

**ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF
FLACOURTIA RAMONTCHI L. HERIT**

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Summary

The alcoholic extract from aerial parts of *Flacourtia ramontchi* L. Herit (Flacourtiaceae) was investigated for analgesic activity by acetic acid induced writhing test, formalin induced paw licking model, tail flick model in Swiss albino mice and anti-inflammatory activity by carrageenin induced rat paw edema and cotton pellet granuloma model in Sprague Dawley rats. The extract at 100, 150 and 200mg/kg body weight doses was found to possess significant ($p < 0.01$) and dose dependent analgesic and anti-inflammatory activity in both acute as well as subacute animal models. Further, the acute toxicity study with the extract showed no sign of toxicity up to a dose level of 3000-mg/kg p.o. The potential to cause ulcer by extract was comparatively less than that of diclofenac. Thus it could be concluded that *Flacourtia ramontchi* alcoholic extract possess significant analgesic and anti-inflammatory activity.

Key words: *Flacourtia ramontchi*; Flacourtiaceae; Alcoholic extract; Analgesic; Antinflammatory

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Introduction

Plants belonging to family Flacourtiaceae are well known for their physiological and medicinal properties (1, 2). *Flacourtia ramontchi* L. Herit (Flacourtiaceae) is a small deciduous thorny shrub, found in scrub forests and rocky hills upto 900 meters throughout India (3, 4). The roots of plant are useful in vitiated conditions of pitta and vata, skin diseases, pruritis, nephropathy and scabies. The fruits are sweet, appetizing and digestible and are used to treat jaundice and enlarged spleen whereas its gum is administered along with other ingredients in cholera (1). The bark is considered astringent, diuretic and used as a tanning material (5). The leaves are useful in pruritis and scabies (3, 4). Phytochemical studies carried out on this shrub showed that its heartwood contains beta-sitosterol, beta-sitosterol-beta-D-glucopyranoside, a butyrolactone lignan disaccharide, ramontoside, triterpenes, a phenolic glucoside ester i. e. flacourtin (6, 7). The ground seeds are applied with Haldi (Turmeric) and Sonth (Dried ginger) on woman body in the form of paste in order to reduce the body pain after delivery. The bark is applied to the body along with that Albizzia at intervals of a day or so during intermittent fever in Chota Nagpur (Campbell). As per the research note of Pankaj Oudhia this plant alone or in the combination with other herbs uses in the treatment of migraine and rheumatic pain in healers of some parts of Chattisgarh (8, 9).

To substantiate this claim, the present study was undertaken to evaluate the anti-inflammatory and analgesic potential of plant extract by using various animal models.

Methods

Plant material:

F. ramontchi was collected from local region of Nagpur, District of Maharashtra, India in the month of July 2005. The botanical identity was confirmed by a taxonomist Dr. Alka Chaturvedi, Department of Botany; RTM Nagpur University, Nagpur where voucher specimen (No. 5561/1) has been deposited for further reference.

Preparation of extracts:

The aerial parts of plant were washed, shade dried and powdered. The powdered material was defatted with petroleum ether (60-80 °C) and then extracted with alcohol in soxhlet apparatus (40 cycles). The extract was concentrated for further studies at reduced pressure and temperature in a rotary evaporator. The defatted alcoholic extract was 14.49% w/w of the starting material. Alcoholic extract was tested for presence of secondary metabolites by different phytochemical tests. Alcoholic extract was dried, crushed to fine powder and suspended in 0.5% gum acacia for oral administration to evaluate anticipated activity.

Drugs and chemicals:

Carrageenin (Sigma chemical Co., St. Louis MO, USA), acetic acid (Ranbaxy laboratories Ltd. Punjab), formalin (S.D. Fine chemicals, Mumbai, India), Diclofenac sodium (Voveran[®] injection, Novartis) and Indomethacin (Gift sample from Glenmark Laboratories Ltd. Mumbai, Maharashtra) were used in this study. Other chemicals used for extraction purpose were of laboratory grade.

Animals:

Swiss albino mice (25-30 gm) and Sprague Dawley (150-200 gm) rats of either sex (National Institute for Laboratory Animal Sciences) were used for present study. They were housed in polypropylene cages in an air-conditioned area at 25±2 °C with 12/12 h light/dark cycle. All animals had free access to standard pellet diet (Goldmohor brand, Lipton India Ltd., Mumbai) and clean water *ad libitum*. The present studies were duly approved by IAEC.

Acute toxicity:

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), (10). Sprague Dawley rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose level of 10 mg / kg body weight by gastric intubation and observed for 7 days. If mortality observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality not observed, the procedure was repeated for further higher doses such as 100, 500 and 3000 mg / kg body weight. They were weighed daily for a week to detect physical alterations.

Pharmacological experimentation:

As per acute toxicity study, alcoholic extract was found to be safe up to 3 gm/kg body weight. Hence the following anticipated activities were screened at 100, 150 and 200 mg/kg body weight orally. Mice or rats were divided into 5 groups consisting of 6 animals each. Group I received vehicle (5 % gum acacia solution in water) i.e. negative control, Group II, III, IV received per oral route alcoholic extract of 100, 150 and 200 mg/kg respectively whereas Group V served as positive control i.e. Indomethacin / Diclofenac sodium (10 mg/kg, p. o.)

Analgesic activity:

Acetic acid induced writhing in mice

Analgesic activity of extract was assessed by employing acetic acid induced writhing method in mice (11) (n=6, each dose). The writhing syndrome was established by intraperitoneal injection of 0.6% v/v acetic acid in normal saline (10 mg/kg). The alcoholic extract (100, 150 and 200 mg/kg, oral) and diclofenac solution (10 mg/kg p.o.) was administered to mice 60 and 30 minutes respectively prior to i.p. injection of algic comp i. e. acetic acid. The analgesic activity is expressed as percent inhibition of writhing. The no. of writhes was counted for 15 minutes.

Formalin induced paw licking in mice

The procedure was similar to that described by Hunskaar et al. (12) and Gorski et al. (13). The formalin possesses two distinctive phases, which possibly reflecting different types of pain. Mice were treated orally with alcoholic extract at 100, 150 and 200mg/kg or 0.5% gum acacia in water or diclofenac solution (10mg/kg, p.o.). 1 h later, 20 μ l of 1 % formalin was injected subcutaneously under the dorsal surface of hind paw. Mice were observed in chambers. The number of licks in injected paw was counted till 5 minutes (early phase) and from 20 to 30 minutes (later phase) after formalin injection. The early phase represents neurogenic pain while latter phase is of inflammatory pain.

Tail flick method in mice

The prescreened animals (reaction time: 6- 7 seconds) were divided into I-V groups as described above. After oral administration of extract or vehicle or standard, the tail flick latency was assessed at 0, 1, 2 and 3 h by analgesiometer (INCO, Ambala India). The strength of current passing through naked nichrome wire was kept constant at 4 amps. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut off time was fixed 15 seconds to avoid any tissue damage (14)

Anti-inflammatory activity:

Carrageenin-induced rat paw edema

The anti-inflammatory activity of extract was evaluated by carrageenin-induced rat paw edema method (15). The suspension of the alcoholic extract was orally administered to rats (100, 150 and 200 mg/kg) (n=6, each dose). After 1 h of administration of extract, 0.1 ml of the carrageenin suspension (0.1% in normal saline) was injected into subplantar region of the right hind paw. The paw edema volume was measured by means of water displacement technique using plethysmometer before and 1 and 3 h after the carrageenin injection. The control rats were received equal volume of the vehicles of the extract and comparable group received Indomethacin (10mg/kg) as a standard anti-inflammatory agent. The results are expressed in terms of mean increase in paw volume at 1 and 3 h and anti-inflammatory activity is expressed in terms of percent inhibition of paw edema at 1 and 3 h.

Cotton pellet- induced granuloma

After shaving the hairs on its back, rats were anesthetized with light ether and granulomatous lesions were induced by surgically implanting two cotton pellets (10 ± 1 mg) subcutaneously in the dorsal region of the rats, one near each axial as described by Swingle and Shideman (16). Alcoholic extract (100, 150 and 200 mg/kg) or vehicle (10 ml/kg body wt.) or diclofenac sodium (10 mg/kg, p.o.) was given orally once daily for 7 days at fixed time of day. On 8th day, the rats were sacrificed by over dose of anesthetic ether, and the pellets covered by granulomatous tissue were dissected and dried to a constant weight at 50°C for 20 hr. The mean weight for different groups were determined and compared to the control group. Ulcer scores were also calculated by examining the changes observed at the stomach, microscopically.

Statistical analysis

The results are expressed as mean \pm SEM. Parametric data were assessed by the method of analysis of One- way ANOVA followed by Dunnett's test. Ulcers score were statistically tested using Kruskal- Wallis test followed by Dunnett's test. $P < 0.05$ was considered as statistically significant.

Results**Phytochemical screening:**

Phytochemical screening revealed the presence of sterol, triterpenoid, flavonoid, lignan, saponin and carbohydrate in alcoholic extract.

Acute toxicity:

Alcoholic extract did not show any toxicity and mortality up to maximum dose of 3 gm/kg of body weight and weight of mice had a normal variation after 7 days of observations. Common side effects such as mild diarrhea, loss of weight and depression were not recorded.

Analgesic activity:

ANOVA revealed that pretreatment with alcoholic extract (100, 150 and 200 mg/kg) showed significant ($p < 0.05$) reduction in number of writhes in dose dependent manner (Table 1). Alcoholic extract at doses 100, 150 and 200 mg/kg caused a significant inhibition of the neurogenic (early phase) and inflammatory phases of formalin induced licking in mice (Table 2). Alcoholic extract showed significant increase in latency time for thermal stimulation in tail flick test (Figure 1). The standard drug, diclofenac sodium (10 mg/kg) also significantly inhibited all the responses evoked by the noxious stimuli except early phase of formalin induced paw licking.

Table 1: Analgesic effect of alcoholic extract in writhing induced by acetic acid in mice

Treatment	Dose mg/kg, p.o.	No. of writhings	% Inhibition
Control (5% gum acacia)	10 ml/kg	63.88 \pm 3.82	-
Alcoholic extract	100	41.83 \pm 3.77**	34.47
	150	28.33 \pm 3.06***	55.61
	200	22.17 \pm 2.12***	65.27
Diclofenac sodium	10	18.50 \pm 1.54***	71.03

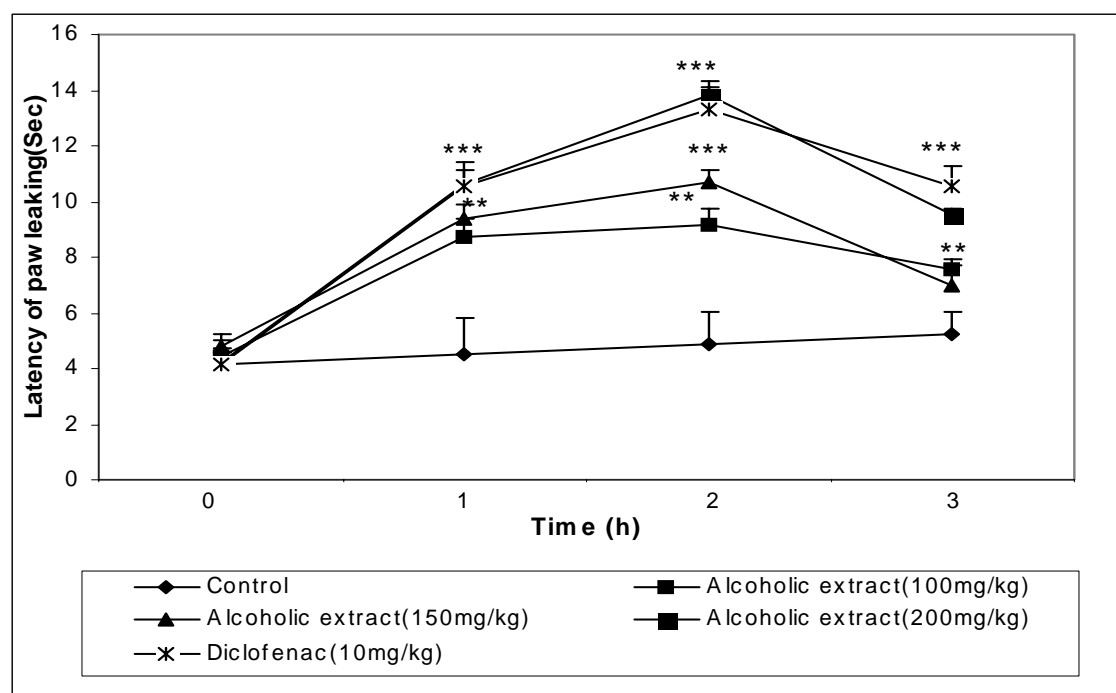
The results are mean \pm SEM from 6 animals; ** $P < 0.01$, *** $P < 0.001$ when compared to vehicle control

Table 2: Analgesic effect of alcoholic extract on formalin induced pain in mice

Treatment	Dose mg/kg, <i>p.o.</i>	Total time in spent in licking (s)			
		0–10 min	% Inhibition	15–30 min	% Inhibition
Control (5% gum acacia)	10 ml/kg	79.3 ± 5.4	-	113.3 ± 5.6	-
Alcoholic extract	100	58.2 ± 4.4	26.68	88.5 ± 5.2*	21.9
	150	48.0 ± 4.8**	39.49	47.5 ± 4.5**	58.1
	200	31.8 ± 4.1***	59.87	22.5 ± 1.5***	80.2
Diclofenac sodium	10	73.3 ± 6.8	7.56	28.8 ± 2.6***	74.6

The results are mean ± SEM from 6 animals; * P< 0.05, **P< 0.01, ***P< 0.001 when compared to vehicle control

Figure 1: Effect of alcoholic extract in tail flick test in mice



Each data represents mean ± SEM from 6 animals; **P< 0.01, ***P< 0.001 when compared to vehicle control

Anti-inflammatory activity:

The results of carrageenin induced rat paw edema, which reveals the anti-inflammatory activity of alcoholic extract of *F. ramontchi*, are shown in Table 3. Pretreatment with alcoholic extract at 100, 150 and 200mg/kg orally, reduced the edema formation of rat paw at 1 and 3 h after carrageenin administration, with significant reduction ($p < 0.01$) at 3 h in all dose range. In cotton

pellet granuloma model, there was statistically significant reduction in the dry weight of granuloma in alcoholic extract (150 and 200 mg/kg) as well as diclofenac sodium (10 mg/kg, p.o.) treated rats as compared to control group (Table 4). Alcoholic extract treated animals showed reduced ulcer score as compared to diclofenac sodium treated group (Table 4). The potential to cause ulcers by alcoholic extract at all doses (100, 150 and 200 mg/kg, oral) was comparatively less than that of diclofenac.

Table 3

Anti-inflammatory Effect of alcoholic extract in carrageenin induced paw edema

Treatment	Dose mg/kg, p.o.	Edema volume	
		1h	3h
Control (5% gum acacia)	10 ml/kg	0.62 ± 0.04	0.76 ± 0.05
	100	0.54 ± 0.03 (12.90%)	0.58 ± 0.03* (23.68%)
Alcoholic extract	150	0.46 ± 0.03 (25.81%)	0.35 ± 0.02** (54.02%)
	200	0.45 ± 0.04 (27.42%)	0.26 ± 0.03** (65.38%)
Indomethacin	10	0.26 ± 0.01** (58.06%)	0.16 ± 0.02** (78.95%)

Figure in parenthesis indicate the % inhibition.

The results are mean ± SEM from 6 animals; * P< 0.05, **P< 0.01 when compared to vehicle control

Table 4

Anti-inflammatory Effect of alcoholic extract on cotton pellet granuloma and ulcer scores

Treatment	Dose mg/kg, p.o.	% Increase in granuloma weight (mg/100g)	% Inhibition	Ulcer scores
Control (5% gum acacia)	10 ml/kg	27.37 ± 2.79	-	No ulcer
Alcoholic extract	100	15.48 ± 1.57**	43.46	0.3 ± 0.2*
	150	8.69 ± 1.35***	68.24	1.7 ± 0.98*
	200	6.25 ± 1.19***	77.18	2.2 ± 0.77*
Diclofenac sodium	10	5.23 ± 0.69***	80.87	6.4 ± 0.97**

The results are mean ± SEM from 6 animals; * P< 0.05, **P< 0.01, ***P< 0.001 when compared to vehicle control

Discussion

The purpose of the present study was to establish scientific evidences for the usage of this plant in inflammatory conditions. Preliminary phytochemical screening of *F. ramontchi* showed presence of phytosterol, triterpenoid, flavonoid, lignan, saponin and carbohydrate in alcoholic extract.

The alcoholic extract was studied for its modulatory effects on pain and inflammation induced by chemical and thermal stimuli. The analgesic effect of alcoholic extract was tested in three different models of analgesia: acetic acid induced writhing test, formalin induced paw licking model and tail flick model in mice whereas anti-inflammatory effect was observed in two models: carrageenin induced paw edema and cotton pellet granuloma formation.

According to acute toxicity study it found that alcoholic extract of *F. ramontchi* did not cause any mortality up to 3gm/kg of body weight and was considered as safe.

In present study, results showed that alcoholic extract had reduced the intensity of acetic acid induced abdominal constriction in mice. Acetic acid causes algesia by liberating endogenous substances including serotonin; histamine, prostaglandin, bradykinin and substance P, which excite pain nerve endings (17). Although the test is nonspecific model (e.g. Anticholinergic and antihistaminic and other agents show activity in this test), it is widely used for analgesic screening and predominately involves induction of prostaglandins. So mechanism of action for alcoholic extract might involve blockade of capillary permeability or inhibition of synthesis or release of these endogeneous substances.

Formalin test has advantage that it involves biphasic pain, with an early pain representing neurogenic and late phase of inflammatory reaction (12). In the present study alcoholic extract was more efficacious in inhibiting neurogenic pain (early phase), while standard drug did not inhibit the neurogenic exhibited comparable potency. Inhibition of both these phases was found in formalin induced licking by alcoholic extract.

Drugs that act centrally inhibit pain produced by thermal stimuli (18). The alcoholic extract produced anti-nociceptive effect against thermal induced pain stimuli in mice in tail flick method at various points indicates that it might be centrally acting. In the present study, diclofenac also inhibited the pain produced by tail flick method. Although, this model is specific for centrally inhibited pain, there are certain evidences that support; NSAID's also inhibit the pain induced by thermal stimuli (19, 20). The observations from writhing test; formalin test and tail flick model suggests that alcoholic extract inhibited the pain induced by chemical and thermal stimuli.

In addition to these analgesic models, alcoholic extract was investigated in acute and subacute inflammatory models for their anti-inflammatory activity. Acute inflammation in rats was induced by sub-plantar injection of carrageenin (phlogistic agent). The carrageenin induced paw edema has been accepted as preliminary screening model for investigating systematic anti-inflammatory agent. The edema formation by subplantar injection of carrageenin is a biphasic response; initial phase (1h) is being due to the release of serotonin and histamine (21) whereas the later phase (over 1h) is attributed to the release of prostaglandins, the cyclooxygenase products and the continuity between two phases is provided by kinins (22, 23). Table 3 showed that alcoholic extract significantly ($p < 0.01$) inhibited later phase of edema so it seems possible that *F. ramontchi* blocks prostaglandins and cyclooxygenase release in later phase of acute inflammation.

In the cotton pellet granuloma model, inflammation and granuloma developed during a period of several days. The dry weight of the pellets correlates with the amount of granulomatous tissue (24). Protein synthesis is necessary for the formation of granuloma. Inflammation involves infiltration of macrophages, neutrophils and proliferation of fibroblasts, which are the basic sources for granuloma formation (25). Consequently decrease in granuloma weight indicates the suppression of the proliferative phase, which was effectively inhibited by the alcoholic extract of *F. ramontchi*. Alcoholic extract (200 mg/kg) appears to be equally effective to that of diclofenac sodium (10 mg/kg) in inhibiting the dry weight of cotton pellets.

Mucosal erosion and ulceration are produced by most NSAID's of varying degree. Inhibition of synthesis of gastric protective prostaglandin's (PGE₂ and PGI₂) is clearly involved (26, 27). To assess the mucosal erosion and ulceration produced, the animals that undergone subchronic therapy with the drug is autopsied and the stomachs are examined for mucosal injury and ulcer formation. Treatment with alcoholic extract increased the ulcer scores dose dependently indicates that the drug possesses prostaglandin inhibitory activity. Further it also suggests that the risk of production of ulcer increases as the dose increases with little change in anti-inflammatory activity. However the damage produced to gastric mucosa by alcoholic extract is comparatively less than diclofenac sodium at dose showing similar anti-inflammatory activity.

Anti-inflammatory activity of flavonoids, triterpenoids and phytosterols have been reported by several researchers (28, 29, 30, 31, 32, 33), so it might possible that particular these phytoconstituents from alcoholic extract of *F. ramontchi* could have contributed to this activity. Further studies intended to confirm these activities, as well as the isolation of active biomolecules responsible, are being conducted.

Conclusion

The results of present study revealed anti-inflammatory and analgesic activity of alcoholic extract of *F. ramontchi*. Thus it substantiates the traditionally proven effectiveness of this plant in painful and rheumatic conditions.

Acknowledgement

Authors are thankful to the Head, Department of Botany, Nagpur University, Nagpur for identification and authentication of plant and also grateful to AICTE for providing grants to present research work.

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