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# An Evaluation of Analgesic and Anti-Inflammatory Activity of *Ficus racemosa* in Rat Model

**Navila Nawjesh Lima [a], Nur-Neasha Dolon [a], Fahmida Maliha [b], Md. Rahmat Ullah [a], Fairuz Humayra [c], Md. Mustafiz Chowdhury [d], Md. Abdullah Hil Baky Rupak [e], Juliana Aditi Baroi [a], F. M. Sharifuzzaman Shohan [c] and Rafat Tashin [a*]**

*[a] Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh.*
*[b] Department of Pharmacy, East West University, Dhaka 1212, Bangladesh.*
*[c] Department of Pharmacy, Primeasia University, Bangladesh.*
*[d] Department of Pharmacy, University of Chittagong, Bangladesh.*
*[e] Department of Pharmaceutical Sciences, North South University, Plot # 15, Block # B, Bashundhara R/A, Dhaka, 1229, Bangladesh.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

When we talk about "herbal medicine," we imply the utilisation of plants with medicinal capabilities to either prevent illness or alleviate its symptoms. This idea encompasses a wide range of practises, from the widespread and frequent usage of traditional treatments in all cultures to the standardisation and tritation of plant extracts. In this study, rats were administered an extract of Ficus racemosa to assess the plant's analgesic and anti-inflammatory properties. To investigate the analgesic and anti-inflammatory properties, the carrageenan-induced acute inflammation

**Corresponding author: Email: whitefang229@gmail.com;*

methodology, as well as the acetic acid writhing and tail flick procedures, were applied. There were no significant effects observed in any of the groups for anti-inflammatory action. Only the high dosage of 1000 mg/kg showed statistically significant ($p< 0.05$) effects when the analgesic efficiency of the acetic acid writhing technique was compared to that of the positive control group. The only dosage that achieved statistically significant ($p <0.05$) results across all groups in the tail flick test was the very high dose of 1000 mg/kg. As our extract does not operate at low or moderate doses, this might be related to seasonal differences or differences in extraction procedures. Collecting the plant in different seasons and altering the extraction procedure may aid in identifying the plant's analgesic and anti-inflammatory effects.

*Keywords: Herbal medicine; F. racemose; analgesic; acetic acid writhing; aspirin.*

## 1. INTRODUCTION

Pain and inflammation are two immunological reactions that cause discomfort, redness, immobility, edema, and heat in response to injury, irritants, or pathogens. "An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [1] was the original definition of pain. Vasodilation (redness, heat, swelling), inflammation, and pain are the major symptoms of inflammation, all of which are caused by the production and physiological activity of prostaglandins (e.g., PGE2) in injured tissue [2]. Common pain relievers and inflammation fighters available without a prescription include acetaminophen, diclofenac, ketorolac, opioids, and many more. Common analgesics include aspirin, codeine, and morphine [3,4]. Aspirin, ibuprofen, naproxen, and indomethacin are examples of commonly used anti-inflammatory medications. To reduce pain and inflammation, NSAIDs function by blocking the production of prostaglandins (PGs) by inhibiting the enzyme cyclooxygenase (COX) [4, 5]. The aforementioned symptoms are often managed with steroidal and non-steroidal anti-inflammatory medicines (NSAIDs), but long-term use of these medications may have negative consequences for the kidney, liver, GIT, CVS, CNS, and lungs [5]. At least 100,000 individuals each year are killed by these toxins, and 8% of all U.S. hospital admissions are due to negative drug responses [6]. Therefore, novel analgesics and anti-inflammatory medicines with minimal side effects are needed despite the abundance of existing options. The abundance of useful phytochemicals in medicinal plants makes them a promising resource for finding novel therapeutic agents. Because of the rich diversity of compound they contain, plants have been used as a source of medicine for thousands of years. In addition to phenols, glycosides, alkaloids, saponins, terpenoids, tannins, polysaccharides, flavonoids, plant lipids, resins, and essential oils, plants may contain a broad range of other chemically active chemicals [7,8]. Once again, the desired medical effect may be achieved by increasing or lowering the concentration of the plant's chemical components through genetic manipulation. Reverse genetics has promise for improving secondary metabolite production, including alkaloid production [9]. Leaves of the *Persea schiedeana* plant, seeds of the *Nigella sativa* plant, and oil extracted from the Eucalyptus tree are only a few of the traditional herbs used to treat pain and inflammation [10–12].

*Ficus racemose,* cluster fig, red river fig, or gular [13] is a plant species in the family Moraceae. Its original habitat was either Australia or tropical Asia. Typically reaching the size of a big shrub, older examples may grow extremely enormous and twisted. The plant grows quickly and has large, very rough leaves. The cauliflorous growth pattern of its figs sets it apart from other trees. After the seeds are removed, the fruit is often used as a vegetable in dishes like stir-fries and curries.

The Ovambo people make ombike, a traditional beverage, from the fruit of the cluster fig, which they name eenghwiyu [14]. *Ficus racemosa* is a tree whose bark has medicinal properties. In India, the paste made by rubbing the bark on a stone with water is used to treat boils and insect bites. After the paste has dried on the skin, a second application may be made. The plant's tough leaves may also be used to scrape out embedded caterpillar hairs. The stinging hairs may be removed by gently rubbing the afflicted region with a leaf, a popular folk cure. Sterols, triterpenoids (Lanosterol), tetracyclic triterpene-glauanol acetate alkaloids, tannins, and flavonoids may all be found in this plant [15]. Antioxidant, antihelmintic, analgesic, and anti-inflammatory properties have been observed in

this plant [16–22]. The antinociceptive impact of flavonoids has been shown [23, 24], with most of the study concentrating on the inhibition of prostaglandin formation as the mechanism of action. The crude ethanol extract of *Ficus racemosa* leaves included flavonoids, tannins, an alkaloid, and sterols, all of which may contribute to the plant's peripheral antinociceptive activity. The methanolic extract of *F. racemosa* has been shown to have anti-inflammatory activities due to its high flavonoid content (169.37 mg quercetin equivalent per g of dry extract). Bioflavonoids, also called flavonoids, are a group of plant-derived compounds. These compounds have been found to have anti-inflammatory properties in both in vitro and in vivo investigations [25,26].

Isolating the active chemical responsible for analgesic and anti-inflammatory action, which might lead to novel medicines for the treatment of pain and inflammation, would require extensive research. The objective of the present study is to investigate the analgesic and anti-inflammatory properties of Ficus racemosa in Rat.

## 2. MATERIALS AND METHODS

### 2.1 Drugs, Chemicals and Instruments

The acetic acid, ethanol, and carrageenan were all purchased from Sigma Aldrich in Germany. The ibuprofen and aspirin were given out at no cost thanks to Healthcare Pharmaceutical Limited (UK). A plethysmometer and an analgesia metre were used to assess the drug's anti-inflammatory and pain-relieving properties, respectively.

### 2.2 Plant Collection and Extract Preparation

The *Ficus racemosa* fruit used for authentication and taxonomic characterization was sourced from the medicinal plant garden of the University of Dhaka's Faculty of Pharmacy. The guidelines of the Bangladesh National Herbarium were followed for archiving the plant samples. The herbarium officials on 11-2-2019 assigned accession number 47380 to the 7-10 days shade-dried and then coarsely pulverised leaf for future reference. The powdered leaves were steeped in 70% ethanol for 96 hours while being vigorously shaken. After the extraction process was complete, the resultant liquid was filtered and stored. The rotary evaporator was then used to filter the concentrated extract. The concentrated extract was then dried and stored.

### 2.3 Experimental Animal Handling

From the Jahangirnogor University Zoology Department in Bangladesh, we got male Wistar rats weighing 125–200 g. These rats were maintained at the University of Dhaka's Institute of Nutrition and Food Science on a 12:12 light:dark cycle with a constant temperature of 25 °C. The rats were housed there for acclimatisation purposes before the trial began; therefore, regular pellet food and clean water were provided on a daily basis. All rat tests were performed within the guidelines set out by the Institutional Animal Ethics Committee (IEAC). Both the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT) have established guidelines for the treatment and use of animals in scientific study.

### 2.4 Experimental Design

Each rat's body weight was recorded, and the animals were then divided into groups (Table 1) with five rodents in each group, evenly distributed over the weight range.

### 2.5 Evaluation of Anti-Inflammatory Activity

In order to test the anti-inflammatory properties of the *Ficus racemose,* we utilized Carrageenan to induce inflammation.

**Table 1. Group specification for anti-inflammatory activity**

| Group Number | Group Specification | Treatment species | Dose Treatment species (mg/kg) | Abbreviation of Groups |
|---|---|---|---|---|
| 1 | Carrageenan Control | N/A | N/A | Car |
| 2 | Carrageenan + Ibuprofen | Ibuprofen | 10 | $Car+Ib_{10}$ |
| 3 | Carrageenan + *Ficusracemosa* | *Ficus racemosa* | 500 | $Car+MZ_{500}$ |
| 4 | Carrageenan + *Ficus racemosa* | *Ficus racemosa* | 750 | $Car+MZ_{750}$ |
| 5 | Carrageenan + *Ficus racemosa* | *Ficus racemosa* | 1000 | $Car+MZ_{1000}$ |

### 2.6 Carrageenan-Induced Acute Inflammatory Model

The effectiveness of anti-inflammatory agents is often evaluated by observing their effects on carrageenan-induced paw edema in rodents. A plethysmometer, a specialized instrument, was used to measure the anti-inflammatory effect. The next thing that had to be done was to determine how big each rodent's paw was. To induce edema, we injected subplanar tissue from the left rear paw of rats at a dose of 0.1 mL per 100 g of body weight with a 1% solution of freshly synthesized carrageenan. After that, an additional hour was provided. Then, the test drugs and extracts were given to rats in a range of doses to see what effects they had. Between 0 and 6 hours after Carrageenan infusion, the paw volume was measured using a plethysmometer. In order to determine the rate of edema blockage, the following formula was used [27,28].

$$\text{Percentage Inhibition} = \frac{V_{Pc} - V_t}{V_{pc}} \times 100$$

Here,

VPC = volume of animals' paw in Positive/carrageenan Control rat
$V_0$=volume of animals' paw in Treatment Group

### 2.7 Evaluation of Analgesics Activity

The rodent is stimulated with pain through the acetic acid-induced writhing test and tail-flick method.

### 2.8 Acetic Acid-Induced Writhing Test

To evaluate peripheral analgesic activity, we employed the acetic acid writhing test. Plant and marketed drugs were administered taken 30 minutes before the acetic acid was injected intraperitoneally. While the rats were experiencing painful stimuli, they received an intraperitoneal injection of 0.9% acetic acid (10 ml/kg). The number of writhes (muscle contraction ions) was measured over a period of 20 minutes commencing immediately after acetic acid administration. The number of times an animal arched its back, flexed its abdominal muscles, brought its hind limbs towards its abdominal walls, and extended its hind limbs during the course of twenty minutes was used to calculate the percentage of writhing inhibition. To calculate the fraction of writhes attributable to the analgesic effect, we employed equation [29].

$$\left\{\frac{A.\ Control\ mean - Treatment\ mean}{A\ Control\ mean}\right\} \times 100$$

Where

$T\ Control$ = the mean number of the writhing of each test group
$A\ Control$ = The mean number of the writhing of acetic acid control group

The analgesic activity of the extract is then also assessed via the "Tail Flick Method" on the same experiment rat model after giving a break for seven days. The effect of injected acetic was terminated by this time.

### 2.9 Tail Flick Method

The tail-flick experiment is a nociceptive test that was initially described by Love and Smith in 1941 [30]. It is used to assess how animals respond behaviorally to painful stimuli. The rats were treated with drug/ extract 30 minutes before starting the experiments. The delay between stimulus presentation and the commencement of the avoidance response was measured using a tail-flick analgesia meter (UGO BASILE®, Germany) pre-programmed with radiant heat. The heat controls helped bring the exposed nichrome up to temperature by maintaining a continuous current of 4 Amps across it. Pain may be induced in the rats by directing radiant heat towards their tails in the middle of the cage. The time it took for treated and untreated rats to show a tail-flick response was measured. Animals were subjected to tests at 0, 15, 30, 45, and 60 minutes after receiving test chemicals.

### 2.10 Statistical Analysis

All of our findings (raw data) fell into many categories, encompassing a broad variety of research parameters, and were recorded and analyzed on a spreadsheet in Microsoft Excel. The data was analyzed using descriptive statistics, and the findings are presented as a mean and standard deviation. Using SPSS 1600's "One Way Anova Test" tool, we analyzed the statistical significance of the differences we found between the groups. At our institution, we consider events to be statistically significant if their associated 'p' value is less than 0.05 ($p < 0.5$).

## 3. RESULTS

The data was showed as time and percent inhibition. Though all the groups decreased the paw edema but no group showed statistically

significant results compared to the positive/carrageenan control group.

## 3.1 Analgesic Activity of *Ficus racemosa*

### 3.1.1 Writhing test

The result of acetic acid writhing test is shown below in Table 2. Only the high dose 1000mg/kg showed statistically significant ($p < 0.05$) results among all the groups.

### 3.1.2 Tail flick test (TFS)

Table 3 shows the outcomes of the experiment. Treatment with FR improved the pain threshold in a dose-dependent way. However, the impact was less than that of the gold standard medication, aspirin. Only high dose 1000mg/kg showed statistically significant results ($p < 0.05$) among all the groups.

## 4. DISCUSSION

Herbal remedies are the art or practice of utilizing herbs and herbal mixtures to preserve health and prevent, treat, or cure illness. Herbal remedies have been used for thousands of years. Researchers that study medicinal plants believe that the one-of-a-kind chemical compounds that are produced by medicinal plants may have potential therapeutic applications [31]. In this particular piece of research, we looked at the analgesic and anti-inflammatory capabilities of the fruits of the *Ficus racemosa* tree. All of the groups were successful in reducing paw edema, which is an indicator of anti-inflammatory effectiveness; however, none of the treatments produced statistically significant results in comparison to the positive control group. Only the high dose of 1000 mg/kg exhibited statistically significant ($p<0.05$) effects when comparing analgesic efficiency in acetic acid writhing method to the positive control group. This was determined by comparing the effects to those of the positive control group. The results of two further studies [32, 33] came to the same conclusion. In the tail flick test, only the very high dose of 1000 mg/kg yielded results that were statistically significant ($p < 0.05$) across all of the groups. Three further investigations [34, 35, 36] came to the same conclusions as the first study. Previous studies [37, 38] have shown that the high alkaloid and flavonoid content is to blame for the effects that are capable of reducing pain.

Additional research is necessary in order to identify the molecule that is responsible for the analgesic and anti-inflammatory effects.

**Table 2. Group specification for analgesic activity by acetic acid writhing method**

| Group Number | Group Specification | Treatment species | Dose Treatment species (mg/kg) | Abbreviation of Groups |
|---|---|---|---|---|
| 1 | Acetic Acid Control | Physiological Saline | 10ml/kg | Ace |
| 2 | Aspirin +Acetic Acid | Aspirin | 100 | $As_{100}$+Acetic Acid |
| 3 | *Ficus racemosa* + *Acetic acid* | *Ficus racemosa* | 500 | $FR_{500}$+Acetic Acid |
| 4 | *Ficus racemosa* + *Acetic acid* | *Ficus racemosa* | 750 | $FR_{750}$+Acetic Acid |
| 5 | *Ficus racemosa* + *Acetic acid* | *Ficus racemosa* | 1000 | $FR_{1000}$+Acetic Acid |

**Table 3. Group specification for analgesic activity by tail flick method**

| Group Number | Group Specification | Treatment species | Dose Treatment species (mg/kg) | Abbreviation of Groups |
|---|---|---|---|---|
| 1 | Tail Flick Stress (control) | Physiological Saline | 10ml/kg | TFS |
| 2 | Aspirin + Tail Flick Stress | Aspirin | 100 | $As_{100}$+TFS |
| 5 | *Ficus racemosa* + Tail Flick Stress | *Ficus racemosa* | 500 | $FR_{500}$+TFS |
| 6 | *Ficus racemosa* + Tail Flick Stress | *Ficus racemosa* | 750 | $FR_{750}$+TFS |
| 7 | *Ficus racemosa* + Tail Flick Stress | *Ficus racemosa* | 1000 | $FR_{1000}$+TFS |

**Table 4. Anti-inflammatory activity of *Ficus racemosa* extract and Ibuprofen through paw edema test in a rat model (* presents the level of significance of result)**

| Group | Time µL | | | | |
|---|---|---|---|---|---|
| | 0 Minute(Just before carrageenan injection) | 1 hour (just before treatment) | 2 Hours | 3 Hours | 4 Hours |
| Car | 109.45±3.74 | 118.22±5.31 | 130.51±4.93 | 136.55±5.36 | 142.32±4.80 |
| Car+$Ib_{10}$ | 112.34±4.57 | 126.75±3.99 | 120.80±5.43 | 117.35±4.86 | 114.64±5.31 |
| Car+$FR_{500}$ | 110.53±5.80 | 125.67±4.30 | 129.22±5.620.99% | 127.46±4.596.66% | 123.30±4.36 13.36% |
| Car+$FR_{750}$ | 113.34±3.48 | 130.91±5.42 | 133.39±6.4-2.21% | 132.47±4.812.99% | 128.34±5.10 9.82% |
| Car+$FR_{1000}$ | 112±4.89 | 129.20±5.40 | 135.35±5.5-3.71% | 130.10±4.304.72% | 126.30±5.11 11.26% |

**Table 5. Analgesic effect of different doses of *Ficus racemosa* and Aspirin by acetic acid writhing test (*presents the level of significance of result)**

| Group specification | Dose | Number of writhing | % Inhibition |
|---|---|---|---|
| Ace | N/A | 97.39±4.80 | |
| $As_{100}$+Acetic Acid | 100 | 78.40±5.34 | |
| $FR_{500}$+Acetic Acid | 500 | 95.39±5.46 | 2.05% |
| $FR_{750}$+Acetic Acid | 750 | 93.10±4.71 | 4.40% |
| $FR_{1000}$+Acetic Acid | 1000 | 90.21±6.34* | 7.37% |

** indicates significant difference (p<0.05)*

**Table 6. Analgesic activity of *Ficus racemosa* and Aspirin by the tail-flick test method**

| Group No | Group Specification | Basal Reaction | Reaction time in second | | | |
|---|---|---|---|---|---|---|
| | | | After 30 minutes | After 1 Hour | After 2 Hour | After 4 Hour |
| 1 | TFS | 3.91±0.86 | 4.10±0.75 | 4.36±0.91 | 5.04±0.91 | 5.48±0.480 |
| 2 | $As_{100}$+TFS | 2.40±0.77 | 3.91±0.88 | 4.46±0.91 | 6.05±1.06 | 6.57±1.80 |
| 3 | $FR_{500}$+TFS | 3.41±0.84 | 4.46±0.83 | 5.04±0.73 | 5.28±0.86 | 5.39±0.48 |
| 4 | $FR_{750}$+TFS | 2.3±0.94 | 2.3±0.71 | 2.5±0.84 | 2.6±0.79 | 2.5±0.67 |
| 5 | $FR_{1000}$+TFS | 3.60±0.94 | 4.19±0.83 | 4.80±0.81 | 5.31±0.89 | 5.67±0.99* |

** indicates significant difference (p<0.05)*

## 5. CONCLUSION

The analgesic and anti-inflammatory efficacy of *Ficus racemosa* was evaluated in a rat model in this work. Although the extract reduced inflammation in terms of anti-inflammatory action, it was not statistically significant. It suppressed pain feelings in the event of analgesic action, which is statistically significant. Further research into the extraction and modification of anti-inflammatory and analgesic components from *F. racemosa* extracts may result in more specific therapeutic ingredients in the illness management system.

## ETHICAL APPROVAL

All studies were conducted in conformity with the 2013 Declaration of Helsinki's ethical guidelines [26].

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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