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ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE ACTIVITIES OF METHANOLIC EXTRACT OF THE LEAVES OF *FRAXINUS FLORIBUNDA* WALLICH

Sutharson Lingadurai*[1], Lila Kanta Nath[1], Prasanna Kumar Kar[1], Shila E.Besra[2], Rajan Vedasiromani Joseph[2]

[1]Department of Pharmacology, Himalayan Pharmacy Institute, Majhitar, Rangpo, Sikkim-737136, India. [2]Drug Development Division, Indian Institute of Chemical Biology, Kolkata-700032, India.
***E-mail:** apjak_son@yahoo.co.in **Tel.:** +91 9932232464; **Fax:** +91 3592 246247

Abstract

Fraxinus floribunda Wallich (Family-Oleaceae) is a wide green tree in the sub-alpine region of Sikkim, India. The methanolic extract of the leaves of *Fraxinus floribunda* (MEFF) at 100, 200 and 400mg/kg/p.o was screened in rats for anti-inflammatory activity by acute-carrageenan induced paw edema, sub-acute cotton pellet induced granuloma and chronic Freund's adjuvant induced arthritis models. In all the three models of anti-inflammatory studies 200 and 400mg/kg/p.o doses of the extract showed significant effect ($P<0.001$). Anti-nociceptive evaluation was performed by writhing and tail-immersion tests in mice. Anti-nociceptive evaluation revealed that MEFF at the dose of 400mg/kg/p.o had significant activity against the control. The relieving effect was through the peripheral and central mechanism of action of the extract. This study rationalized the ethno medicinal use of the plant for relieving pain in inflammatory pathological conditions like fracture and dislocation.

Key Words: *Fraxinus floribunda*, Carrageenan, Cotton pellet, Freund's adjuvant, Writhing test, Tail immersion test.

Introduction

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) (Baeu et al.,1998). *Fraxinus floribunda* Wallich is a tree, occurring in eastern Himalayas and Khasi hills. Leaves are pinnate, leaflets 7-9, opposite, stalled, ovate-oblong. Manna is obtained by incision from the stem of the tree and it is used as laxative (Kritikar and Basu, 1988). The barks and leaves of the plant have been traditionally used for the treatment of fracture and dislocation (Bijoy, 2002). The leaves are employed as diuretic and for the treatment of gout (Anonymus, 1956). Some coumarins have been isolated from the leaves (Nagarajan et al., 1980). The literature survey revealed that there are no research studies carried out related to anti-inflammatory and anti-nociceptive activities on the leaves of this plant, hence in the present study anti-inflammatory activities in acute, sub-acute and chronic models as well as anti-nociceptive activity by writhing test and tail-immersion tests were determined.

Materials and Methods

Collection of plant material

The leaves of *Fraxinus floribunda* were collected from Pakyong region of Sikkim, India in the month of September 2005. The plant material was identified and authenticated at Botanical Survey of India (BSI), Sikkim. A herbarium numbered as LS/FF/04/RPS was also kept in the parent institute for future reference.

Preparation of plant extract

The collected leaves of *F. floribunda* was shade dried for 15 days and reduced to coarse powder using laboratory grinder. It was stored in a well-closed container to protect from light and moisture till used. The powdered leaves (2.5 kg) was extracted with methanol in soxhlet apparatus. After exhaustive extraction, the extract was concentrated *in vaccuo* and freeze dried to yield a solid extract (9.2g).The dried extract was suspended in 2% Carboxy Methyl Cellulose (CMC) and used as test drug sample for the animal studies. Similarly, aspirin was suspended in 2% CMC and used as standard drug

Phytochemical analysis

The dried extract was subjected to phytochemical analysis for constituent identification using standard protocol (Harborne, 1984).

Animals

Wistar Albino rats (150-200g) and Swiss Albino mice (20-35g) of either sex were used in the studies. They were housed in large propylene cages and kept at 22±2°C in 12 h dark-light cycle. The animals were fed with rat pellet food and water *ad libitum.* All animals were acclimatized for at least one week before the experimental session. All the experimental procedures were done following the guidelines of Institutional Animal Ethics Committee (IAEC).

Drugs and Chemicals

Aspirin, carrageenan, Freund's adjuvant were purchased from Sigma, Pentazocine was purchased from Ranbaxy Lab Ltd, New Delhi, India. All other chemicals were of analytical grade and procured locally.

Anti-inflammatory activity
Carrageenan induced Paw Edema (Acute Model)

Acute inflammation was produced by injecting 1% solution of carrageenan in to plantar surface of rat hind paw at the dose of 0.1ml per 100g body weight (Winter et al., 1963). Wistar albino rats were divided in to five groups of six in each. A 2% solution of CMC at a dose of 0.1ml/100g/p.o was administered to group 1. The test drug sample was administered to the animals of group 2, 3 and 4 at the dose range of 100, 200 and 400mg/kg/p.o respectively against the standard drug aspirin at 100mg/kg/p.o to the 5. After 30 minutes carrageenan solution was injected to the animals of all the groups. The paw edema was measured at the intervals of 1, 2, 3 and 4h using Plethysmometer (Model-520-R,IITC Life science, USA). The paw edema among the different group of animals was compared, the percentage inhibition of paw edema was determined.

$$\% \text{ Inhibition of Paw Edema} = \frac{Vc - Vt}{Vc} \times 100$$

Vc----Paw edema of control animals
Vt----Paw edema of drug treated animals

Cotton pellet induced granuloma (Sub-acute model)

Two autoclaved cotton pellets weighing 10±1mg were implanted in both sides of the groin region of each rat (D'Arcy et al., 1960). The animals were divided into five groups of six each. The Control group received 2% CMC solution at the dose of 0.1ml/100g/p.o. The test groups were treated with test drug samples for seven consecutive days at the dose of 100, 200 and 400mg/kg/p.o. The standard group received aspirin at the dose of 100mg/kg p.o for seven days. After seven days animals were sacrificed by cervical dislocation and the cotton pellets along with the granuloma tissues were dried in an oven at 60°C, weighed and resulted weights were compared with the control. The percentage inhibition of granuloma by the test drug was determined.

Freund's adjuvant induced arthritis (Chronic Model)

Male albino rats were divided in to five groups. On day one 0.1ml of Freund's adjuvant was injected in to the plantar pad of each rat. The control group received 0.1ml/100g/p.o of 2% CMC solution consecutively for 21 days. The three test groups were treated with the test drug samples at the dose of 100, 200 and 400mg/kg/p.o for 21 days. The standard group received aspirin at 100mg/kg/p.o for 21 days (Newbould, 1963). The paw edema of each group was measured using Plethysmometer (Model-520-R,IITC Life sciences, USA) on day 1 before and on day 22 after drug administration. The percentage inhibition of arthritis (Paw edema) was calculated.

Anti-nociceptive activity
Tail-immersion test

Swiss albino mice of either sex (20-35g) were used in the study. Animals were divided into five groups of six each. Group 1 received 0.1ml of 2%CMC solution as control. The test drug MEFF was administered at the dose of 100, 200 and 400mg/kg p.o to the groups 2, 3 and 4 respectively against the standard drug Pentazocine administered to group 5 at the dose of 5mg/kg i.p .The animals were held in a suitable restrainer with tail extending out. The tail up to 5cm was then dipped into a pot of water maintained at 55±0.1°C (Periyanayagam et al., 2004). The time taken for the mouse to withdraw the tail in seconds was considered as the reaction time. The reading was recorded after 30, 60 and 120 min of administration of drugs and control.

Writhing test

Animals were divided in to five groups of six each. The control group received 0.1 ml of 2% CMC solution. The test groups were treated with 100, 200 and 400 mg/kg/p.o. of test drug samples. The standard group received aspirin at the dose of 100mg/ kg/p.o. After 30 min of drug administration 0.7% acetic acid was given to each mouse at the dose of 0.1 ml/10g body weight i.p. (Collier et al.,1968). Number of writhing was counted for 15 minutes. The percentage inhibition of writhing offered by the drug samples to the animals was calculated and compared with the control.

Statistical analysis

The values are represented by mean±SEM; Student's t-test was performed. $P<0.05$ was considered as significant.

Results

Phytochemical analysis

Phytochemical study showed that MEFF tested positive for alkaloid, steroid, saponin and glycosides.

Anti-inflammatory activity
Carrageenan induced Paw Edema

The test drug MEFF at the dose of 100, 200 and 400 mg/kg p.o showed significant reduction in paw edema ($P<0.001$) after carrageenan administration. It was observed that MEFF at the dose of 400mg/kg /p.o produced 55.14 % percentage inhibition of paw edema (Table-1) at the 4^{th} hr of drug administration, whereas, 64.48% was produced by aspirin.

Cotton pellet induced granuloma

In granuloma induced sub-acute inflammation model, the test drug MEFF at the dose of 200 and 400 mg/kg/p.o. had significant anti-inflammatory activity ($P<0.01$) (Table-2). The percentage inhibition of granuloma after drug administration was found to be 35.72% for MEFF at the dose of 400mg/kg/p.o and 41.88% for the standard drug aspirin.

Table 1. Anti-inflammatory activity of MEFF on carrageenan induced paw edema in rats. Data represent mean ± SEM of 6 animals. ***P<0.001 compared to control (Student's t-test), MEFF-Methanolic Extract of leaves of *Fraxinus floribunda.*

Treatment (mg/kg/p.o.)	Paw volume in ml (% Inhibition of Paw Edema)			
	1h	2h	3h	4h
Control	1.52±0.036	1.84±0.061	2.11±0.008	2.14±0.073
MEFF-100	1.41±0.036*** (7.20)	1.34±0.005*** (27.17)	1.26±0.120*** (40.28)	1.24±0.020*** (42.05)
MEFF-200	1.32±0.004*** (13.15)	1.25±0.017*** (32.06)	1.10±0.034*** (42.86)	1.08±0.025*** (51.40)
MEFF-400	1.24±0.057*** (18.42)	1.14±0.028*** (38.04)	1.08±0.011*** (48.81)	0.96±0.002*** (55.14)
Aspirin-100	1.02±0.012*** (32.89)	0.86±0.022*** (53.26)	0.79±0.017*** (62.35)	0.76±0.052*** (64.48)

Table 2. Anti-inflammatory activity of MEFF on Cotton pellet induced granuloma and Freund's adjuvant induced arthritis in rats. Data Represent mean±SEM of 6 animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control (student's t-test).MEFF-Methanolic Extract of leaves of *Fraxinus floribunda.*

Treatment (mg/kg p.o)	Weight of dried cotton pellet	%Inhibition of granuloma	Paw volume in ml	%Inhibition of arthritis
Control	37.03±1.92	-------	1.94±0.075	----------
MEFF-100	31.90±0.64*	13.86	1.75±0.020*	9.79
MEFF-200	29.01±0.78**	21.65	1.71±0.024**	11.85
MEFF-400	23.80±0.77**	35.72	1.67±0.013**	13.91
Aspirin-100	21.52±0.82***	41.88	1.06±0.030***	45.36

Table 3. Antinociceptive activity of MEFF on thermally induced nociception in mice Data represent mean±SEM of 6 animals. NS Non Significant, **P<0.01 and ***P<0.001 compared to control (student's t-test).MEFF-Methanolic Extract of leaves of *Fraxinus floribunda.*

Treatment (mg/kg)	Tail flick after 30 minutes (sec)	Tail flick after 60 minutes(sec)	Tail flick after 120 minutes(sec)
Control (p.o.)	2.78±0.451	2.59±0.335	2.43±0.314
MEFF -100 (p.o.)	3.15±0.336NS	4.18±0.456**	4.78±0.142***
MEFF- 200 (p.o.)	5.38±0.336**	6.45±0.830**	8.08±0.405***
MEFF- 400 (p.o.)	8.54±0.356***	12.28±1.260***	13.18±0.493***
Pentazocine- 5 (i.p.)	12.12±2.275***	14.43±0.805***	15.46±0.890***

Table 4. Antinociceptive effect of MEFF on acetic acid induced writhing in mice. Data represent mean±SEM of 6 animals.*P<0.05,**P<0.01 and ***P<0.001 compared to control. MEFF-Methanolic Extract of leaves of *Fraxinus floribunda*

Treatment(mg/kg/p.o)	Number of writhing in 15 minutes	% Inhibition of Writhing
Control	32.51±3.14	----------
MEFF- 100	25.17±1.42*	22.57
MEFF- 200	22.03±1.93**	32.23
MEFF- 400	16.62±1.84***	48.87
Aspirin -100	12.13±1.46***	62.68

New Delhi. p.61-63.

2 Baue, A.E., Durham.R., Faist.E. (1998). Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF) arewe winning the battle? Shock, **10:79.**

3 Bijoy, G. (2002). The Medicinal plants of the Sikkim Himalaya. Jasmin Bejoy Gurung, Maples, Chakung, West Sikkim.p.22.

4 Cirino,G. (1998).Multiple controls in inflammation: extracellular and intracellular phospholipaseA2 inducible and constitutive cyclooxygenase and inducible nitric oxide synthase. Biochem Pharmacol. **55:** 111.

5 Collier,H.O.J., Dinneen,L.C., Johnson, C.A., Scheider.C.(1968).The abdominal contraction response and its suppression by antinociceptive drugs in the mouse. British Journal of Pharmacology and Chemotherapy **32:**295.

6 D'Arcy, P.F., Haward, E.M., Muggleton,P.W., Townsend,S.B.(1960).The anti-inflammatory action of griseofulvin in experimental animals. Journal of Pharmacy and Pharmacology **12:** 659.

7 Ferreira, S.H., Vane,S.R.,1974.New aspects on the mode of action of non-steroid anti-inflammatory drugs. Annual Review of Pharmacology **14:**57-73.

8 Harborne,J.B., (1984).Phytochemical Methods. Chapman and Hall, London-New York.p.120

9 Kritikar, K.R., Basu,B.P . (1988).Indian Medicinal Plants. International Book distributors, Dehradun.p.1529.

10 Mossa,J.S., Rafatullah,S., Galal,A.M., Al-Yahya,M.A.,(1995). Pharmacological studies of *Rhus retinorrhaea.* International Journal of Pharmacognosy **33:**242-246.

11 Nagarajan, G.R.,Rani,U.,Parmar,V.S.(1980).Coumarins from *Fraxinus floribunda* leaves.Phytochemistry.**19:**2494-2495.

12 Newbould,B.B.(1963).Chemotherapy of arthritis induced in rats by mycobacterial adjuvants. Brit J Pharmacol **21:** 127.

13 Olajide,O.A., Awe,S.O., Makinde,J.M.,Ekhelar,A.I., Olusola,A.,Morebise,O., Okpako,D.T.,(2000).Studies on the anti-inflammatory,antipyretic and analgesic properties of *Alstonia boonei* stem bark .Journal of Ethnopharmacology **71:**179-186.

14 Perianayagam,J.B., Sharma, S.K., Joseph,A., Christina ,A.J.M. (2004). Evaluation of anti pyretic and analgesic activity of *Emblica officinalis* Gaertn. J.Ethnopharmacol. **95:**83.

15 Turner,R.A.,(1965).Screening Methods in Pharmacology. Academic Press, New York.p.100.

16 Vane, J.R.(1971).Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nature **231:**232.

17 Winter,C.A., Risely,G.W .(1963). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drug .Proc Soc Exp Biol Med **111:**544.

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

SUTHARSON LINGADURAI*, LILA KANT NATH, PRASANNA KUMAR KAR, SHILA E. BESRA† and RAJAN VEDASIROMANI JOSEPH†
Department of Pharmacology, Himalayan Pharmacy Institute Majhitar, Rangpo, Sikkim-737132, India
Fax: (91)(3592)246247; Tel.: (91)9932232464; E-mail: apjak_son@yahoo.co.in

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 antinflammatory 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, *Bischoia javanica*, Carageenan, 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[1]. 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[2]. 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)[3].

†Drug Development Division, Indian Institute of Chemical Biology, Kolkata-700 032, India.

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[4]. This plant has been utilized significantly for various ailments like topical treatment for ulcer, sores and boils[5]. It possesses antiulcer, antimicrobial, anthelmintic and antidysenteric activities[6]. Tribes of Chhattisgarh and Sikkim use the leaf juice of the plant for the treatment of cancerous wound[7]. *B. javanica* have been reported to contains the phytoconstituents like triterpenoids, glucosides, flavonoids and tannins[8]. Recently betulinic acid and its derivatives from the stem bark of the plant which are having Topoisomerase II inhibitory activities[9]. 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 ± 2°C in 12 h dark-light cycle. The animals were fed with rat pellet food and water *ad libitum*. All animals were aclimatized 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_{50}): 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_{50} was taken as the starting dose for pharmacological screening[10].

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[11]. 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.

$$\% \text{ Inhibition of paw edema} = \frac{Vc - Vt}{Vc} \times 100$$

where, Vc = Paw volume of control animals; Vt = Paw volume of drug treated animals.

Cotton pellet induced granuloma in rats (sub-chronic model): Two autoclaved cotton pellets weighing 10 ± 1 mg were implanted in to the both sides of the groin region of each rat[12]. 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[13] 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 adminstered 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 ± 0.1°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[14].

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[15]. 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 negativecontrol of 2 % CMC were different groups. The percentage of inhibition of writhing movement was calculated.

Statistical analysis: The values are represented by Mean ± 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 ± SEM (% inhibition of paw edema)			
	1 h	2 h	3 h	4 h
Control	1.19 ± 0.0187	1.27 ± 0.0204	1.32 ± 0.0232	1.36 ± 0.0191
MEBJ 200	1.05 ± 0.0588‡ (11.76)	0.99 ± 0.0473‡ (22.04)	0.955 ± 0.0438‡ (27.65)	0.8983 ± 0.0414‡ (33.94)
MEBJ 400	0.995 ± 0.0462‡ (16.38)	0.9766 ± 0.0687‡ (23.10)	0.848 ± 0.0532‡ (35.75)	0.805 ± 0.0508‡ (40.80)
MEBJ 600	1.0266 ± 0.1159‡ (13.78)	0.9333 ± 0.0318‡ (26.53)	0.8166 ± 0.1561‡ (38.13)	0.7916 ± 0.1032‡ (41.83)
Aspirin100	0.9683 ± 0.0409‡ (18.63)	0.9033 ± 0.0194‡ (36.67)	0.77 ± 0.08312‡ (41.66)	0.7366 ± 0.2600‡ (45.83)

All the drugs were given by p.o.; Each value represents Mean ± 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 ARTHITIS 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 m/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^{NS}	3.78 ± 0.4569^{NS}	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, Statistcal 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. Statisatical 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[16]. 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 phytoconstituent responsible for antiinflammatory and antinociceptive action.

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REFERENCES

1. A.C. Zanini and S. Oga. Farmacologia Aplicada. São Paulo: Atheneu, p. 625 (1979).
2. J.I. Gallin, Inflammation, in ed.: W.E. Paul, Fundamental Immunology, New York, Raven Press, edn. 2, p. 721 (1989).
3. A.E. Baue and R. Durham, *Shock*, **10**, 79 (1998).
4. C. Nayar and Chopra, Glossary of Indian Medicinal Plants, CSIR, India, p. 37 (1970).
5. L.M. Perry, Medicinal Plants of East and South East Asia: Attributed Properties and Uses. MIT Press, Cambridge (1980).
6. D.R. Gupta, R.P. Dhiman, S. Naithani and B. Ahmed, *Pharmazie*, **43**, 222 (1988).
7. http://www.botanical.com/site/column_poudhia/publish/journal.cgi
8. T. Tanaka, G.I. Nonaka, I. Neishioka and I. Kouno, *Phytochemistry*, **38**, 509 (1995).
9. S. Wada and R. Tanaka, *Chem. Biodiver.*, **2**, 689 (2005).
10. M.N. Ghosh, Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta, India, edn. 2 (1984).
11. C.A. Winter and G.W. Risely, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
12. P.F. D'Arcy, E.M. Haward, P.W. Muggleton and S.B.Townsend. *J. Pharm. Pharmacol.*, **12**, 659 (1960).
13. B.B. Newbould, *Br. J. Pharmacol.*, **21**, 127 (1963).
14. J.B. Perianayagam, S.K. Sharma, A. Joseph and A.J.M. Christina, *J. Ethnopharmacol.*, **95**, 83 (2004).
15. H.O.J. Collier, L.C. Dinneen, C.A. Johnson and C. Scheider, *Br. J. Pharmacol. Chemother.*, **32**, 295 (1968).
16. G. Cirino, *Biochem. Pharmacol.*, **55**, 111(1998).

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