



RESEARCH ARTICLE

Exploring the therapeutic potential of ethanol extract of *Erythrina fusca* Lour. roots as an analgesic, antipyretic, and anti-inflammatory agent in experimental animals

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ARTICLE HISTORY

Received: 12 December 2023
Accepted: 12 June 2024

Available online
Version 1.0 : 25 June 2024
Version 2.0 : 29 June 2024



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonpublishing.com/journals/index.php/PST/indexing_abstracting

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Nhung TTP, Quoc LPT. Exploring the therapeutic potential of ethanol extract of *Erythrina fusca* Lour. roots as an analgesic, antipyretic, and anti-inflammatory agent in experimental animals. Plant Science Today. 2024; 11(sp1): 235-243.
<https://doi.org/10.14719/pst.3198>

Abstract

Erythrina fusca Lour., renowned in traditional medicine for its soothing, analgesic, anti-inflammatory, and neuroinhibitory properties, is the focus of our investigation. Expanding on its traditional applications, we assessed the analgesic, anti-inflammatory, and antipyretic characteristics of the ethanol extract from *E. fusca* root (REEF). Administered orally to mice at varying doses (50, 100, and 150 mg/kg for pain and fever reduction; 100, 150, and 200 mg/kg for anti-inflammatory experiments), REEF's effects were systematically evaluated through parameters such as reaction time, pain inhibition ratio, COX2 and PGE2 levels, rectal temperature, fever inhibition ratio, paw diameter, paw edema inhibition ratio, and inflammatory cytokines. Results reveal that REEF consistently reduced licking and biting time ($p < 0.05$) in response to formalin-induced pain in mice. Particularly noteworthy was the more pronounced reduction in COX2 and PGE2 during the late phase, with the most significant decrease observed at the 150 mg/kg REEF treatment ($p < 0.05$). The licking time graph substantiated the analgesic efficacy of REEF, illustrating simultaneous and sustained pain reduction over time. Moreover, REEF significantly alleviated fever ($p < 0.05$) in mice, with the most substantial effect observed three hours post-yeast injection. REEF not only efficiently reduced body temperature but also countered the fever-inducing effects of yeast at evaluated time points ($p < 0.05$). In the context of carrageenan-induced inflammation, paw swelling markedly diminished after 4 and 5 hours ($p < 0.05$). Notably, at the 5-hour mark, REEF treatment at 150 mg/kg dose exhibited significantly superior anti-inflammatory effects ($p < 0.05$). Additionally, mice treated with REEF (100, 150, and 200 mg/kg) exhibited a considerable decrease ($p < 0.05$) in serum concentrations of TNF- α , IL-1 β , and IL-6 compared to the control treatment. The ethanol extract from *Erythrina fusca* roots demonstrates substantial potential for pain relief, fever reduction, and anti-inflammatory effects, positioning it as a promising herbal resource for the research and development of natural medicinal products with these therapeutic properties.

Keywords

analgesic effects; antipyretic properties; anti-inflammatory activity; therapeutic potential

Introduction

When the body faces invaders, the immune system deploys cells, signals, and fluids to eliminate the threat and prevent harm. Inflammation, the

body's response to repel bacterial and viral intruders, is often accompanied by fever and pain. Fever, an elevated body temperature triggered by immune cell activity during inflammation, and pain serve as an uncomfortable warning to avoid actions that worsen bodily harm (1). The U.S. Centers for Medicare & Medicaid Services (CMS) predicts healthcare costs to reach 5.7 trillion USD annually by 2026 due to the increasing prevalence of inflammation and related syndromes worldwide (2). Medications like acetaminophen, diclofenac, ketorolac, opioids, etc., function as pain relievers, fever reducers, or anti-inflammatories. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX), an enzyme responsible for producing prostaglandins (PG), but they entail serious side effects (3). Opioids for pain relief can lead to addiction, dependency, and side effects like nausea, constipation, and respiratory depression (4). Hence, research on pain relievers, fever reducers, and anti-inflammatories with minimal side effects is crucial. Herbal researchers propose that newly identified chemical compounds from medicinal plants may have therapeutic potential. Consequently, scientists actively explore herbal remedies for conditions such as pain, fever, and inflammation (5).

Erythrina species are commonly used in traditional medicine for their pharmacological benefits, including sedative effects, pain-relieving, anti-inflammatory, and nerve inhibition. Among the Erythrina genus, known for its pain-relieving and anti-inflammatory properties, *Erythrina fusca* (genus Erythrina, family: Fabaceae) is a perennial plant with medium to large flowers and spreading thorns. This medicinal plant is found along coastlines and rivers in tropical regions across Asia (India, Sri Lanka, Myanmar, Indonesia), Australia, Mascarene Islands, Madagascar, Africa, and South America (6). In traditional medicine, various parts of *E. fusca*, such as leaves, roots, and bark, are utilized for their pain-relieving and fever-reducing properties. A water extract from the bark is used for intermittent fevers like malaria. The inner bark, when cleaned, serves as a dressing for fresh wounds to prevent infection, while a combination of stem bark and roots is used for severe and persistent headaches. The roots exhibit anti-rheumatic properties, pain-relieving, and fever-reducing. Purple-stemmed leaves are used to clean ulcerated wounds and are topically applied for toothache relief, while the flowers possess anti-cough properties (7). Analyzing the traditional uses of *E. fusca* and its valuable components, our study zeroes in on evaluating the pain-relieving, anti-inflammatory, and fever-reducing properties of ethanol extract from *E. fusca* roots. This research could act as a foundation for a more extensive exploration of the pharmacological effects of *E. fusca*.

Materials and Methods

Plant material

Collection plant material: In March 2023, fresh *Erythrina fusca* fruits were harvested in Buon Ma Thuot City, Daklak Province, Vietnam. After selecting large, uniformly colored,

mold-free, and undamaged fruits, they were thoroughly washed. The fruits were then cut into small pieces and sun-dried for 3 days, followed by further drying in a Memmert drying cabinet (Germany) at 60°C until reaching a constant weight. The resulting dried fruits were finely ground into powder and stored in moisture-proof bags at room temperature, protected from light, for subsequent experiments.

Preparation of the extract: 500 grams of this powder underwent a cold soaking process in 2.5 liters of 70% ethanol for 72 hours, with regular shaking to improve extraction efficiency. After soaking, the resulting extract passed through the Whatman No. 4 filter paper to remove impurities. Subsequently, the filtered solution was evaporated using a vacuum rotary evaporator RV 10 Digital V-C (IKA, Germany) at 40°C, creating a concentrated extract solution (named REEF). The extract solution was stored in glass vials at 4°C, shielded from light, for subsequent experiments.

Screening and quantification of phytochemicals in extracts

Screening of phytochemicals: To verify the existence of bioactive compound treatments in REEF, plant chemical screening was conducted using standard methods outlined by Tran et al. (8). The tests covered tannins (Braymer test), flavonoids (acetate lead test), saponins (foam test), steroids (Salkowski test), cardiac glycosides (Keller–Kelliani test), alkaloids (Hager test), proteins (Xanthoproteic test), phenolic compounds (iron chloride test), and terpenoids (Liebermann–Burchard test).

Quantification of phytochemicals: The extract's total phenol and flavonoid content were assessed using standard methods outlined by Tran et al. (8). Total phenol content (TPC) was determined through the Folin–Ciocalteu method, presented as Gallic acid equivalents (GAE) per gram of crude extract. The total flavonoid content (TFC) was determined by forming an aluminum-flavonoid complex, expressed as Quercetin equivalents (QE) per gram of crude extract. Each sample underwent three analyses, and the average values were computed.

Experimental animals

All experiments utilized healthy Swiss albino mice (30 ± 2 g, 6-7 weeks old) obtained from the Pasteur Institute, Ho Chi Minh City. The mice were housed in glass cages for a week to acclimate to the new environment at the Experimental Animal Farm of the East Agriculture and Food Company in Ho Chi Minh City. The animal facility maintained a temperature of 24 ± 2°C and a relative humidity of approximately 60 - 65%. A 12-hour light/12-hour dark cycle was implemented, and the mice had unrestricted access to water, along with standard rodent food trays. Adherence to ethical principles in animal research, outlined in the Basel Declaration on Animal Research (9), was stringent. All animal handling procedures and experimental processes followed the rigorous standards and guidelines established by the Animal Ethics Committee of Ho Chi Minh City University of

Industry, ensuring the ethical treatment and welfare of animals throughout the research.

Analgesic activity

The formalin test: The formalin-induced pain model was created following Nhung and Quoc's experimental protocol with minor adjustments (10). Thirty Swiss albino mice were divided into different treatments (n=5 per treatment) and were administered treatments: normal saline (10 mL/kg), REEF (50, 100, and 150 mg/kg), aspirin (ASA, 150 mg/kg), and tramadol (TRAMA, 10 mL/kg). After 30 minutes, we injected a 50 μ l 2% formalin solution under the skin of the left hind paw. Pain behaviors, such as licking, biting, or shaking of the injected paw, were quantified in terms of frequency and duration. The recorded time (in seconds) spent on these activities reflected the pain response observed within the 0-15 minute interval (early phase, acute phase, and intermediate phase) and the 15-30 minute interval (late phase, sub-chronic phase, and inflammatory phase) following formalin injection. The percentage of pain inhibition (PPI, %)

$$\text{PPI (\%)} = \frac{\text{Latency (test-control)}}{\text{Latency test}} \times 100 \quad (\text{Eq.1})$$

was then calculated using a specific formula.

The measurement of cyclooxygenases-2 (COX-2) and prostaglandin E2 (PGE2) levels in the formalin test:

Mice blood samples were collected at 0 and 30 minutes during the formalin experiment through jugular vein extraction. The serum was obtained by centrifuging at 10,000 rpm for 10 minutes. The initial step involved incubating arachidonic acid, cyclooxygenase, and test samples to generate prostaglandin. Subsequently, COX-2 and PGE2 levels were assessed using the ELISA kit (ADI-901-001, USA). Samples and standards were added to pre-coated ELISA wells with COX-2 and PGE2 antibodies, followed by HRP (Horseradish peroxidase) addition at 37°C. After 1 hour, the wells were washed thrice with a wash solution. Reagents A and B (compounds interacting with COX-2 and PGE2) were added, and incubated at 37°C for 20-25 minutes, and the reaction was halted with a solution. Absorbance at 450 nm was measured using an ELISA reader. COX-2 and PGE2 concentrations were determined by comparing optical density values with a standard curve (11).

Antipyretic activity

Thirty mice were divided into six treatments (5 mice/treatment): Saline treatment (received sterile saline, 10 mL/kg, no fever induction), Yeast treatment (fever induced by yeast injection, 10 mL/kg, no additional treatment), Yeast+PCM treatment (fever induced by yeast injection, oral paracetamol 150 mg/kg), and Yeast+REEF₅₀₋₁₅₀ treatment (fever induced by yeast injection, followed by REEF administration at doses of 50, 100, and 150 mg/kg). Rectal temperature was measured using a Laserliner 082.030A digital thermometer (China). Injection of 44% yeast in saline (10 mL/kg) was confirmed to induce fever in

all mice. Before the experiment, animals fasted overnight with ad libitum access to water. The baseline rectal temperature (time = 0) was measured 18 hours after yeast injection. Animals with a rectal temperature increase of approximately 0.3 - 0.5°C were selected for antipyretic activity assessment. Rectal temperature changes were

$$\text{PFR (\%)} = \frac{(H - \text{In})}{H - M} \times 100 \quad (\text{Eq.2})$$

monitored and recorded hourly for 3 consecutive hours post-dose administration. The fever reduction ratio (PFR) was calculated using a formula (11).

Using the variables: H for post-fever temperature, In for the temperature after 1, 2, and 3 hours, and M for normal body temperature.

Anti-inflammatory activity

Carrageenan-induced paw edema: We explored the anti-inflammatory effects using the carrageenan-induced paw edema model, injecting a 1% carrageenan (CGN) solution (50 μ L per mouse) into the subplantar area of the right hind paw. Mice were treated into six, each with five animals. Before carrageenan injection, various substances were orally administered: REEF (100, 150, and 200 mg/kg),

$$\text{PFR (\%)} = \frac{A - B}{A} \times 100 \quad (\text{Eq.3})$$

saline solution (oral), or indomethacin (IND) (10 mg/kg), 30 minutes beforehand. Paw edema, measured in millimeters as paw diameter, was assessed using a Mitutoyo digital caliper (Japan) at 0, 1, 2, 3, 4, and 5 hours post-carrageenan injection. Edema inhibition percentage (PFR) was then calculated (11).

A represents paw edema in the control treatment, and B represents paw edema in the test treatment.

Assays of cytokines in CGN-induced paw edema: TNF- α , IL-1 β , and IL-6 were assessed via ELISA following Bhowmick *et al.*'s protocol (11). Specific antibodies for TNF- α , IL-1 β , and IL-6 were incubated in individual wells of a 96-well plate overnight. On the subsequent day, a second biotin-labeled antibody was applied to the sample tissues or standard antigens before introducing streptavidin-HRP (Horseradish peroxidase). Quantification of TNF- α , IL-1 β , and IL-6 levels occurred at 450 nm. Concentrations were reported in protein pg/mg.

Statistical analysis

The data underwent analysis and computation using Statgraphics Centurion 19 software (USA) and were presented as mean \pm SD. Significance was assessed through one-way ANOVA, followed by Tukey's post hoc test. A p-value < 0.05 was deemed statistically significant in all instances.

Results

Screening and quantification of phytochemicals in

Table 1. Chemical screening of the ethanol extract from *E. fusca* roots

Plant chemical constituents	Presence in REEF	Plant chemical constituents	Presence in REEF
Alkaloids	+	Saponins	+
Tannins	+	Flavonoids	+
Steroids	+	Phenolics	+
Terpenoids	+	Cardiac glycosides	-

The presence of plant chemicals in REEF: - absent, + present.

Table 2. Quantification of total phenolic and flavonoid content in the ethanol extract from *E. fusca* roots

Sample	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
REEF	73.33 ± 3.18	40.46 ± 1.77

Note: GAE: Gallic acid equivalent, QE: quercetin equivalents

extracts

The ethanol extract from *E. fusca* roots was subjected to chemical screening, indicating the existence of secondary metabolites including saponins, flavonoids, terpenoids, steroids, tannins, alkaloids, and phenolics, while cardiac glycosides were not detected in the extract (Table 1). The TPC in the ethanol extract of *E. fusca* roots is measured at 73.33 mg GAE/g. Similarly, it exhibits a TFC of 40.46 mg QE/g (Table 2).

Analgesic activity

Formalin test

The findings reveal that REEF consistently diminishes licking and biting duration in response to formalin-

induced pain in mice. Across all doses (100, 150, and 200 mg/kg) of REEF, a significant reduction in licking time is observed in both phases ($p < 0.05$) compared to the control *treatment*. This underscores REEF's reliable pain-alleviating impact in both the early (acute phase) and late phases, indicating its capability to inhibit both acute pain perception and inflammation-induced pain. The pain-relief efficacy of REEF is supported by the licking time chart, demonstrating a concurrent and stable reduction in pain over time (Fig. 1).

The plasma levels of COX-2 and PGE2 in formalin test

Results from Table 4 indicate a significant reduction in COX2 and PGE2 concentrations compared to the control *treatment* ($p < 0.05$) in the formalin-induced pain experiment with REEF. Notably, the decrease in COX2 and PGE2 levels in the late phase is even more pronounced than in the early phase ($p < 0.05$), with the most substantial reduction observed in the REEF 150 mg/kg *treatment* ($p < 0.05$). This suggests that REEF can inhibit intermediaries like COX2 and PGE2, thereby alleviating pain sensations in both the early and late phases of the formalin-induced pain experiment.

Antipyretic activity

The findings reveal that root extract of *E. fusca* (REEF) significantly diminishes fever in mice (Table 5). The most pronounced antipyretic effect is seen in *treatments* treated with REEF (50, 100, 150 mg/kg) three hours post-yeast injection ($p < 0.05$). Notably, REEF at 150 mg/kg demonstrates robust antipyretic activity, reducing rectal temperature from 39.71 ± 0.13 °C to 37.24 ± 0.14 °C three hours after administration. The antipyretic impact of the extract (yeast+REEF₅₀, yeast+REEF₁₀₀, and yeast+REEF₁₅₀ *treatment*) at 1 hour, 2 hours, and 3 hours contrasts with the yeast *treatment* (Fig. 2, $p < 0.05$). These results

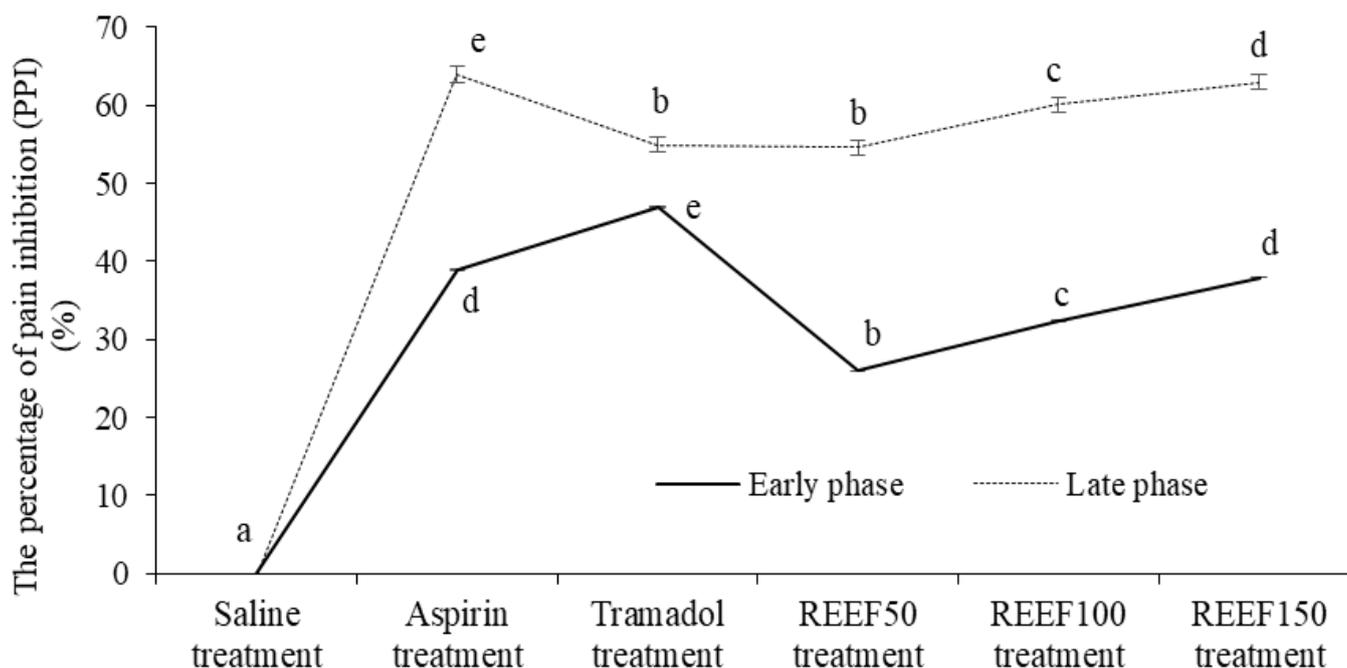


Fig. 1. The percentage of pain inhibition by the ethanol extract from *E. fusca* roots was investigated in a formalin-induced pain model. Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

Table 3. The analgesic effects of the ethanol extract from *E. fusca* roots investigation in a formalin-induced pain model

Experiment phase	Paw licking time (seconds)					
	Saline treatment	Aspirin treatment	Tramadol treatment	REEF ₅₀ treatment	REEF ₁₀₀ treatment	REEF ₁₅₀ treatment
Early phase	20.05 ± 0.41 ^a	32.86 ± 0.11 ^e	31.83 ± 0.11 ^f	27.08 ± 0.21 ^b	29.66 ± 0.21 ^c	32.31 ± 0.15 ^d
Later phase	18.13 ± 0.11 ^a	50.36 ± 0.52 ^e	40.25 ± 0.22 ^b	39.99 ± 0.26 ^b	45.52 ± 0.31 ^c	48.98 ± 0.34 ^d

Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

Table 4. The effect of *E. fusca* root extract on plasma concentrations of COX-2 and PGE2 was investigated in the formalin test

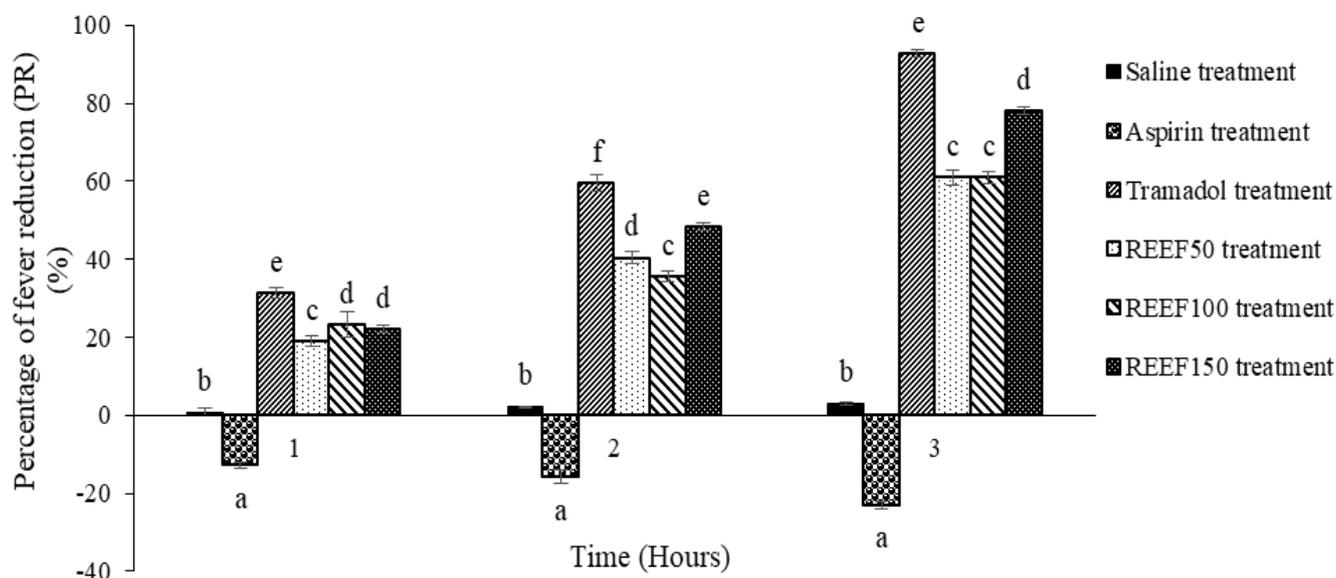
Parameters	Time	Saline treatment	Aspirin treatment	Tramadol treatment	REEF ₅₀ treatment	REEF ₁₀₀ treatment	REEF ₁₅₀ treatment
COX-2 levels (ng/mL)	15 min	6.89 ± 0.12 ^e	4.92 ± 0.13 ^b	4.53 ± 0.11 ^a	5.58 ± 0.11 ^d	5.43 ± 0.12 ^d	5.09 ± 0.12 ^c
	30 min	7.94 ± 0.22 ^f	3.94 ± 0.11 ^a	4.22 ± 0.14 ^b	5.04 ± 0.12 ^e	4.65 ± 0.11 ^d	4.43 ± 0.11 ^c
PGE2 levels (ng/mL)	15 min	0.88 ± 0.09 ^c	0.65 ± 0.04 ^b	0.58 ± 0.05 ^a	0.71 ± 0.06 ^b	0.69 ± 0.04 ^b	0.65 ± 0.04 ^b
	30 min	0.96 ± 0.06 ^d	0.45 ± 0.04 ^a	0.49 ± 0.05 ^{ab}	0.59 ± 0.05 ^c	0.55 ± 0.04 ^{bc}	0.52 ± 0.04 ^b

Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

Table 5. The effect of the ethanol extract from *E. fusca* roots on yeast-induced fever

Treatments	Initial (°C)	Fever (°C)	1 hour (°C)	2 hours (°C)	3 hours (°C)
Saline treatment	36.67 ± 0.12 ^{abc}	36.77 ± 0.12 ^a	36.69 ± 0.19 ^a	36.56 ± 0.14 ^a	36.48 ± 0.14 ^a
Yeast treatment	36.55 ± 0.12 ^a	39.12 ± 0.12 ^f	39.45 ± 0.13 ^e	39.52 ± 0.14 ^f	39.71 ± 0.13 ^f
Yeast+PCM treatment	36.74 ± 0.11 ^{bc}	38.31 ± 0.11 ^b	37.82 ± 0.13 ^b	37.37 ± 0.13 ^b	36.85 ± 0.13 ^b
Yeast+ REEF ₅₀ treatment	36.81 ± 0.13 ^c	39.03 ± 0.11 ^e	38.61 ± 0.14 ^d	38.14 ± 0.13 ^e	37.68 ± 0.13 ^e
Yeast+ REEF ₁₀₀ treatment	36.59 ± 0.12 ^{ab}	38.71 ± 0.11 ^d	38.22 ± 0.14 ^c	37.96 ± 0.13 ^d	37.42 ± 0.14 ^d
Yeast+ REEF ₁₅₀ treatment	36.62 ± 0.11 ^{ab}	38.53 ± 0.13 ^c	38.11 ± 0.16 ^c	37.61 ± 0.13 ^c	37.04 ± 0.14 ^c

Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

**Fig. 2.** The percentage of fever reduction by the ethanol extract from *E. fusca* roots was assessed in a yeast-induced fever model. Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

underscore the significant antipyretic properties of *E. fusca* root extract following yeast injection in mice. The peak antipyretic efficacy of REEF is evident at the 3-hour mark post-yeast injection, particularly at a dosage of 150 mg/kg. This extract not only effectively lowers body temperature but also counteracts the fever-inducing effects triggered by yeast at the assessed time points (1 hour, 2 hours, and 3 hours) when compared to the yeast-treated without the extract. This implies that REEF holds promise in alleviating fever caused by factors like yeast.

Anti-inflammatory activity

Carrageenan-induced paw edema

The data in Table 6 reveals that REEF demonstrates anti-inflammatory properties in the carrageenan-induced paw edema study. This anti-inflammatory efficacy is contingent on both time and concentration. Noticeable variations in paw diameter emerge between the REEF-treated and the

control treatment during the initial 2 hours ($p < 0.05$). The decrease in paw swelling becomes more pronounced at 4 and 5 hours. At the 5-hour mark, the REEF-treated at 150 mg/kg exhibits significantly superior anti-inflammatory effects ($p < 0.05$) with a paw diameter reduction (3.62 ± 0.06 mm) compared to the yeast treatment (9.82 ± 0.05 mm) and is nearly on par with the indomethacin-treated (3.59 ± 0.05 mm). In the early inflammatory phase, the reduction in paw swelling is 81.94% (first hour) and 87.92% (third hour) ($p < 0.05$), with progressive improvement. The peak anti-inflammatory efficacy (92.83%) ($p < 0.05$) is observed after the 5th hour of the experiment (Fig. 3). These findings affirm the substantial anti-inflammatory effects of REEF in the carrageenan-induced paw edema study, particularly highlighted at the 150 mg/kg dosage and reaching maximum efficacy post the 5th hour.

Investigating plasma cytokine levels in the context of

Table 6. Ethanol extract from *E. fusca* roots effects on carrageenan-induced paw edema in mice

Treatments	Diameter paw (mm)					
	0 h	1 h	2 h	3 h	4 h	5 h
Saline treatment	3.41 ± 0.05 ^a	3.37 ± 0.05 ^a	3.37 ± 0.05 ^a	3.38 ± 0.05 ^a	3.37 ± 0.05 ^a	3.36 ± 0.04 ^a
CGN treatment	3.38 ± 0.05 ^a	4.24 ± 0.05 ^f	5.66 ± 0.05 ^f	6.95 ± 0.04 ^f	8.25 ± 0.03 ^f	9.82 ± 0.05 ^f
CGN + IND treatment	3.37 ± 0.06 ^a	3.96 ± 0.07 ^b	3.84 ± 0.04 ^b	3.76 ± 0.04 ^b	3.64 ± 0.04 ^b	3.59 ± 0.05 ^b
CGN + REEF ₁₀₀ treatment	3.38 ± 0.05 ^a	4.22 ± 0.06 ^e	4.14 ± 0.04 ^e	4.09 ± 0.06 ^e	3.97 ± 0.05 ^e	3.87 ± 0.04 ^e
CGN + REEF ₁₅₀ treatment	3.39 ± 0.07 ^a	4.14 ± 0.04 ^d	4.08 ± 0.05 ^d	3.96 ± 0.07 ^d	3.89 ± 0.05 ^d	3.75 ± 0.04 ^d
CGN + REEF ₂₀₀ treatment	3.36 ± 0.04 ^a	4.11 ± 0.06 ^c	3.95 ± 0.04 ^c	3.84 ± 0.04 ^c	3.76 ± 0.03 ^c	3.62 ± 0.06 ^c

Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

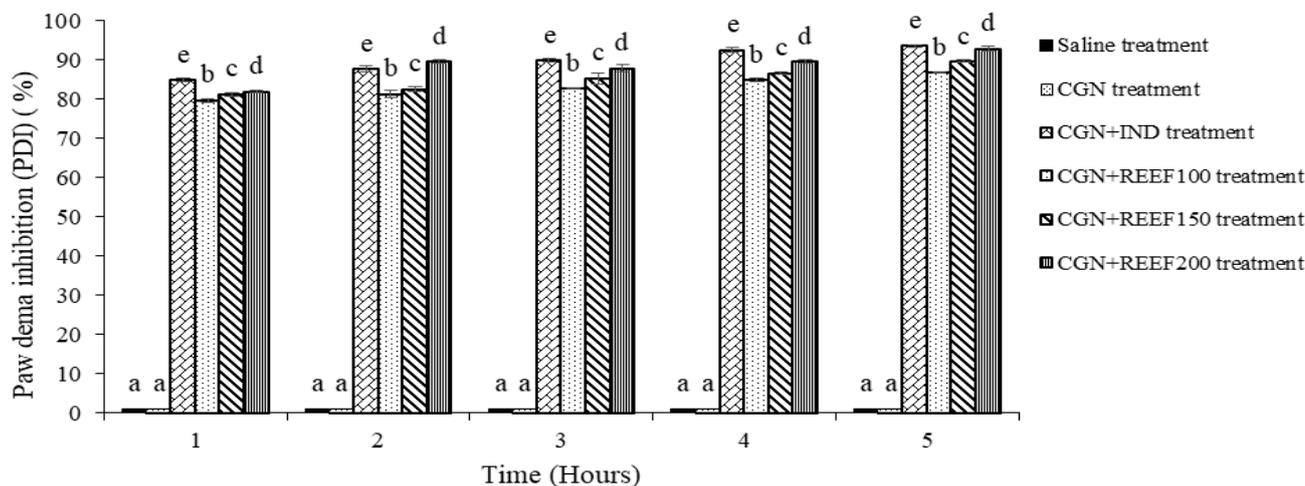


Fig. 3. Paw edema inhibition by the ethanol extract from *E. fusca* roots was assessed in a yeast-induced fever model. Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

Table 7. Effect of the ethanol extract from *E. fusca* roots on cytokines level in the edema paws

Parameters	Saline treatment	CGN treatment	CGN + IND treatment	CGN + REEF ₁₀₀ treatment	CGN + REEF ₁₅₀ treatment	CGN + REEF ₂₀₀ treatment
TNF- α (pg/mL)	138.82 ± 15.05 ^a	289.15 ± 18.05 ^d	165.66 ± 13.98 ^b	230.17 ± 18.11 ^c	223.86 ± 16.17 ^c	185.05 ± 12.97 ^b
IL-1 β (pg/mL)	292.48 ± 13.24 ^a	680.19 ± 18.87 ^f	369.29 ± 14.01 ^b	471.74 ± 15.88 ^e	449.97 ± 14.98 ^d	400.56 ± 16.27 ^c
IL-6 (pg/mL)	26.63 ± 1.03 ^a	98.63 ± 1.11 ^f	32.88 ± 2.09 ^b	51.21 ± 1.14 ^e	46.72 ± 1.18 ^d	38.03 ± 1.02 ^c

Values are shown as Mean ± SD, with letters (a, b, c, d, and e) indicating significant treatment differences ($p < 0.05$).

carrageenan-induced paw edema

Table 7 illustrates the evaluation of inflammatory mediators responsible for carrageenan-induced pain in mice and the impact of *E. fusca* root extract on serum concentrations of inflammatory cytokines. Mice treated with REEF (100, 150, and 200 mg/kg) exhibited a substantial reduction ($p < 0.05$) in serum levels of TNF- α , IL-1 β , and IL-6 compared to the control treatment. The ethanol extract of *E. fusca* root significantly decreased ($p < 0.05$) the concentrations of all these inflammatory mediators.

Discussion

Erythrina fusca a well-known medicinal plant, has been traditionally used in various countries. In Peru, the bark eases migraine pain, while in Thailand, both the bark and leaves are employed for inflammation reduction. In Indonesia, the seeds address skin irritations, and the flowers are consumed as vegetables. The plant exhibits diverse biological activities, including antiviral, anti-inflammatory, central nervous system inhibition, fever reduction, and pain relief (12). Numerous reports emphasize the pain-relieving, fever-reducing, and anti-inflammatory effects of *Erythrina* genus plants. For example, *Erythrina indica* leaves show analgesic and anti-inflammatory properties (13), and *Erythrina variegata* and *Erythraea centaurium* leaves have pain-relieving, fever-reducing, and anti-inflammatory capabilities (14, 15). In this study, the ethanol extract of *E. fusca* roots, containing plant-derived compounds like flavonoids, phenolics, tannins, terpenoids, etc., was examined for its efficacy in pain relief, fever reduction, and anti-inflammatory activities in an animal model.

Plant secondary metabolites, including flavonoids, phenolics, tannins, terpenoids, alkaloids, etc., are synthesized through secondary metabolic pathways following the conversion of primary metabolites like carbohydrates. These compounds play vital and diverse biological roles, with their mechanisms often stemming from intricate interactions. They contribute to the plant's antioxidative, analgesic, antipyretic, and anti-inflammatory effects (16). Specific compounds like flavonoids and polyphenols showcase antioxidative properties, protecting cells from free radical damage, mitigating inflammation, and relieving pain. Certain secondary metabolites can inhibit pro-inflammatory enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing the production of pain- and inflammation-inducing compounds such as prostaglandins and leukotrienes. Others, like alkaloids, may interact with the nervous system, diminishing nerve stimulation and pain perception. Moreover, select metabolites can regulate the immune system, curtailing excessive inflammation and fortifying resistance against viruses and bacteria, aiding in infection control and inflammation reduction (17).

Following formalin injection into the mouse paw, immediate and brief pain responses emerge, characterized by licking, biting, or shaking of the injected paw. The early phase (acute and intermediate) spans 0 to 15 minutes,

during which formalin directly activates pain receptors (pain-sensing nerve fibers). Pain perception temporarily diminishes as descending inhibitory pathways are activated to reduce pain signals. The late phase (sub-chronic and inflammatory) initiates around 15 minutes post-formalin injection, lasting until 30 minutes. Prolonged licking and biting behaviors in this phase indicate persistent pain due to a combination of inflammatory processes and central sensitization. Formalin induces local inflammation at the injection site, releasing inflammatory mediators like prostaglandins, bradykinin, and histamine. These substances sensitize pain receptors, heightening their responsiveness to pain stimuli. Formalin-induced pain is linked to central sensitization, where the central nervous system undergoes changes amplifying pain signals, contributing to prolonged pain reactions during the late phase (18). Following formalin injection, COX-2 (Cyclooxygenase-2) undergoes a robust stimulation, being synthesized in response to inflammatory conditions or environmental damage. COX-2 plays a pivotal role in converting arachidonic acid into prostaglandins, particularly PGE2 (prostaglandin E2), a key contributor to pain sensation and the inflammatory cascade. PGE2 amplifies pain signals, activating nociceptors – nerve fiber sensors for pain perception. Additionally, it fuels the inflammatory process and heightens the central nervous system's sensitivity to pain (central sensitization). The escalated COX-2 activity and PGE2 production in the damaged environment post-formalin injection critically drive the pain induction process, leading to prolonged pain responses in the subsequent experimental period (19). Our research demonstrates a significant decrease in biting and licking behaviors induced by formalin at all dosage levels (Table 3) during both phases with the *E. fusca* root extract. This indicates that the *E. fusca* root extract (REEF) effectively mitigates pain by addressing both early nociceptive pain and, notably, late-phase inflammatory pain, showcasing its dual impact on peripheral and central pain transmission pathways. Additionally, there is a noteworthy reduction in COX-2 and PG2 levels in treatments with the extract and standard medication compared to the saline control treatment (Table 4). The inhibition ratio of licking responses in the late phase exceeds that of the early phase across all treatment methods (Fig. 1), potentially linked to the decrease in the production or inhibition of inflammatory mediators such as prostaglandin, adenosine, and histamine in the periphery (20). These groundbreaking findings emphasize the dual pain-relieving effects of REEF on both peripheral and central pain transmission pathways.

Fever, often resulting from cellular infection, tissue damage, malignancy, graft rejection, or other pathological conditions, is characterized by an excessive formation of inflammatory mediators (IL-1 β , TNF- α , etc.). These mediators trigger the synthesis of prostaglandin E2 (PGE2) and PGI2 in the hypothalamus, leading to an increase in body temperature (21). The induction of fever by fungi is a common method to evaluate the antipyretic effects of substances. Fungi contain various proteins linked to fever

through the synthesis of diverse fever-inducing cytokines, interleukins, and prostaglandin intermediates. Inhibiting prostaglandin (PG) synthesis, achieved by reducing cyclooxygenase enzyme activity, may contribute to antipyretic effects (22). REEF at doses of 50, 100, and 150 mg/kg exhibits significant antipyretic effects in mice (Table 5 and Fig. 2), with a body temperature reduction comparable to the standard indomethacin drug. Hence, we propose that REEF may possess antipyretic properties through the discussed mechanism.

The carrageenan-induced paw edema model in mice represents a two-phase inflammation process. In the initial stage (0–2 hours), histamine and bradykinin release predominantly induce paw edema by widening blood vessels and increasing permeability. The subsequent inflammatory stage (2–6 hours) relies on prostaglandin release facilitated by intermediaries like leukotrienes and bradykinin, sustaining inflammation (23). Post-REEF administration, there is a notable reduction in paw edema diameter, emphasizing the extract's enduring anti-inflammatory effects. The study also illustrates REEF's ability to diminish inflammation in both stages, suggesting its potential to impede the production of aforementioned inflammatory mediators. Elevated TNF- α concentration, resulting from external damage, can potentially trigger inflammation and pain while concurrently stimulating the expression and synthesis of nitric oxide synthase (iNOS) and interleukins like IL-6 and IL-1 β . Hence, TNF- α plays a pivotal role in body protection, anti-inflammatory responses, and immune reactions (24). REEF significantly diminishes TNF- α , IL-6, and IL-1 β concentrations, demonstrating its potential anti-inflammatory activity by inhibiting iNOS expression and interleukin synthesis through TNF- α pathway inhibition.

Conclusion

The root extract of *E. fusca* (REEF) consistently mitigates nociceptive responses in formalin-exposed mice, characterized by a notable reduction in COX2 and PGE2 levels. Moreover, REEF demonstrates a significant antipyretic effect in mice, reaching its zenith three hours post-administration. After REEF treatment, paw edema exhibits a marked decrease at the 4 and 5-hour intervals, particularly at 150 mg/kg. Furthermore, REEF significantly reduces serum concentrations of TNF- α , IL-1 β , and IL-6. These findings underscore the therapeutic potential of *E. fusca* root extract in alleviating pain, reducing fever, and exerting anti-inflammatory effects, underscoring its pivotal role in developing herbals with these attributes.

Authors' contributions

TTPN designed and conducted this research. This author also wrote the manuscript. LPTQ revised the final version of the manuscript and was responsible for working with the Journal.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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