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Research Article

Evaluation of analgesic, anti-inflammatory and antipyretic properties of the *Flacourtia indica* extract in laboratory animal

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Abstract

Worldwide attention on plant study has expanded, and a wealth of data has accumulated to demonstrate the enormous potential of medicinal plants employed in diverse traditional systems. In the traditional medical system, *Flacourtia indica* (FI) has been used to cure a variety of illnesses, including cancer, diabetes, hepatic disorders, and snakebites. Due to the presence of a variety of phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, glycosides, it may have antioxidant and anti-inflammatory activity. Thus, this study was designed to assess the analgesic, anti-inflammatory and antipyretic effects of the ethanolic extract of *Flacourtia indica*. *Flacourtia indica* was extracted using 100% ethanol followed by assessing acute toxicity and doses selected for the studies were 500 and 1000 mg/kg body weight. *Swiss albino* mice of either sex weighing 25-30 gm and *Sprague dawley* rats weighing 180-200 gm were used in this study. Analgesic activity was evaluated by using acetic acid induced writhing test, formalin induced paw licking and hot plate test. Anti-inflammatory effect was assessed using xylene and croton oil induced ear edema test and carrageenan induced paw edema test. Also the antipyretic effect was investigated. The *Flacourtia indica* extract exhibited significant effect against pain in acetic acid test ($p<0.01$), formalin test ($p<0.01$) and insignificantly in hot plate test. Inflammation was reduced by FI extract in xylene test ($p<0.05$), croton oil test ($p<0.01$) and significantly reduced the paw edema ($p<0.001$) in carrageenan-induced paw edema test. In the yeast-induced antipyretic test, administration of *Flacourtia indica* at both doses significantly ($p< 0.001$) reduced pyrexia. Within these two doses, higher doses (1000 mg/kg body weight) had better aptitudes in the reduction of pain, inflammation and pyrexia. The FI extract shown strong analgesic, anti-inflammatory, and antipyretic capabilities, according to the study results, and more research is needed to assess these effects and the potential of the plants.

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1. Introduction

As a sensory modality, pain is generally defensive in nature yet frequently causes suffering. Pain is the most significant symptom that prompts the patient to see a doctor. Analgesics treat the outward signs of pain but do little to address the underlying cause. A complex biological reaction of vascular tissues to adverse stimuli like infections, damaged cells, or irritants called inflammation. Inflammation is an organism's protective attempt to get rid of injurious stimuli and start the tissue's healing process [1]. The current synthetic analgesic and anti-inflammatory drug supply pose a number of health issues for which safer and more efficacious analgesic and anti-inflammatory drugs are urgently needed. The use of

herbal medicine has grown in popularity throughout the world, and it is thought that medicinal plants represent a significant source of novel chemicals with potential therapeutic benefits [2]. Plant secondary metabolites play an imperative role in health care for about 80% of the world's population [3].

Flacourtia indica is a spiny shrub having numerous branches, attaining a height of 2-3 m. Bark is greyish in color with a rough surface where the leaves and shoots are reddish. This leafy shrub is covered with a great deal of long, pointed spines. In Bangladesh, it can be found in village thickets, deep forest, and along waterways [4]. *Flacourtia indica* (Burm.f.) Merr. is considered as nutraceutical plant belonging to the member of the Flacourtiaceae family. It is one of the rarest plants, not much has scientifically explored, but its medical benefits have been described in Ayurveda, where they have been shown to be a successful cure for a number of ailments. Numerous studies on the plant's phytochemistry revealed the presence of a variety of phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, glycosides, etc [5]. The plant may have excellent pharmacological qualities like antibacterial, antioxidant, antimalarial, anti-asthmatic, anticancer, and hepatoprotective effects, according to a number of investigations. The plant has a stellar folkloric reputation and is used to treat a variety of ailments, including scabies, jaundice, enlarged spleen, fever, poisonous snake bites, skin diseases, pruritus, erysipelas, strangury, nephropathy, psychopathy, nephritic colic, as well as cholera, rheumatic pain, malaria, cancer and diabetes [5-9].

In order to determine the potential use of herbal medicine, it is crucial to place an emphasis on the study of medicinal plants that were identified in folklore. In light of this *Flacourtia indica* usage, research was conducted to see whether the plant extract has analgesic and anti-inflammatory properties.

2. Materials and methods

2.1 Plant collection and Extraction

Flacourtia indica was collected from medicinal plant garden of Pharmacy department, Jahangirnagar University, savar, Dhaka and identified, and authenticated by the department of Botany of same University, Savar, Dhaka. Identification of the plant specimen was performed by Sarder Nasir Uddin, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur Botanical Garden, Dhaka, Bangladesh. A voucher specimen (DACB No 46494) was deposited in the herbarium for future reference. The collected materials were thoroughly cleaned in water, cut into smaller pieces and shed dried at 35–40 ^{0}C for a week and pulverized into a coarse powder using an electric grinder. Then powders were extracted with ethanol. Finally, a solid mass was obtained and preserved in a Petridis in the refrigerator for further investigation.

2.2 Experimental animals

Female *Swiss albino* mice, aged 6-7 weeks, weighing between 25 and 30 g, and *Sprague Dawley* rats, weighing 180 to 200 g, were collected from the animal research lab in the Department of Pharmacy, Jahangirnagar University, Savar for the experiment. Animals were kept in typical habitat settings (temperature: 27°C ± 1.0 °C, relative humidity: 55-65% and 12 h light/12 h dark cycle) with unlimited access to food and water *ad libitum*. Prior to the experiments, the animals were acclimatized to laboratory environment for one week. All protocols for animal experiments were approved by the institutional animal ethical committee (Permission no is BBEC, JU/M 2020 (3)1).

In each type of analgesic test, mice were employed as experimental subjects. With the exception of the xylene-induced ear edema test, rats were utilized to determine the anti-inflammatory and antipyretic activity of plant extract.

2.3 Toxicity studies

Swiss Albino mice of either sex and weighing between 20 and 25 g underwent toxicity tests on the extracts. No fatalities were discovered until 5000 mg/kg per-oral. i.e. LD_{50} of the extract were > 5000 mg/kg body weight [10].

2.4 Analgesic activity evaluation

2.4.1 Acetic acid-induced writhing test

The method according to Koster *et al.* (1959) [11] was employed for this test. Four groups of eight mice each were pretreated with *Flacourtia indica* extracts. Each mouse was injected intraperitoneally with 0.7% acetic acid at a dose of 10 mL/kg body weight after forty-five

minutes of extract administration. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 minutes of acetic acid administration and the mean number of abdominal writhes for each group was calculated.

2.4.2 Hot plate test

The hot plate test was used to measure response latency according to the method described by Eddy and Leimbach [12] with slight modification. The temperature of the hot plate (model 7280; Ugo Basile, Italy) was maintained at 55°C ± 2°C. The mice was placed in a Perspex cylinder on a heated surface and the time from placing the animal on the hot plate to the onset of discomfort indicated by licking the paw or jumping off the surface was recorded as the response latency. Annoyance latencies were measured at 0, 30, 60, 120, and 180 minutes after administration of the test solution [12].

2.4.3 Formalin-induced Paw licking test

The method of Hunskaar and Hole [13] was used for the study. 2.7% formalin was injected into the left hind paw's dorsal surface an hour after the medication was given. Time spent licking the injected paw was recorded. Mice were observed for five minutes after formalin (the acute phase), then for five minutes beginning twenty minutes after formalin (delayed phase) [13].

2.5 Anti-inflammatory activity evaluation

2.5.1 Carrageenan Induced Leukocyte migration test

The test was conducted to find out whether the extract has anti-inflammatory properties or not by the method of Vinegar *et al.* [14]. After one hour of administration of *Flacourtia indica* extract, 0.25 mL of 0.75% carrageenan solution was injected intra-pleurally. Four hours after the injection of carrageenan, the mice were killed by excess of chloroform. From each rat, 20 µL of the exudates and washing solution were collected with a micropipette from the opening pleural cavity, and the number of mobilized leukocytes in the exudates was measured. Reduction in the number of leukocytes migrated in the cavity as compared to the vehicle-treated control rat was considered as anti-inflammatory response [14].

2.5.2 Xylene-Induced Ear Edema test

The Xylene-induced ear edema test was performed as described in Dai *et al.* [15]. Each mice received 20 µL of xylene on the anterior and posterior surfaces of the right ear lobe one hour later of administration time. One hour after Xylene application circular sections were taken and weighed. The percentage of ear edema was calculated as inflammation based on the weight of the left ear without xylene [15].

2.5.3 Cotton pellet-induced granuloma formation test

The method of Swingle and Shideman [15]. was used. Sterilized cotton pellets of 10±1 mg of each weight was impregnated subcutaneously, one on each side of the abdomen of the rat, under light chloroform anesthesia and sterile technique. Test drugs were administered orally to male mice weighing 25-30 g in once-daily dose regimen for 7 days; the control group received vehicle only. The mice were killed on the eighth day, their cotton pellets removed, dried at 60 °C for 24 hours, and their dry cotton weight measured. Cotton pellet weight loss suggests that inflammation has been inhibited [16].

2.6 Antipyretic activity evaluation

The antipyretic study was done by using the brewer's yeast-induced pyrexia model in rats [17]. Fever was induced by injecting 20 ml/kg body weight of 20 % suspension of brewer's yeast subcutaneously. In our set up the rats developed fever after 10 hours of yeast injection. Only those animals which developed fever were taken for further study and rest were rejected. Following the onset of the initial pyrexia, both the standard and test medications were administered intra-peritoneally, with the injection volume remaining constant at 0.5 mL per rat. For comparison, 100 mg of paracetamol per kg of body weight was used as the reference medicine. FI was intra-peritoneally administered to various groups of rats at dosages of 500 mg and 1000 mg/kg body weight. Following the drug treatment, the rectal temperatures were taken 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours later.

2.7 Statistical Analysis

Statistical analysis for animal experiments was carried out by one-way ANOVA following Dunnet's post hoc test using SPSS 23.0. Data were presented as Mean±SEM. The results obtained were compared with the control group. $p<0.05$, $p<0.01$, and $p<0.001$ were considered to be statistically significant, highly significant, and very highly significant respectively.

3. Results

3.1 Analgesic test

The current study looked into the ethanol extract of FI's analgesic, anti-inflammatory, and antipyretic properties. The analgesic activities were evaluated by two animal models, which could provide response to two different grades of noxious stimuli (in the thermal stimulus and chemically induced tissue damage) [18]. With respect to the acetic acid-induced abdominal writhing which is the visceral pain model [19], the result presented in Table-1 showed that in contrast to the control, FI extract reduced the number of writhes. When compared to the control group, the FI extract at a dose of 1000 mg/kg significantly ($p<0.01$) decreased the number of writhing at a rate of 53%. Diclofenac at 100 mg/kg exhibited higher antinociceptive power at 78% indicating that the extracts have a lower antinociceptive effect than the reference drug used in this study.

The hot-plate test is helpful in understanding centrally mediated antinociceptive responses since it primarily focuses on alterations above the spinal cord level [20] that possibly operate on a descending inhibitory pain pathway [29]. On the hot-plate test paradigm, the data demonstrated that the FI extract increased the latency time in heated plates up to 120 minutes of study time without yielding any appreciable differences from the control group. Tramadol significantly lengthened the latency (Figure 1).

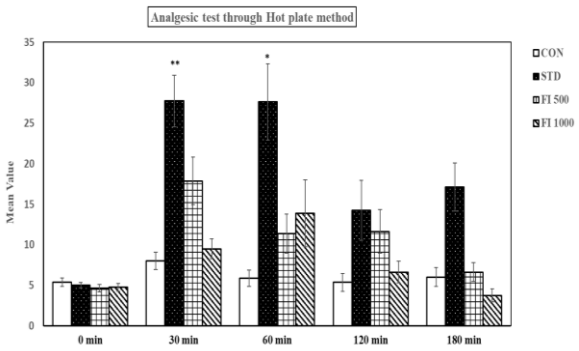

Figure 1. Effect of FI in latency time in hot plate test.

A more reliable analgesic model that is more linked with clinical pain is thought to be the formalin test [21, 22]. Figure 2 of the study's findings showed that the extract FI, at doses of 500 mg/kg and 1000 mg/kg, significantly ($p<0.01$) reduced the licking time and licking frequency by the mice injected formalin. At the late phase, this inhibitory effect is more pronounced ($p<0.001$). Higher doses of FI extract decreased licking time highly significantly ($p<0.01$) and very highly significantly ($p<0.001$) at both phases (Figure 2).

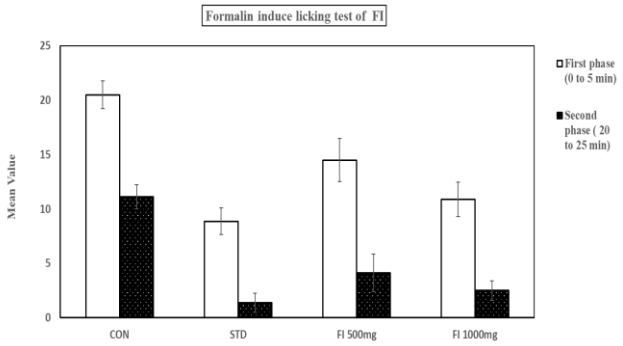

Figure 2. Evaluations of analgesic effect of FI through formalin induce licking test

3.2 Anti-inflammatory test

Administration of FI at both doses showed significant reduction ($p<0.05$) of paw volume at 60- minute time period compared to control group. Administration of FI at100mg/kg reduced the paw volume very highly significantly ($p<0.001$) at 120, 180 and 240-minute of administration as compared to the control group. Similar results were obtained for 500 mg/kg dose of FI except 120-minute time period where the result was highly significant ($p<0.01$). So, paw edematous response induced by carrageenan injection was markedly inhibited by FI at both doses (Table 2).

Table 1: Effect of *Flacourtia indica* in acetic acid induced writhing test.

Group	Total writhing (Mean ± SEM)	Inhibition (%)
CON	38.375 ± 3.615	
STD	8.125 ± 2.039***	**78.83**
FI 500mg	28.750 ± 2.883	**25.08**
FI 1000mg	18.000 ± 2.044**	**53.09**

*= $p<0.05$, **= $p<0.01$ and ***= $p<0.001$

The application of mouse models of ear edema induced by different irritant agents (Croton oil, xylene, capsaicin, AA, phenol, histamine) has been widely used to identify the probable topical anti-inflammatory effect of the substance in study and to propose its possible mechanism of action [23]. The results showed that FI extract at the dose of 1000 mg/kg, suppressed xylene induced ear swelling in

Table 2. Evaluation of anti-inflammatory effects of FI through Carrageenan-Induced Acute Inflammatory test.

Group	0 min (Mean±SEM)	60 min (Mean±SEM)	120 min (Mean±SEM)	180 min (Mean±SEM)	240 min (Mean±SEM)
CON	0.100± 0.000	0.383 ± 0.017	0.767 ± 0.033	0.983 ± 0.040	1.117 ± 0.031
STD	0.100± 0.000	0.300 ± 0.037	0.350 ± 0.067**	0.400 ± 0.052***	0.433 ± 0.042***
FI 500mg	0.100± 0.000	0.267 ± 0.021*	0.383 ± 0.060**	0.483 ± 0.048***	0.517 ± 0.060***
FI 1000mg	0.100± 0.000	0.200 ± 0.037*	0.283 ± 0.048***	0.333 ± 0.021***	0.350 ± 0.022***

*= p<0.05, **= p<0.01 and ***= p<0.001

mice significantly (p<0.05), with 53.85%, of the inhibition rate and Diclofenac (100 mg/kg, p.o.) showed marked anti-inflammatory activity with a 76% reduction compared to the control, and indicted it might reduce the release of substance P or antagonize its action (Table 3).

Table 3: Evaluation of anti-inflammatory effects of FI through Xylene induce ear edema test.

Group	Ear inflammation(mg) (Mean ± SEM)	Inhibition (%)
CON	0.013 ± 0.001	
STD	0.003 ± 0.000**	76.92
FI	0.008 ± 0.000	38.46
FI	0.006 ± 0.001*	53.85

*= p<0.05, **= p<0.01 and ***= p<0.001

Cotton pellet granuloma model was used to evaluate the anti-inflammatory activity of FI in sub-acute inflammation. The dry weight of cotton pellet granuloma was highly significantly reduced (P<0.01) by FI 500 mg/kg dose and also very highly significantly (p<0.01) at 1000 mg/kg dose with 33.79% and 40.74% inhibition compared to control group (Table 4 and Figure 3).

Table 4: Evaluation of anti-inflammatory effects of FI through Cotton pellet method.

Group	Inflammation (mg) (Mean ± SEM)	Inhibition (%)
CON	31.500 ± 1.035	
STD	12.833 ± 0.600***	59.26
FI 500mg	20.857 ± 1.405**	33.79
FI 1000mg	18.667 ± 1.820***	40.74

*= p<0.05, **= p<0.01 and ***= p<0.001

3.3 Antipyretic effect

In the antipyretic test, FI at 500mg/kg and 1000mg/kg demonstrated a very highly significant (p<0.001) reduction of temperature after 120 minutes which was up to 240 minutes of administration compared to control group. FI at 500 mg/kg also deceased body temperature after 30 and 60 minute-time period significantly (p<0.05 and p<0.01) (Table 5).

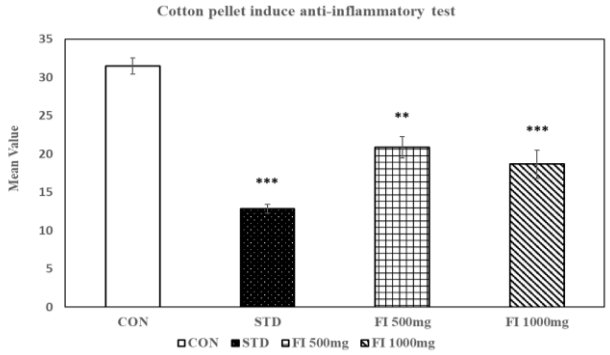

Figure 3. Anti-inflammatory effects of FI through cotton pellet method.

4. Discussion

According to the current study, the Flacourtia indica plant has analgesic and anti-inflammatory properties and is utilized for these purposes by traditional practitioners. Reactive oxygen species (ROS) produced either endogenously or exogenously are linked to the pathogenesis of a number of illnesses, including arthritis, cancer, diabetes, and the aging process [24]. ROS are involved in the pathophysiology of inflammatory diseases and are scavenged by antioxidants, which are predicted to alleviate these conditions [25].

Acetic acid, a model frequently used for testing peripheral analgesics, induces pain by releasing endogenous chemicals that activate nerve terminals, including serotonin, histamine, prostaglandins (PGs), bradykinins, and substance P. [26, 27]. The procedure has also been linked to lipoxygenase products and prostanoids in general, such as elevated levels of PGE2 and PGF2 in peritoneal fluids [27, 28]. The

Table 5: Antipyretic effect of Flacourtia Indica on yeast induced pyrexia

Group	Before 30 min (Mean±SEM)	After 30 min (Mean±SEM)	After 60 min (Mean±SEM)	After 120 min (Mean±SEM)	After 180 min (Mean±SEM)	After 240 min (Mean±SEM)
CON	85.00 ± 0.267	85.83±0.349	85.60 ±0.295	86.54±0.224	86.96±0.228	87.74±0.159
STD	84.71 ± 0.311	83.81± 0.297**	82.85±0.419**	82.54±0.455***	79.40± 0.327***	76.34± 0.129***
FI 500mg	84.00 ± 0.423	82.85± 0.633*	83.24± 0.435**	82.15± 0.488***	81.80± 0.466***	81.00± 0.353***
FI 1000mg	84.13 ± 0.398	84.75± 0.350	84.00± 0.598	82.03± 0.435***	81.33± 0.470***	80.20± 0.338***

*= p<0.05, **= p<0.01 and ***= p<0.001

significant reduction in acetic acid-induced writhes by FI suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances. The hot-plate test is helpful in under tanding centrally mediated antinociceptive responses since it primarily focuses on alterations above the spinal cord level [20] possibly acting on a descending inhibitory pain pathway [29]. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems [30-33].

The biphasic formalin test assesses pain that is both neurogenic (first phase) and of inflammatory origin (second phase). The first phase (0-5 min) being a result of direct stimulation of nociceptors measures centrally mediated effects while the second phase (15 - 30 min) is dependent on peripheral inflammation and changes in central procession due to chemical mediator release from damaged cells that stimulate nociception and thus induced pain [13]. In general, this test is recommended as a tool in basic pain research because it measures the response to a long-lasting nociceptive stimulus [21]. The ability of FI extract to inhibit late phases of the formalin test more prominently indicates its involvement in peripherally mediated activity, probably by prostaglandin synthesis inhibition.

The inflammation induced by carrageenan involves cell migration and plasma exudation mediated by the production of inflammatory mediators which were previously shown to recruit leukocytes, such as neutrophils, in several experimental models [34, 35]. Additionally, it is known that 3 h after carrageenan administration, cyclooxygenase-2 (COX-2) activation results in an increase in prostaglandin E2 synthesis, which is important for cell migration and the development of paw edema [36]. FI reduced the paw edema volume at 500 and 1000 mg/kg very highly

significantly may be by affecting cyclooxygenase (COX) 1 and 2, the enzymes participating in the prostaglandin biosynthesis from arachidonic acid [37].

Xylene-induced neurogenous swelling is a common inflammatory model. This model was selected for evaluating vascular permeability which was partially associated with substance P [38]. Xylene causes instant irritation of the mouse ear and leads to fluid accumulation and edema. Edema is the characteristic of the acute inflammatory response [39].

Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in the rats, a transudative phase, that occurs during the first 3 h, an exudative phase, occurring between 3 and 72 h after implanting the pellet and a proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation have been described [16]. The suppression of proliferative phase of sub-acute inflammation could result in decrease in the weight of granuloma formation [40].

The antipyretic efficacy of the extract FI in the present study is indicative of similarity in mechanism of action. Like other non-steroidal anti-inflammatory/analgesic drugs, FI works by preventing the synthesis of prostaglandins, which cause pain and pyrexia [41]. Therefore, FI was a good antipyretic agent because it was able to decrease the body temperature in albino rats even below its baseline temperature.

5. Conclusions

Overall, the results of the current experimental evaluation show that the FI extract significantly

reduces both cerebral and peripheral pain. Additionally, the extract had an antipyretic effect on rodents whose pyrexia was brought on by yeast. Consequently, the FI extract may play a crucial role in the clinical management of pain, inflammation, and fever. To determine the mechanism behind these behaviors, more research is needed.

Author Contributions

Conceptualization, M.A.F.I. and R.M.; Methodology, M.A.F.I. and R.M.; Validation, M.A.F.I. and R.M.; Formal Analysis, M.A.F.I. and R.M.; Investigation, M.A.F.I., A.M.R.R., M.S.H. and A.J; Resources, M.A.F.I. and M.N.H.; Data Curation, M.A.F.I., M.N.H.; Writing – Original Draft Preparation, M.A.F.I., A.M.R.R., and M.S.H.; Writing – Review & Editing, M.A.F.I. and R.M..; Supervision, R.M.

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Conflict of Interest

Authors has no conflict of interest

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