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

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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±0.1ºC (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

Freund's adjuvant induced arthritis

In chronic inflammation induction model, the MEFF reduced the arthritis by 11.85% and 16.66% at the doses of 200 and 400mg/kg/p.o. respectively compared to the standard drug aspirin (100 mg/kg/p.o.) which reduced the arthritis by 31.28% (Table-3).

Anti-nociceptive activity
Tail-immersion test

Tail-immersion analgesic method revealed that MEFF at all the doses significantly delayed the time of tail withdrawal response by thermal induction of pain at 120 min ($P<0.001$). MEFF at the dose of 400mg/kg/p.o. showed significant protection from nociception at 30, 60 and 120 min similar to the standard drug pentazocine 5mg/kg i.p. (Table-3).

Writhing test

The nociception induced by 0.7% acetic acid was significantly reduced by the MEFF in dose dependent manner (Table 4).

Discussion

Inflammatory events involve micro-vascular changes with increased vascular permeability, flow of exudation, including plasmatic protein and amplification of endogenous chemical mediators (Ciarino, 1998). Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are the common drugs against superficial nociception and inflammation. NSAIDs alleviate the hyperalgesic symptoms associated with inflammation by inhibiting the COX enzyme and the resultant inhibition of Prostaglandins synthesis from arachidonic acid (Vane, 1971). In this study a positive step was put forward to investigate the anti-nociceptive and anti-iflammatory actions of *F. floribunda* utilized traditionally for nociception and inflammation. The methanolic extract of the leaves of *F. floribunda* (MEFF) was found to have significant ($P<0.001$) anti-inflammatory property in all the dose level in acute carrageenan induced paw edema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa et al., 1995). In sub-acute and chronic studies, the inflammatory granuloma and arthritis are the typical features (Olajide et al., 2000) which have been reduced significantly ($P<0.01$) by MEFF at the dose level of 200 and 400mg/kg. The percentage protections of inflammation at the dose level of 400mg/kg in acute, sub-acute and chronic model were 55.14 (at 4^{th} hr), 35.72 and 13.91 respectively. It provided the feedback that MEFF was more effective in acute than sub-acute and chronic inflammation.

The writhing induced by chemical substances is due to sensitization of nociceptors by Prostaglandins. This test is useful for evaluation of mild analgesic non-steroidal anti-inflammatory compounds (Ferreira and Vane, 1974).The test drug at the dose level of 400mg/kg showed significant ($P<0.001$) inhibitory activity on the writhing induced by acetic acid when compared to control. Opioid type analgesics can be differentiated from NSAIDs by their effectiveness in the tail-immersion test (Turner, 1965). The tail immersion results depicted that the test drug MEFF at the dose level of 100, 200 and 400mg/kg gave significant response ($P<0.001$) at 120 min. Anti-nociceptive study by tail-immersion test provided the evidence for central mechanism which is also exhibited by the test drug for relieving the pain. The studies have rationalized the ethno-medicinal utility of the leaves of *F. floribunda* for various ailments related to inflammatory disorders.

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