ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *FERONIA LIMONIA* FRUIT PULP

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Summary

Fruits of *feronia limonia* (Fam: Rutaceae) have been used traditionally as tonic. The methonlic extract was found to anti-inflammatory, antipyretic and analgesic activity. Acute toxicity studies revealed that the extract up to dose of 1g/kg intraperitoneal was non-toxic. The extract did not inhibit arachidonic acid induced paw inflammation thus indicating cyclo-oxygenase pathway of arachidonic acid metabolism.

Key words: *Feronia limonia*, anti-inflammatory, analgesic, anti-pyretic.

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Introduction

Feronia limonia (Fam: Rutaceae) commonly known as wood apple or elephant apple , is a moderate size tree which is native of India and occurs up to an elevation of 1500 feet in western Himalayas. Ripe fruit of F.limonia contain tyramine derivative acicissimol, acidissiminin, sepxide, N.benzoyl tyramine and stigmasterol. [1] It is useful as tonic in diarrhoea, dysentery, stomatitis, tumors, cough, asthama, leucorrhoea, wounds and ulcers. Fruits, leaves and stem bark of F.limonia have been studied for anti-tumor [2], larvicidal [3] and antimicrobial activity [4, 5]. However, so for no study has been reported to evaluation anti-inflammatory analgesic and antipyretic potency of *F. limonia* fruit pulp extracts.

Materials and Methods

Plant material.
The fresh ripe fruit of *F. limonia* were collected by a local supplier from around the Bangalore in the month of march-April The Fruit material was Taxonomically identified by the Regional Research Institute, Karnataka, India and Voucher specimen RRI/BNG/DSRU/F54/2006-07. The fruits were dried under shade with and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of extracts.
The coarse powder of shade dried fruit pulp of *feronia limonia* (2kg) was extracted with methanol (AR grade) in a Soxhlet extractor. The methnolic extract was then concentrated on rotary flash evaporator. The extract was subjected to preliminary phytochemical investigation [6]. When needed the extract was suspended in a gum acacia or DMSO and administered intraperitoneally to animals in different doses. In control animals, equivalent amounts of the vehicle were administered intraperitoneally.

Animals.
Experiments were carried out on sprague-dawley rats weighing 125-150 g and Balb/C mice weighing 20-25 g. these were bred in college animals house. The animals were kept in room maintained at 27±1º C and at relative humidity between 35 to 60 % with a 12hrs light/dark cycle. They were fed with standard rat feed (Gold Mohr Lipton India Ltd.) and water *ad libitum* was provided. The litter in cages was renewed thrice a week to ensure hygeinicity and maximum comfort for animals. Ethical Clearance for handling the animals was obtained from the Institutional Animals Ethical committee (MMU/IAEC/O8/2007) prior to the beginning of the project work. (131/1999/CPCSEA)

Chemicals.
Acetyl salicylic acid, carraageenan, croton oil ibuprofen, indomethacine brewers yeast and arachidonic were purchased locally.

ANTI-INFLAMMATORY STUDIES:

a) Carrageenan-induced oedema.
First 0.1 ml of carrageenan (10 mg/ml) was injected into the plantar aponeurosis of right hind paw of the rats.methanolic extracts or vehicle was administered at doses of 50,100 and 150 mg/kg I.P. 30, mins prior to carrageenan administration and the paw volume was measured after 4 hrs using plethismograph [7] .

b) Croton oil-induced ear inflammation.
Croton oil irritant solution prepared [8].was applied (0.1 ml) to the inner surface of ear of mice .the mice were sacrificed after 4hrs and 7 mm punches were made in the ear by cork borer. Each ear disc was weighed and compared with control.

c) Cotton pellet-induced granuloma.
Two autoclaved cotton pellets weighing 10±1 mg were implanted subcutaneously into both sides of the groin region of each rat [8]. The animals were divided into five groups. Group 1 served as control and received the vehicle .extract at concentrations

of 50,100, and 250 mg/kg was injected (I.P) daily to three groups of animals for 7 days. Another group received ibuprofen daily at a dose of 100mg/kg orally for 7 days. After 7 days the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in oven at 60º C, weighed and compared with control.

d) Arachidonic acid-induced paw oedema.
Paw oedema was induced by single injection of 0.1 ml off 0.5% arachidonic acid in 0.2 M carbonate buffer (PH 8.4) into the right hind paw (subplantar) of rats.Drugs and extracts were administered either ip or orally at different doses 2 h prior to arachidonic acid injection.Hind paw volume was measured 1 hr after arachidonic acid[9]

ANALGESIC ACTIVITY

a) Writhing in mice.
Four groups of six mice each were injected (I.P) with 0.1 ml of 1% acetic acid. One group received vehicle and other three groups received extract (50, 100 and 250 mg/kg I.P) 30 min prior to acetic acid. Number of writhing movements was counted for 15 min. the percentage of inhibition of writhing movement was calculated.

b) **Thermal noicieption**.
Mice were kept on an eddy's hot plate having a constant temperature of 55±0.1 ºC. The times taken for either paw licking or jumping were recorded before and after administration of extract. Mice were divided into four groups. While one group served as control and received the vehicle, the other groups received extract at doses of 50,100, &250mg/kg (I.P) 30 min prior to placement on the hot plate.

ANTIPYRETIC EFFECT

Groups of rats received 1 ml s.c of 20% Brewer's yeast at the nape of neck. The animals were fasted and after 18h their body temperatures were recorded for 1 min using a telethermometer (Aplab) with rectal probe. Animals with 1º for more elevation in body temperature were selected. The selected animals were divided into five groups. One groups received the vehicle and four groups received extract (50,100,500 mg /Kg I.P). Body temperatures were recorded 1,2,3,4 h after administration.

EFFECT OF EXTRACT ON NORMAL PERITONEAL CELLS.
Three groups of normal mice (n=6) were chosen for the study. Two groups were treated with extract at a dose of 100mf/kg and 200 mf/kg respectively. One group was treated with equivalent amount of DMSO. Total peritoneal exudates cells were counted from each group (3-5 at a time) at 6, 12, 24 and 48 h of treatment and compared (Hudson and Hay 1989). The number of phagocytes was also determined by staining with 1% neutral red solution and counting in a haemocytometer.

ACTUE TOXICITY
For acute toxicity study, different doses of extract were given i.p to groups of mice (n=10) and 24 h mortality recorded.

Statistical Methods.

Values reported are mean±S.E.M. student's t-test and probability level of $p<0.05$ were chosen as the criterion significance.

Results And Discussion

The extract inhibited carrageenan-induced paw oedema and cotton pellet granuloma significantly. It failed to show any activity against croton oil-induced ear inflammation in mice. The extract produced a dose-dependent inhibition of carrageenan oedema which was comparable with known anti-inflammatory drugs. The percent inhibition at dose levels of 50, 100, and 250 mg/kg were 41%, 56.98% and 45 %, respectively (Table 1). The extract significantly inhibited cotton pellet granuloma.The percent inhibition was 42% and 54% at doses of 50 and 100mg/kg, respectively, and this inhibition was more than that produced by ibuprofen (23%) (Table 1). In mice the extract did not significantly inhibit croton oil-induced ear inflammation as compared to acetyl salicylic acid (Table 1). The failure of the extract to inhibit croton oil-induced ear inflammation may be due to the large animal variance encountered in this model.

The injection of arachidonic acid (AA) into hind paw, produced significant oedema after 1 h. extract, at a dose of 100 mg/kg did not inhibit the arachidonic acid induced paw volume (Table 2).Indomethacine, the anti-inflammatory drug, also did not inhibit rat paw odema significantly,. hence, it is plausible that the extract reduced inflammation by blocking the cylooxygenase and not the lipooxygenase pathway of arachidonic acid metabolism .The extract, at doses of 50,100, and 200 mg/Kg (i.p), reduced acetic acid induced writhing in mice (Table 3).the extract did not exhibit any significant analgesic activity when tested using the hot plate method .since non-narcotic analgesics can be differentiated from narcotics ones by their ineffectiveness in the hot plate method . It can be assumed that the analgesic activity of the extract is of the type produced by non-narcotic analgesics.The extract also showed antipyretic activity against Brewer's yeast-induced pyrexia (Table 4). At dose levels of 50 to 100mg/kg i.p. it significantly reduced the temperature. This effect lasted for 1-2 h. at higher dose levels (0.5 and 1 g/kg) its effect persisted for up to 4 h.Normally each mouse contains about 5×10^6 intraperitoneal cells. Extract treatment enhanced the peritoneal cell count and also the number of macrophages significantly after 6 h, 12 h, and 48 h (Fig. 1). The maximum enhancement in the number of macrophages was found to be between 12-24 h after extract administration.Non-specific accumulation of macrophages occurs in the peritoneal cavity after injection of certain materials such as casein, freund's complete adjuvant thiogycolate, etc. mature macrophages in the untreated peritoneal cavity are mostly residential.intraperitoneal injection of different agents lead to exudation and i.p accumulation of new macrophages. It has been reported that generally the excaudate macrophages are more active than the residential mature ones in their ability to spread on the surface to which the cells are attached, receptor size of cell coat, response to chemotactic stimuli, composition of cell wall, etc though the actual role of extract in the enhancement of peritoneal cell count and macrophage count cannot also alter the immune response along with its anti-inflammatory effect.

The present study revealed that the methnolic extract of *Feronia limonia* possessed anti-inflammatory, analgesic and antipyretic activity in experimental animals. It is possible that the non-polar compounds present in the methanolic extract may be responsible for the observed effects in this study.

Table 1. Effect of methanolic extracts of *feronia limonia* on three different models of inflammation.

Drug	Dose (mg/kg)	Carragenan-induced oedema (volume.ml)	Croton oil-induced ear inflammation (mg)	Increase in cotton pellet weight (mg)
Control	Vehicle i.p	0.3487±0.02 (n=12)	15.16±1.2066 (n=6)	40.75±2.63 (n=12)
feronia limonia	50 mg/kg i.p	0.2056±0.0287* (n=8)	13.18±1.8519(n=6)	25.58±2.79* (n=12)
feronia limonia	100mg/kg i.p.	0.15±0.0169*(n=12)	14.57±1.7669(n=6)	18.63±0.98* (n=11)
feronia limonia	250mg/kg i.p	0.1912±0.03226* (n=8)	12.26±1.9065(n=6)	_____
Ibuprofen	100mg/kg p.o	0.1042±0.0087* (n=10)	_____	3122±1.85*(n=9)
Acetyl salicylic acid	100mg/kg p.o	0.2100±0.0144*(n=10)	5.6±1.0216*(n=6)	_____

n. Denotes number of experiments. * Denotes significant reduction from control ($p<0.05$)

Table 2. Comparison of effect of extract and reference drugs on arachidonic acid-induced increased paw volume

Drugs	Dose	%Inhibition as compared with control (n=4)
Indomethacine	10mg/kg p.o	12
Feronia limonia	250mg/kg i.p	3.4

n. denotes number of experiments. * Denotes significant increase from control ($p<0.05$)

Table 3. Effect of extract on acetic acid- induced writhing in mice.

Dose (mg/kg i.p)	Mean writhing /15 sec (±S.E.)
Feronia limonia (50mg/kg)	26.85±4.8
Control (n=7)	38.42 ±7.75
Feronia limonia (100 mg/kg)	15.7±7.41 *
Control (n=8)	44.5±6.93
Feronia limonia (200 mg/kg)	15.6±1.85 *
Control (n=6)	43.5 ±2.4

* Denotes significant reductions compared to respective control ($p<0.05$)

Table 4. Effect of Different Doses of Extract on Brewer's Yeast-Induced Pyrexia in Rats

No.	. Dose (mg/kg	Temperature (º F) after 18 h of Brewer's yeast injection	Temperature after vehicle			
			1h	2h	3h	4h
1	Vehicle i.p	101.4±0.2996	101.5±0.2616	101.9±0.0856	101.3±0.5431	101.6±0.5330
2	50 mg/kg i.p	101.7±0.1341	100.3±0.3088*	100.6±0.2683*	101.9±0.260	101.1±0.4405
3.	100 mg/kg i.p	101.7±0.134	100.5±0.4893*	100.0±0.4272*	100.9±0.434	101.4±0.535
4.	500 mg/kg i.p	102.2±0.145	97.6±0.379*	96.8±0.409*	97.9*±0.599	99.1±0.632
5.	1g/kg i.p	100.9±0.246	98.2±0.298	97.1±0.786	97.0*±0.882	98.2±0.9026

*Denotes significant reduction as compared to vehicle control (P <0.05).

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