Article

Pharmacological Investigation of the Active Fractions of *Ficus benjamina* Leaf Extract

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Abstract: Objective: *Ficus benjamina*, commonly known as the weeping fig, is valued for its medicinal properties and potential health benefits. Due to its antibacterial, anti-inflammatory, and antioxidant effects, it can treat infections, reduce inflammation, and minimize oxidative damage. This study aimed to investigate the analgesic, anti-inflammatory, and antipyretic effects, along with the acute toxicity, of ethyl acetate (EA) and n-hexane (n-H) fractions derived from ethanolic leaf extract. Methods: In vivo evaluations were conducted to assess the analgesic, anti-inflammatory, antipyretic, and acute toxicity effects of the extracts. The acetic acid-induced writhing method was used to evaluate analgesic activity, while the formaldehyde-induced paw edema method was employed to assess anti-inflammatory effects. Antipyretic activity was determined by monitoring changes in rectal temperature in mice. Acute oral toxicity testing was performed according to OECD Guideline 423 (Organization for Economic Co-operation and Development) using the Fixed Dose Procedure. Results: The extract of *F. benjamina* exhibited significant analgesic, anti-inflammatory, and antipyretic activities. In analgesic tests, the ethyl acetate fraction (250 and 500 mg/kg) inhibited pain by 36.78% and 48.27%, respectively, while the n-hexane fraction showed 37.93% and 49.42% inhibition. Anti-inflammatory assays confirmed significant activity, with the ethyl acetate fraction reducing inflammation by 34.24% and 36.98%, and the n-hexane fraction by 27.84% and 29.17% at the same respective doses. Both fractions demonstrated antipyretic effects, with the ethyl acetate fraction (500 mg/kg) showing the highest efficacy. Acute toxicity tests indicated no toxic effects at doses up to 5000 mg/kg. Conclusion: *F. benjamina* leaf extract demonstrated notable analgesic, anti-inflammatory, and antipyretic properties.

Keywords: *F. benjamina*; analgesic; anti-inflammatory; antipyretic; acute toxicity

1. Introduction

Traditional plant-based medicines have long been used in human and animal medicine to treat a variety of conditions, especially pain and inflammation [1]. Native and alternative medicinal plants offer promising avenues for safer and more accessible treatment options, as synthetic drugs are often associated with adverse side effects and high costs. Plant cells harbor a wide variety of metabolites essential for cellular survival, communication between cells, defense against pathogens and herbivores, and adaptation to abiotic stresses [2]. Phytochemicals are natural chemical compounds in plants that offer health benefits to humans beyond basic nutrients. Phytochemicals are classified as primary and secondary metabolites, based on their function in plant metabolism. Primary metabolites are necessary for plant life and include carbohydrates, amino acids, proteins, lipids, purines, and pyrimidines of nucleic acids. On the contrary, secondary metabolites are the remaining plant chemicals produced by the cells through metabolic pathways derived from the primary metabolic pathways. These chemical components have been described as an antiviral, antifungal, and antibiotic, which are responsible for protecting plants from pathogens [3]. Medicines are described as the science of healing, which comprises the treatment and prevention of disease, the practice of diagnosis and promotion of health. It is also referred to plant substances, drugs, medications which are used to cure many diseases and to promote health [4]. Antipyretic medications typically work by preventing or reducing the expression of COX, which helps lower elevated body temperatures by inhibiting the production of prostaglandin E2 (PGE2) [5]. Anti-inflammatory medications work by blocking the COX enzyme, which in turn stops the production of prostaglandins. This decrease in prostaglandin levels helps

alleviate pain and inflammation [6]. Analgesia involves multiple mechanisms beyond COX inhibition, including opioid receptor activation, inhibition of pro-inflammatory cytokines, ion channel modulation, and suppression of neurogenic inflammation. Plant-derived compounds often act through several of these pathways, providing both central and peripheral analgesic effects [7–9]. The adverse effects of synthetic drugs have increased global interest in plant-based medicines, which are considered more suitable for long-term use. In developing countries, traditional plants offer affordable alternatives with fewer side effects [10].

F. benjamina is a tree from the Moraceae family, native to Asia and Australia [11]. With its antibacterial, anti-inflammatory, and antioxidant qualities, it may be helpful in managing inflammation, treating infections, and mitigating oxidative damage [12]. It is employed in the treatment of several ailments such as malaria, influenza, dysentery, bronchitis, acute enteritis, pertussis, and febrile seizures in pediatric patients [13]. *F. benjamina* leaf extracts show promise as α-glucosidase and α-amylase inhibitors and can therefore be utilized in the development of anti-diabetic functional diets/nutra-pharmaceuticals [14]. Furthermore, this plant posssess antiseizure and antidiabetic properties [15], as well as its antioxidant and hemolytic activities [16], and its antidiarrheal activities [17].

Previous studies on *F. benjamina* primarily focused on chemical profiling and in vitro evaluations, including antioxidant and antimicrobial activities, as well as DPPH, hydrogen peroxide (H_2O_2), and nitric oxide (NO) scavenging activities, along with in vivo antidepressant studies. These previous studies utilized a broad range of solvent systems, such as methanol, chloroform, butanol, and water [16,18,19]. One particular study evaluated the anti-inflammatory effects of aqueous extracts of *F. benjamina* (beringin) and *Muntingia calabura* (kersen) leaves in rats by measuring the reduction in edema volume and the percentage of inflammation inhibition following carrageenan-induced paw inflammation, using diclofenac sodium as a positive control. The study also suggested that flavonoids present in the extracts may be responsible for the observed anti-inflammatory activity [20]. However, a thorough literature review found no reports on the analgesic, antipyretic, or anti-inflammatory activities of the fractions derived from the ethanolic extract of *F. benjamina* leaves. Therefore, the novelty and originality of our study lie in the in vivo evaluation of analgesic, anti-inflammatory, antipyretic, and acute toxicity activities using the ethyl acetate and n-hexane fractions of the ethanolic extract. Our research uniquely employs OECD Guideline 423 for acute oral toxicity testing and utilizes multiple animal models, including acetic acid-induced writhing, formaldehyde-induced paw edema, and yeast-induced pyrexia. Additionally, this study is distinctive in including an OECD 423-compliant acute toxicity assessment, confirming the safety of the extract up to 5000 mg/kg, which provides regulatory-level validation. Another original aspect is the specific pharmacological comparison between the ethyl acetate and n-hexane fractions in vivo, which has not been reported in prior literature.

This study aims to evaluate the therapeutic potential of the fractions of *F. benjamina* leaf extract by assessing its analgesic, anti-inflammatory, and antipyretic properties, as well as its acute toxicity, to explore any potential benefits it may offer for healthcare.

2. Materials and Methods

2.1. Chemical

Laboratory-grade reagents, including acetic acid (Merck, Germany), formaldehyde (Merck, Germany), Nacl (Merck, Germany). All standard medications used for pharmacological assessments in vivo were acquired from Square Pharmaceuticals Ltd. and Beximco Pharmaceuticals Ltd. in Bangladesh.

2.2. Plant Collection

Leaves of the *F. benjamina* plant were collected from the Mohanagar Project, Hatirjheel, Rampura, Dhaka, Bangladesh, in April 2024. The plant specimen was identified by Dr. Mohammad Sayedur Rahman, a senior scientific officer at the Bangladesh National Herbarium in Mirpur, Dhaka, Bangladesh, and was assigned the authentication number DACB-94759.

2.3. Phytochemical Test

Using the methodology of Ayoola et al. (2008), a qualitative phytochemical analysis of the *F. benjamina* extract was conducted to ascertain the existence or non-existence of various secondary metabolites, including reducing sugars, flavonoids, glycosides, alkaloids, terpenoids, saponins, tannins, and so forth [21].

2.4. Extraction and Fractionation

The collected leaves were shade-dried, ground into a coarse powder, and extracted using the maceration method, in which 565 g of the powdered leaves were soaked in 2000 mL of 96% ethanol for 14 days to maximize the yield of bioactive constituents. Maceration is a gentle, non-thermal extraction technique that is particularly suitable for preserving thermolabile compounds that may degrade under heat-based methods such as reflux or Soxhlet extraction. The extended extraction time of 14 days allows for sufficient solvent–plant matrix interaction, enabling effective diffusion of both polar and non-polar phytochemicals into the solvent. This prolonged contact time enhances the extraction efficiency, especially for compounds present in lower concentrations or embedded deep within the plant cell matrix. Thus, the selected method and duration were intended to optimize both the quality and quantity of the extracted phytoconstituents [22,23]. The yield of the ethanolic extract was 13.70%. Fractionation was performed on crude ethanolic extract using water, n-hexane, and ethyl acetate [24]. A total of 77.45 g of *F. benjamina* extract was suspended in 400 mL of distilled water and successively partitioned with n-hexane (150 mL, then another 150 mL, then 100 mL), collecting and pooling the organic layers. The defatted aqueous phase was then extracted in the same way with ethyl acetate. The collected organic layers were concentrated under reduced pressure using a rotary evaporator to yield n-hexane (30.98%) and ethyl acetate (60.68%) fractions.

2.5. Animals

Young Swiss albino mice (*Mus musculus*), aged 4 to 6 weeks and weighing between 20 to 25 g, were obtained from Jahangirnagar University in Bangladesh. They were kept in the pharmacology laboratory's animal facility at the Pharmacy Department of Dhaka International University for 2 to 3 weeks to allow them to acclimate to their new environment. The facility is located at Dhaka International University in Bangladesh. All experiments were conducted in a quiet, private, and controlled setting. The animal experiments adhered to the ethical guidelines set by the Committee of Clinical Pharmacy & Pharmacology at the Department of Pharmacy, Dhaka International University, Satarkul, Badda, Dhaka-1212. [Ref No. CPP/DIU/EC/0010].

2.6. Experimental Design

For the analgesic, anti-inflammatory, and antipyretic studies, Swiss albino mice were randomly divided into six groups (n = 5 per group), designated as Group I to Group VI for each pharmacological test. Each group received a specific treatment, i.e., control, standard, and two different doses of the extract. For the acute toxicity experiments, experimental animals were randomly selected and divided into seven groups (n = 5 per group), denoted as Group I to Group VII. Each group received a specific treatment, i.e., control and three different doses of the extract. Each mouse was properly weighed, and the doses of the test samples and control substances were adjusted accordingly. The doses were calculated based on the body weight of each mouse.

2.7. Evaluation of Analgesic Activity

The analgesic activity was assessed using a model in which mice were induced with writhing by the administration of acetic acid, as described by Debnath [21,25]. In animal models, the acetic acid-induced writhing technique is commonly used to assess analgesic activity on the peripheral nervous system. The study included six groups with every group containing 5 mice; Group I (negative control): 1% Tween-80 in distilled water, 10 mL/kg orally given, Group II (positive control): Diclofenac Na in distilled water, 25 mg/kg orally given, Group III: Test extract fraction (EA) in distilled water, 250 mg/kg orally given, Group IV: Test extract fraction (EA) in distilled water, 500 mg/kg orally given. Group V: Test extract fraction (n-H) in distilled water, 250 mg/kg orally given, Group VI: Test extract fraction (n-H) in distilled water, 500 mg/kg orally given [25,26]. After intraperitoneal injection of 0.7% acetic acid, which induced writhing (characterized by abdominal constriction, trunk twisting, and hind leg extension) in groups of five mice each, the treatments were administered orally using a feeding needle. The number of writhes was counted for each mouse during a five-minute period starting fifteen minutes after administration. The analgesic effect was evaluated by calculating the percentage inhibition of writhing in treated groups compared to the control group.

2.8. Evaluation of Anti-Inflammatory Activity

The anti-inflammatory activity was assessed using a model in which mice were induced with paw edema by administration of formaldehyde, as described by Debnath [21,27]. Each group contained five animals. The groups were as follows Group I (control group, treated orally with 1% Tween-80 in distilled water), Group II (standard

group, administered Ibuprofen at a dose of 100 mg/kg body weight), Group III (test group-I, given the EA fraction of *F. benjamina* leaf extract at 250 mg/kg body weight), Group IV (test group-II, given the EA fraction of *F. benjamina* leaf extract at 500 mg/kg body weight), Group V (test group-III, given the n-H fraction of *F. benjamina* leaf extract at 250 mg/kg body weight), Group VI (test group-IV, given the n-H fraction of *F. benjamina* leaf extract at 500 mg/kg body weight), respectively, via oral gavage [25,26]. The linear circumference of the right hind paw was measured 30 min before formaldehyde injection using a sliding caliper. Next, each mouse received an injection of 0.1 mL of 2% formaldehyde solution into the right hind paw. The linear diameter of the injected paw was measured at 1, 2, 3, and 4-h intervals following the injection. The anti-inflammatory activity was assessed by measuring paw thickness and determining the percentage inhibition of paw edema in treated groups compared to the control group.

2.9. Evaluation of Antipyretic Activity

Fever is a complex physiological response triggered by infections or aseptic stimuli. Pyrexia, or fever, is typically a secondary effect of infection, tissue damage, inflammation, graft rejection, malignancy, or other disease states. The antipyretic activity was assessed using a model in which pyrexia was induced in mice by the administration of Brewer's yeast, as described by Subedi [28]. The experiment consisted of six groups, each with 5 mice; Group I (negative control) received saline (10 mL/kg, orally), Group II (positive control) received paracetamol (150 mg/kg, orally), Group III received the EA fraction of *F. benjamina* leaf extract (250 mg/kg, orally), Group IV received the EA fraction of *F. benjamina* leaf extract (500 mg/kg, orally), Group V received the n-H fraction of *F. benjamina* leaf extract (250 mg/kg, orally), Group VI received the n-H fraction of *F. benjamina* leaf extract (500 mg/kg, orally) [25,26]. Distilled water was used to dissolve the materials during preparation. The mice were weighed to establish the appropriate dosage, and their normal body temperature of each mouse was recorded using a digital thermometer. Pyrexia was induced in all mice by subcutaneous injection of a 15% aqueous suspension of Brewer's yeast (10 mL/kg body weight). After an overnight fast with free access to water, rectal temperatures were measured 24 h after injection [29]. Animals exhibiting a temperature increase of less than 0.5 °C were excluded, while those with an increase exceeding 0.5 °C were confirmed to be pyrexia. Each group was administered its respective treatment, and rectal temperatures were monitored at 1, 2, 3, and 4 h after treatment. The antipyretic effect was determined by recording rectal temperatures and calculating the percentage reduction in temperature in treated groups compared to the control group.

2.10. Evaluation of Acute Toxicity

Acute oral toxicity was performed by using OECD guidelines-423 (Organization of Economic Co-Operation Development)—Fixed Dose Procedure [30]. The purpose of this study was to determine an appropriate starting dosage for the primary investigation. The acute oral toxicity of *F. benjamina* Linn was evaluated in Swiss albino mice. Prior to the experiment, all the animals were housed in an overnight fasting state with unrestricted access to water and their body weights were recorded. Mice weighing between 180–250 g were commonly used for acute toxicity studies. There were seven groups, each consisting of five animals. Group I received an oral administration of a normal saline solution containing 0.9% sodium chloride (NaCl). The mice in this group were given a solution of (0.01 times their body weight) milliliters on the first day. Groups II to Group VII received the fractions of *F. benjamina* leaf extract orally at doses of 300, 2000, and 5000 mg/kg body weight (dissolved in purified water). The mice in this group were given a solution of (0.01 times their body weight) milliliters on the first day. Food was provided one to two hours after drug administration. For the first four hours post-administration, the animals were closely observed for any noticeable behavioral changes. Subsequently, routine monitoring continued for the next 24 to 48 h. Observations included behavioral changes as well as parameters such as body weight, urine output, food consumption, body temperature, and changes in skin and eye coloration.

2.11. Statistical Analysis

All experimental results were expressed as means ± standard error of the mean (SEM). Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's test. Statistical analysis was performed using Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). A $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Phytochemical Test

The phytochemical analysis of *F. benjamina* leaf extract identified different classes, including tannins, saponins, steroids, terpenoids, flavonoids, and reducing sugars (Table 1). These phytochemicals are believed to be the primary contributors to the plant's major biological activities.

Table 1. Presence or absence of different phytochemical groups in *F.benjamina* leaves.

Phytochemical Group	Result	
	Ethyl Acetate	n-Hexane
Reducing sugar (Benedict's test)	−	−
Reducing sugar (Fehling's test)	+	+
Combined reducing sugar	−	−
Tannins (Ferric chloride test)	+	+
Flavonoids	+	+
Saponin	−	−
Gums	+	+
Steroids	+	+
Alkaloids	+	+
Glycoside	−	-
Proteins	+	+
Terpenoids	+	+
Acidic compounds	−	−

'+' indicates presence and & '−' indicates absence.

3.2. Evaluation of Analgesic Activity

The fractions of *F. benjamina* extract, administered at doses of 250 and 500 mg/kg body weight, demonstrated a dose-dependent reduction in acetic acid-induced writhing reflex in mice, with statistical significance set at ($p < 0.05$). In the analgesic test, intraperitoneal administration of a 0.7% acetic acid solution induced notable body contractions. The ethyl acetate fraction reduced writhing by 36.78% at 250 mg/kg and 48.27% at 500 mg/kg, while the n-hexane fraction showed inhibition rates of 37.93% and 49.42% at the same respective doses. In comparison, the standard drug Diclofenac Na produced 63.21% inhibition at a dose of 25 mg/kg body weight. The n-hexane fraction at 500 mg/kg yielded statistically significant results, indicating greater analgesic activity than the ethyl acetate fraction (Figure 1).

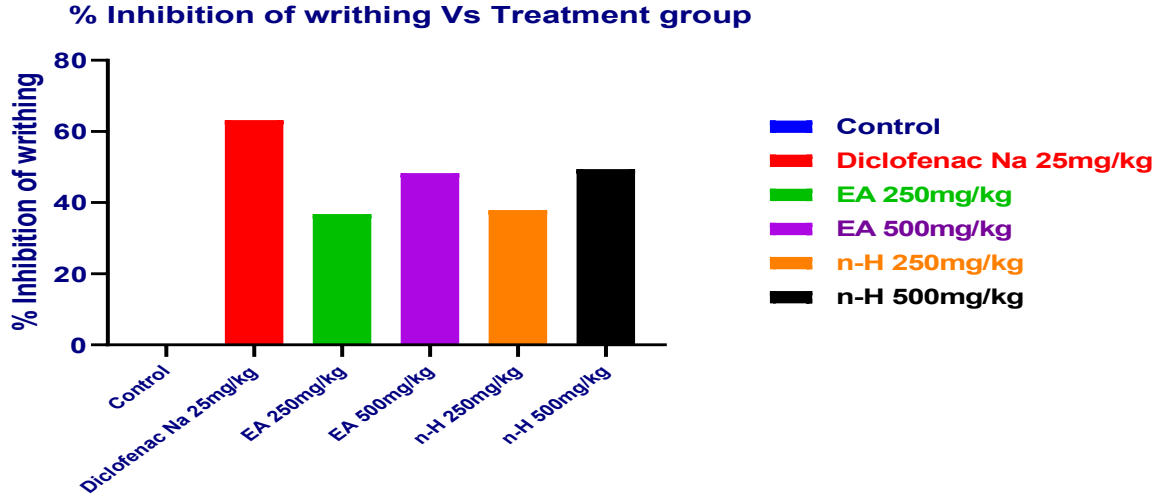

Figure 1. Percentage of inhibition writhing in case of acetic acid-induced writhing. EA and n-H at 250 and 500 mg/kg reduced writhing by 36.78%/48.27% and 37.93%/49.42%, respectively. In comparison, Diclofenac Na, used as the standard drug, showed 63.21% inhibition at a dose of 25 mg/kg body weight. Among the two fractions, the n-hexane fraction at a 500 mg/kg dose exhibited the highest analgesic activity. Experimental data are presented as the mean values along with their standard errors (mean ± SEM).

Analgesics alleviate pain by acting on the sensory nervous system, either centrally or peripherally, without significantly affecting consciousness. They are broadly classified into two categories: non-opioid (non-narcotic) analgesics, which possess antipyretic and anti-inflammatory properties, and opioid (narcotic) analgesics, which act by depressing the central nervous system. Non-opioid analgesics primarily target peripheral pain pathways but also contribute to increasing the pain threshold within the central nervous system [31]. The mechanism of action of analgesic drugs involves the inhibition of cyclooxygenase (COX) enzymes, which stops prostaglandin synthesis. Less prostaglandin is produced, which lessens inflammation and pain [6]. Flavonoids suppress enzymes like cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), leading to reduced synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes [32]. Tannins inhibit cyclooxygenase (COX) enzymes and reduce the production of pro-inflammatory cytokines [33]. Phenolic compounds inhibit cyclooxygenase (COX) enzymes [34]. Flavonoids, tannins, and phenolic compounds help reduce pain [35]. The analgesic effect of *F. benjamina* extract, demonstrated by a decrease in the writhing reflex in acetic acid-induced models, suggests that bioactive compounds such as flavonoids, tannins, and phenolic compounds may be responsible. Therefore, these compounds may contribute to the analgesic mechanism and explain the traditional uses of *F. benjamina* in pain relief. Our findings from the acetic acid-induced abdominal constriction test showed a substantial reduction in the writhing response. Among the two fractions, the n-hexane fraction at a dose of 500 mg/kg exhibited the highest analgesic activity.

In the literature review, no reports were found on the analgesic activity of fractions derived from the ethanolic extract of *F. benjamina* leaves. Therefore, the novelty and originality of our study lie in the in vivo evaluation of the analgesic activity of the ethyl acetate and n-hexane fractions of the ethanolic extract.

3.3. Evaluation of Anti-Inflammatory Activity

The *F. benjamina* extract fractions, administered at doses of 250 and 500 mg/kg body weight, exhibited a dose-dependent reduction of inflammation in formaldehyde-induced paw edema in mice. In the anti-inflammatory test, inflammation was induced by injecting 0.1 mL of a 2% formaldehyde solution into the right hind paw of the mice. Throughout the observation period, paw edema was significantly reduced by *F. benjamina* extract fractions at both doses. After four hours, inflammation was suppressed by 34.24% and 36.98% with the ethyl acetate fraction and by 27.84% and 29.17% with the n-hexane fraction at 250 mg/kg and 500 mg/kg, respectively. In comparison, the standard drug Ibuprofen demonstrated 59.89% inhibition at a dose of 100 mg/kg body weight (Figure 2).

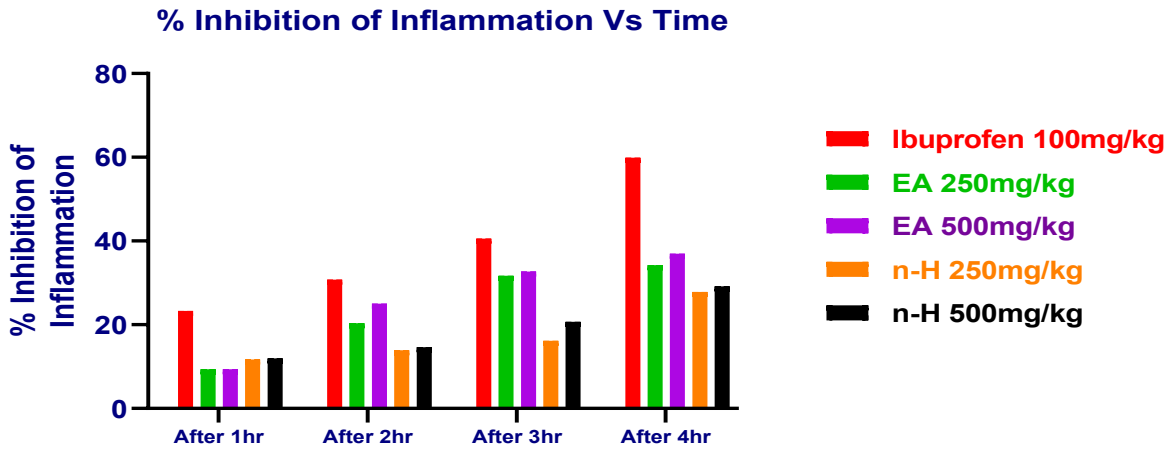

Figure 2. Percentage inhibition of inflammation in the formaldehyde-induced paw edema model. After four hours, EA and n-H (250/500 mg/kg) inhibited inflammation by 34.24%/36.98% and 27.84%/29.17%, respectively. In comparison, the reference drug Ibuprofen showed 59.89% inhibition at a dosage of 100 mg/kg body weight. In both instances, the 500 mg/kg dose showed enhanced anti-inflammatory efficacy compared to the 250 mg/kg dose. The experimental results are expressed as means ± standard error of the mean (SEM).

Inflammation is the local response of living mammalian tissues to injury in response to a stimulus. It is a defense mechanism of the body aimed at eliminating or limiting the spread of the injurious agent. Inflammation is triggered by the release of chemicals from damaged tissues and migrating cells. The most strongly implicated mediators include prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, and, more recently, platelet-activating factor (PAF) and interleukin-1 [36]. Anti-inflammatory medications work by blocking the COX enzyme,

which in turn stops the production of prostaglandins. This decrease in prostaglandin levels helps alleviate pain and inflammation [6]. Gallocatechin directly inhibits cyclooxygenase (COX) enzymes, reducing prostaglandin production, which contributes to inflammation [32]. Catechin inhibits the activity of COX enzymes (COX-1 and COX-2), reducing prostaglandin synthesis [32]. Gallocatechin and catechin reduce inflammation. Therefore, these compounds may play a role in the anti-inflammatory mechanism. This study also supports the traditional use of the plant parts in medicine for their anti-inflammatory effects [37].

The previous study mainly investigated the anti-inflammatory effects of aqueous extracts of *F. benjamina* L. (beringin) and *Muntingia calabura* L. (kersen) leaves in rats. When comparing the aqueous extract of *F. benjamina* with the fractions of its ethanolic extract, the aqueous extract showed moderate anti-inflammatory activity, with a maximum inhibition of 44.36% at a dose of 264 mg/kg in the carrageenan-induced edema model. In contrast, the ethyl acetate fraction of the ethanolic extract demonstrated a stronger, dose-dependent effect, achieving 36.98% inhibition at 500 mg/kg in the formaldehyde-induced edema model. While both extracts significantly reduced inflammation, the ethanolic fractions, particularly the ethyl acetate fraction, were more potent, suggesting that the ethanol extraction concentrated more effective anti-inflammatory compounds than the aqueous method [20].

The fractions of the *F. benjamina* extract showed a significant reduction of paw edema. The results indicate that the 500 mg/kg dose demonstrated stronger anti-inflammatory effects compared to the 250 mg/kg dose in both cases. Additionally, the ethyl acetate fraction showed greater anti-inflammatory activity than the n-hexane fraction.

3.4. Evaluation of Antipyretic Activity

The *F. benjamina* extract fractions, administered at doses of 250 and 500 mg/kg body weight, exhibited antipyretic effects by reducing yeast-induced fever in mice. The ethyl acetate fraction lowered body temperature to 96.70 ± 0.48 °F at 250 mg/kg and 96.26 ± 0.34 °F at 500 mg/kg, while the n-hexane fraction reduced fever to 97.60 ± 0.20 °F and 96.92 ± 0.26 °F at the respective doses (Figure 3).

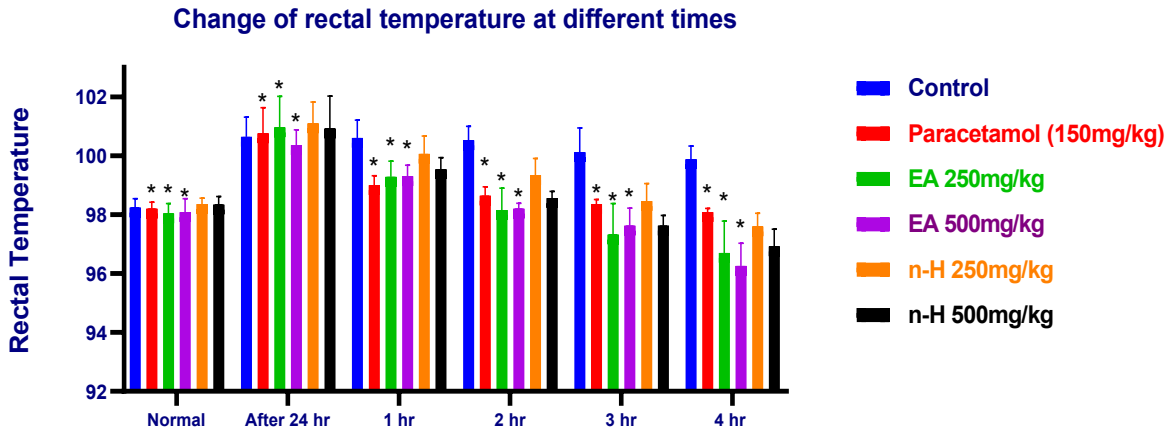

Figure 3. Change of rectal temperature at different times (for different samples) EA reduced pyrexia to 96.70 ± 0.4827 °F and 96.26 ± 0.3444 °F, and n-H to 97.60 ± 0.2000 °F and 96.92 ± 0.2596 °F at 250 and 500 mg/kg, respectively. In comparison, the reference drug Paracetamol exhibited 98.08 ± 0.05831 °F inhibition at a dose of 150 mg/kg body weight. Among the two fractions tested, the ethyl acetate fraction at 500 mg/kg showed the most pronounced antipyretic activity. The experimental results are expressed as means ± standard error of the mean (SEM) (Significance: * $p < 0.05$).

The study was carried out to examine the antipyretic effects in mice over a specified duration. In this experiment, a 15% Brewer's yeast solution was administered subcutaneously to induce fever by increasing prostaglandin production. This method is widely used to assess the antipyretic potential of both plant-based and synthetic drugs [38]. Yeast-induced pyrexia, or pathogenic fever, is thought to be caused by increased prostaglandin production. The antipyretic effect likely occurs through the inhibition of prostaglandin synthesis, resembling the mechanism of paracetamol. This process is achieved by suppressing the activity of the cyclooxygenase (COX) enzyme [39]. After 4 h, at doses of 250 and 500 mg/kg body weight, the ethyl acetate fraction reduced body temperature to 96.70 ± 0.48 °F and 96.26 ± 0.34 °F, respectively. The n-hexane fraction reduced body temperature to 97.60 ± 0.20 °F and 96.92 ± 0.26 °F at the same doses. In comparison, the standard

drug paracetamol showed a body temperature of 98.08 ± 0.06 °F at a dose of 150 mg/kg body weight. Among the two fractions, the ethyl acetate fraction at the 500 mg/kg dose demonstrated the most significant antipyretic effect.

The fractions of *F. benjamina* extract showed significant inhibition of pyretic activity, which may be attributable to the presence of antipyretic compounds such as stigmasterol [40]. Stigmasterol exhibits antipyretic properties primarily by inhibiting the synthesis of prostaglandins, which are key mediators of fever. This effect is achieved through the suppression of cyclooxygenase (COX) enzyme activity, similar to the mechanism of action of conventional antipyretic drugs like paracetamol [41]. Therefore, this compound may be responsible for the observed antipyretic effect. The study reinforces the traditional use of plant parts for their fever-reducing properties. Oral administration of the extract effectively decreased rectal temperature in yeast-induced febrile mice, indicating that the ethyl acetate and n-hexane fractions possess pharmacologically active components that may suppress prostaglandin release.

A literature review found no reports on the antipyretic activity of fractions derived from the ethanolic extract of *F. benjamina* leaves. Therefore, the novelty and originality of our study lie in the in vivo evaluation of the antipyretic activity of the ethyl acetate and n-hexane fractions of the ethanolic extract.

3.5. Evaluation of Acute Toxicity

In the control group (Group-I), the average weight of mice slightly increased from 30.6 ± 1.89 g to 31.3 ± 1.87 g, indicating normal growth. Group-II (EA 300 mg/kg) maintained a stable body weight. In comparison, Groups III (EA 2000 mg/kg) and Group IV (EA 5000 mg/kg) exhibited modest weight increases, from 30.6 ± 1.89 g to 31.6 ± 1.208 g and 30.6 ± 1.89 g to 32.00 ± 1.304 g, respectively. Meanwhile, Group-V (n-H 300 mg/kg) showed moderate weight gain, from 29.20 ± 0.5831 g to 30.6 ± 1.89 g. Group VI (n-H 2000 mg/kg) experienced a smaller increase, from 33.20 ± 1.241 g to 33.80 ± 1.281 g, while Group VII (n-H 5000 mg/kg) showed a more notable gain, from 28.80 ± 2.596 g to 31.00 ± 1.673 g (Figure 4).

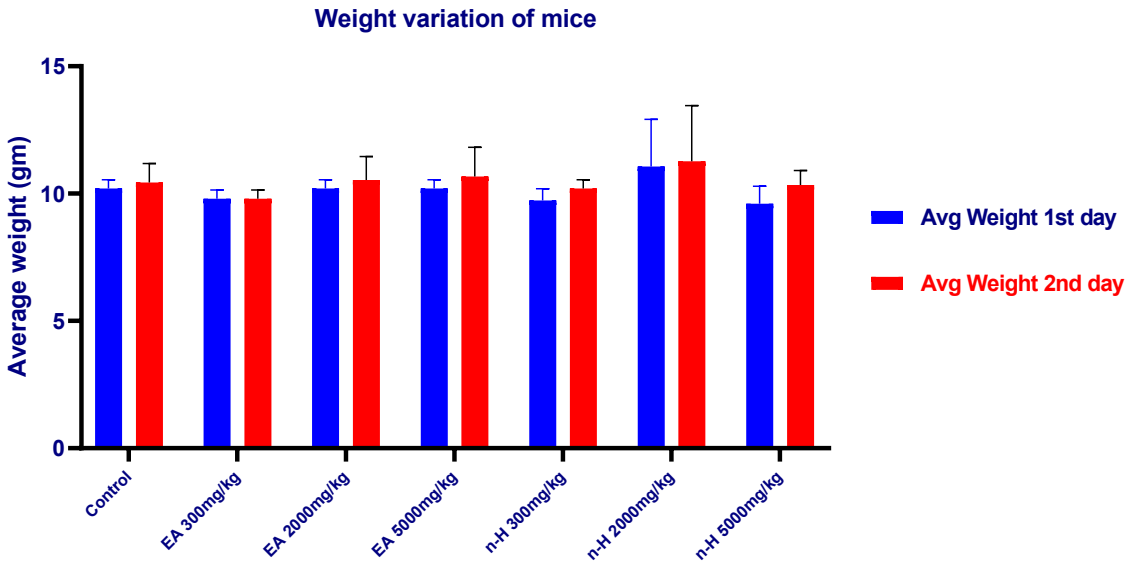

Figure 4. Effects of ethanolic extract fractions of *F. benjamina* leaves on the body weight of mice. The control group showed normal growth. EA-treated mice had slight to moderate gains, while n-H groups showed dose-dependent increases, lowest at the mid-dose.

Evaluating acute toxicity is a crucial step in determining the safety of both synthetic and natural compounds for human and animal use, as well as their potential environmental impact. Acute toxicity testing involves examining the adverse effects of a substance following a single or short-term exposure, typically for 24 to 48 h [42]. The average body weight of the control group considerably dropped. Meanwhile, the group treated with *F. benjamina* leaf extract showed no changes in appearance or behavior, and their average body weight increased, suggesting the extract had no harmful effects. On the second day, the average body weight of mice administered the ethyl acetate fraction of *F. benjamina* extract at doses of 300 mg/kg, 2000 mg/kg, and 5000 mg/kg was 29.40 ± 0.5099 g, 31.6 ± 1.208 g, and 32.00 ± 1.304 g, respectively. Meanwhile, for the n-hexane fraction at the same doses, the recorded average weights were 30.6 ± 1.89 g, 33.80 ± 1.281 g, and 31.00 ± 1.673 g, respectively. In toxicological research, establishing the safety of specific doses is often accomplished by conducting studies at

higher dose levels to identify potential adverse effects. If higher doses, such as 2000 mg/kg or 5000 mg/kg, do not result in significant adverse effects, this provides a safety margin indicating that lower doses, like 250 mg/kg or 500 mg/kg, are likely to be non-toxic. In this study, doses up to 2000 mg/kg did not produce any observable adverse effects, further suggesting that lower doses, such as 250 mg/kg and 500 mg/kg, are likely to be safe [43]. Behavioral changes and other parameters such as urination, food intake, temperature, changes in eye and skin colors were normal (Table 2). Any alterations or irregularities noted could be a sign of toxicity. However, no significant behavioral changes were noted in the test animals at any of the administered dose levels. In the acute toxicity study, the fractions of the ethanolic extract of *F. benjamina* leaves were found to be safe, indicating that this plant is non-toxic. Consequently, this plant is regarded as safe for use in conventional medicinal practices.

Through a literature review, we found no reports investigating the acute toxicity of fractions derived from the ethanolic extract of *F. benjamina* leaves. Therefore, the novelty and originality of our study lie in the in vivo evaluation of the acute toxicity of the ethyl acetate and n-hexane fractions of the ethanolic extract.

Table 2. General appearance and behavioral observations of acute toxicity study for control and treated groups. The fractions of *F. benjamina* extract showed no behavioral changes, indicating it is safe and non-toxic.

Observation	Group-I (Control)	Group-II EA (300 mg/kg)	Group-III EA (2000 mg/kg)	Group-IV EA (5000 m/kg)	Group-V n-H (300 mg/kg)	Group-Vl n-H (2000 m/kg)	Group-Vll n-H (5000 mg/kg)
Food intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Drowsiness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eye color	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal	Normal	Normal	Normal

4. Conclusions

In this study, we carried out extensive pharmacological tests on living organisms to evaluate the medicinal effectiveness of the *F. benjamina* plant, which is widely used in traditional medicine. The results suggest that the plant has significant pharmaceutical potential, showing strong antipyretic, anti-inflammatory, and analgesic properties. Furthermore, no harmful effects were observed from its use. These findings emphasize the plant's potential as a natural remedy for various ailments. Chemical analysis is essential for linking extract composition to biological activity. However, due to limited resources, detailed phytochemical profiling (e.g., GC-MS, LC-MS, or HPLC) of the *F. benjamina* extract was not performed in this study. In future studies to conduct comprehensive chemical characterization to better elucidate the underlying mechanisms. However, further research is advocated to fully understand its mechanisms of action and promote its application in medical fields.

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Data Availability Statement: All data generated in this study are available from the corresponding author upon request. Results are expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism (version 9.0), with one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$ considered statistically significant). Raw data are securely maintained for verification.

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Abbreviation

PGE2	Prostaglandin E2
COX-2	Cyclooxygenase-2

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