The time taken for the mouse to withdraw the tail in seconds was taken as the reaction time. The reading was taken after 0.5, 1.0 and 2.0 h of administration of the test drugs<sup>14</sup>.

**Writhing test in mice (NSAID screening method):** This method was performed by administering intraperitoneally 0.7 % acetic acid at the dose of 0.1 mL/10 g body weight<sup>15</sup>. Writhing (stretching episode of belly and touching on the floor when moving) rate was counted for 15 min. MEBJ at the dose of 200, 400, 600 mg/kg p.o were administered initially before the administration of acetic acid, also positive control of aspirin 100 mg/kg and negative control of 2 % CMC were different groups. The percentage of inhibition of writhing movement was calculated.

**Statistical analysis:** The values are represented by Mean  $\pm$  SEM, Students 't' test was performed.  $p < 0.05$  was considered as significant.

## RESULTS AND DISCUSSION

### Antiinflammatory activity

**Carrageenan induced paw edema in rats:** Methanolic extract of *B. javanica* at the dose of 200, 400 and 600 mg/kg p.o shown significant reduction in paw volume ( $p < 0.001$  vs. control) after carrageenan administration. It was observed that *B. javanica* at 600 mg/kg the percentage of inhibition of paw edema was 41.83 % (Table-1) at 4 h of drug administration and it was 45.83 % for the standard aspirin drug.

TABLE-1  
EFFECT OF METHANOLIC EXTRACT OF LEAVES OF *Bischofia javanica* ON CARRAGEENAN INDUCED PAW EDMA IN RATS

Treatment (mg/kg) (p.o)	Paw volume in mL Mean $\pm$ SEM (% inhibition of paw edema)			
	1 h	2 h	3 h	4 h
Control	1.19 $\pm$ 0.0187	1.27 $\pm$ 0.0204	1.32 $\pm$ 0.0232	1.36 $\pm$ 0.0191
MEBJ 200	1.05 $\pm$ 0.0588‡ (11.76)	0.99 $\pm$ 0.0473‡ (22.04)	0.955 $\pm$ 0.0438‡ (27.65)	0.8983 $\pm$ 0.0414‡ (33.94)
MEBJ 400	0.995 $\pm$ 0.0462‡ (16.38)	0.9766 $\pm$ 0.0687‡ (23.10)	0.848 $\pm$ 0.0532‡ (35.75)	0.805 $\pm$ 0.0508‡ (40.80)
MEBJ 600	1.0266 $\pm$ 0.1159‡ (13.78)	0.9333 $\pm$ 0.0318‡ (26.53)	0.8166 $\pm$ 0.1561‡ (38.13)	0.7916 $\pm$ 0.1032‡ (41.83)
Aspirin100	0.9683 $\pm$ 0.0409‡ (18.63)	0.9033 $\pm$ 0.0194‡ (36.67)	0.77 $\pm$ 0.08312‡ (41.66)	0.7366 $\pm$ 0.2600‡ (45.83)

All the drugs were given by p.o.; Each value represents Mean  $\pm$  SEM, (n = 6). Statistical significance test with control was one † $p < 0.01$ , ‡ $p < 0.001$ .

**Cotton pellet induced granuloma in rats:** In granuloma induced sub-chronic inflammation methanolic extract of *B. javanica* at the dose of 400, 600 mg/kg had significant antiinflammatory ( $p < 0.001$  vs. control) activity (Table-2). The percentage of inhibition of granuloma after drug administration shown that 600 mg/kg of methanolic extract of *B. javanica* had 50.27 % and aspirin the standard drug had 60.83 %.

TABLE-2  
EFFECT OF METHANOLIC EXTRACT OF LEAVES OF *Bischofia javanica* ON COTTON PELLET INDUCED GRANULOMA IN RATS

Treatment (mg/kg) (p.o)	Weight of dry cotton pellet granuloma (mg)	Inhibition (%)
Control	36.0 ± 2.37	–
MEBJ-200	29.1 ± 1.39*	19.16
MEBJ-400	22.2 ± 1.23‡	38.33
MEBJ-600	17.9 ± 1.01‡	50.27
Aspirin 100	14.1 ± 0.646‡	60.83

All the drugs were given by p.o.; Each value represents Mean ± SEM (n = 6). Statistical significance test with control was done \* $p < 0.05$ , ‡ $p < 0.001$ .

**Freund's adjuvant induced arthritis in rats:** The result from acute and sub chronic models of antiinflammatory activities were significant manner that encouraged the present workers to perform chronic model by Freund's adjuvant induced arthritis in rats. The antiinflammatory effect of methanolic extract of *B. javanica* from acute, subchronic. In chronic inflammation induction methanolic extract of *B. javanica* had reduced the arthritis by 29.95, 50.24 at the doses of 400, 600 mg/kg, respectively (Table-3).

TABLE-3  
EFFECT OF METHANOLIC EXTRACT OF LEAVES OF *Bischofia javanica* ON FREUND'S ADJUVANT INDUCED ARTHRITIS IN RATS

Treatment (mg/kg) p.o.	Paw volume (mL)	Inhibition (%)
Control	2.070 ± 0.0543	–
MEBJ-200	1.810 ± 0.0445†	12.56
MEBJ-400	1.450 ± 0.0583‡	29.95
MEBJ-600	1.030 ± 0.0416‡	50.24
Aspirin-100	0.803 ± 0.0462‡	61.20

All the drugs were given by p.o.; Each value represents Mean ± SEM (n = 6). Statistical significance test with control was done † $p < 0.01$ , ‡ $p < 0.001$  vs. control.

### Antinociceptive activity

**Tail-immersion test (opioid screening method):** In opioid analgesic method of screening the result (Table-5) depicted that methanolic extract of *B. javanica* at all the dose level increased the tail flick response by thermal induction at 2 h in significant level ( $p < 0.001$ ). 600 mg/kg of methanolic extract of *B. javanica* shown significant ( $p < 0.001$ ) protection from nociception for 0.5, 1.0 and 2.0 h. Pentazocine the standard drug had tail flick response at 15 s compared to solvent treated had 3 s.

**Writhing test in mice (NSAID screening method):** The nociception induced by 0.7 % acetic acid was reduced by the methanolic extract of *B. javanica* in dose dependent manner in significant level (Table-4). 200 mg/kg had significantly ( $p < 0.05$ ) reduced the writhing, where as 600 mg/kg shown highly significant ( $p < 0.001$ ) as compared to control. This study usually undertaken to screen NSAID type of analgesics. Antinociception of methanolic extract of *B. javanica* was by both central and peripheral mechanism.

TABLE-4  
EFFECT OF METHANOLIC EXTRACT OF LEAVES OF *Bischofia javanica* ON THERMALLY INDUCED NOCICEPTION IN MICE

Treatment (mg/kg)	Tail flick at 0.5 h (s)	Tail flick at 1.0 h (s)	Tail flick at 2.0 h (s)
Control p.o.	2.98 ± 0.4612	2.78 ± 0.3259	2.75 ± 0.3343
MEBJ 200 p.o.	4.05 ± 0.3365 <sup>NS</sup>	3.78 ± 0.4569 <sup>NS</sup>	4.73 ± 0.1429‡
MEBJ 400 p.o.	5.38 ± 0.3369†	6.45 ± 0.8307†	8.08 ± 0.4052‡
MEBJ 600 p.o.	7.54 ± 0.3564‡	12.28 ± 1.2602‡	13.18 ± 0.4934‡
Pentazocine 5 i.p.	11.12 ± 2.2754†	15.43 ± 0.8051‡	15.46 ± 0.8940‡

Pentazocine was given by i.p., others by p.o.; Each value represents Mean ± SEM, n = 6, Statistical significant test was done by Student t test. \* $p < 0.05$ , † $p < 0.01$ , ‡ $p < 0.001$ .

TABLE-5  
EFFECT OF METHANOLIC EXTRACT OF LEAVES OF *Bischofia javanica* ON ACETIC ACID INDUCED WRITHING IN MICE

Treatment (mg/kg) p.o.	Number of writhing for 20 min	Inhibition (%)
Control	35.53 ± 3.09	–
MEBJ 200	25.17 ± 1.42*	29.15
MEBJ 400	20.00 ± 1.93†	43.70
MEBJ 600	17.67 ± 1.84‡	50.26
Aspirin 100	15.17 ± 1.56‡	57.30

All the drugs were given orally, Each value represents mean ± SEM, n = 6. Statistical significant test with control was done \* $p < 0.05$ , † $p < 0.01$ , ‡ $p < 0.001$ .

## Conclusion

Inflammatory events involve micro-vascular changes with increased vascular permeability, flow exudation, including plasmatic protein and amplification of endogenous chemical mediators<sup>16</sup>. NSAIDS are the more common drugs against superficial nociception and inflammation. The adverse effect of these NSAIDS are like gastric ulceration, gastritis, allergic reaction. In this study a positive step was put forward to find a plant source medicine for nociception and inflammation which has been traditionally utilized. The methanolic extract of leaves of *Bischofia javanica* had proven its anti inflammatory property by acute, sub chronic and chronic models at significant level ( $p < 0.001$ ). In antinociception analysis both NSAID and opioid screening methanolic extract of *Bischofia javanica* had shown analgesic effect in significant level. This rationalize the medicinal utility of *B. javanica* leaf. The future direction is to isolate the phyto-constituent responsible for antiinflammatory and antinociceptive action.

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